

Relationship between copper exposure duration, tissue copper concentration, and rainbow trout growth

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Abstract

Rainbow trout fry were exposed in soft water to sublethal concentrations of copper for 60 days under controlled laboratory conditions. At 20-day intervals, fish were sampled for weight, length, and whole-body copper and metallothionein concentrations. Exposures to waterborne copper concentrations as low as $4.6 \mu\text{g l}^{-1}$ resulted in significantly reduced growth and significantly elevated whole-body copper concentrations after 20 days. Whole-body metallothionein concentrations did not differ significantly from controls. Fish did not recover or return to control growth rates throughout the entire exposure period; a 45% reduction in mean weight relative to controls observed on day 40 in the $9.0 \mu\text{g l}^{-1}$ Cu exposure was sustained through day 60. Whole-body accumulation rates of copper in fish exposed to $4.6 \mu\text{g l}^{-1}$ and higher levels of Cu increased significantly between 0 and 40 days and appeared to reach steady-state after 40 days. Copper accumulation was found to depend on dose and time. Trout exposed to higher copper concentrations accumulated more whole-body copper, with longer times to reach steady-state. Our data suggest that both accumulation capacity and copper depuration rates from a slowly exchangeable pool are concentration-dependent. A linear model was developed for the relationship between exposure duration, copper accumulation, and fish weight: $\ln(\text{wet wt., } \mu\text{g}) = 4.8 + 0.03(\text{exposure duration, days}) - 0.04(\text{whole-body copper, mg g}^{-1} \text{ dry wt.})$ ($P < 0.01$, $R^2 = 0.94$). Thus, growth reductions are predictable from tissue residues if exposure durations are known. Although exposure times may not be known for field-collected fish, residues may have utility in evaluating adverse effects on fish sampled in the wild because residues integrate exposure over time, space, and exposure route.

Keywords: Copper; Rainbow trout; Growth; Accumulation

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1. Introduction

Various studies have demonstrated that Cu tissue accumulation in fish can be associated with adverse physiological responses (e.g. Benoit, 1975; Dixon and Sprague, 1981a; Collvin, 1985; Marr et al., 1995). Exposure to Cu has been shown to cause reduced growth, often with impacts to specific growth rates most pronounced during initial exposure periods (Lett et al., 1976; Waiwood and Beamish, 1978; Dixon and Sprague, 1981b; Collvin, 1984; Seim et al., 1984). Confounding factors such as acclimation, feeding efficiency or behaviors, feed conversion, diet composition, and exposure regime may explain inconsistencies among Cu exposure studies showing initial growth reductions followed by complete or partial recovery of growth to control levels (e.g. Lett et al., 1976; Dixon and Sprague, 1981b; Collvin, 1984; Seim et al., 1984; Lanno et al., 1989). Metallothionein (MT) induction has been related both to Cu accumulation and growth reductions (Dixon and Sprague, 1981a; Marr et al., 1995). This had led to the use of MT as a biomarker of Cu exposure and/or adverse effects (Hogstrand et al., 1991).

Alternatively, tissue residues have been used as a means of determining metals exposure associated with adverse response measurements in field-collected fish (Miller et al., 1992; Farag et al., 1995). For example, Farag et al. (1995) found elevated tissue concentrations of metals in field-collected trout to be associated with tissue abnormalities and cellular damage. Using Cu tissue residues to predict reduced growth responses could offer advantages over exposure concentrations, because the metabolic demands associated with detoxifying or acclimating to Cu may be more directly related to internal accumulation of Cu. Yet, relationships between tissue Cu and adverse responses have not been adequately quantified. However, a recent toxicokinetic study of Cu in rainbow trout suggested that long-term waterborne Cu exposure resulting in continued Cu accumulation in slowly exchangeable pools should not result in toxicological consequences (Carbonell and Tarazona, 1994).

In this study we examined whether growth responses in rainbow trout fry exposed to waterborne Cu could be related to whole-body Cu accumulation and/or exposure duration. The specific objectives of our laboratory study were to determine the sensitivity of growth responses in rainbow trout exposed to sublethal Cu concentrations and to determine whether prolonged exposure resulted in increased whole-body Cu accumulation and/or MT concentrations.

2. Methods

The experiment was conducted at the Red Buttes Environmental Biology Laboratory, University of Wyoming, Laramie, Wyoming, USA.

2.1. *Experimental design, exposure water and system*

A randomized block design was used to assign control and Cu exposure dilutions

to aquaria. Exposure treatments consisted of six total Cu levels (nominal 0.0, 0.6, 1.2, 2.5, 5.0, or 10.0 $\mu\text{g l}^{-1}$). Each block contained one replicate of each exposure dilution and a control; six blocks were used, for a total of 36 aquaria. Cu concentrations were achieved by metering (Fluid Metering, Inc., QG-20 Laboratory pump) a Cu stock solution via Mariotte bottles into the dilution box of a continuous-flow, proportional diluter. The stock solution was prepared from reagent grade Cu salt ($\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$) dissolved in deionized water.

The proportional diluter delivered exposure and control waters to each aquarium at 0.2 l min^{-1} , providing a flow rate of 288 l day^{-1} (36 volume renewals day^{-1}) and a 90% volume replacement time of less than 1.5 h (Sprague, 1969). Waters were formulated by continuously mixing well and deionized water (well water treated with sediment filtration, reverse osmosis, and separate-bed deionization) to attain the following nominal water quality conditions: hardness, 25 mg l^{-1} as CaCO_3 ; alkalinity, 25 mg l^{-1} as CaCO_3 ; pH, 7.5; temperature, 10°C. Fiberglass headtanks (190 l; Frigid Units, Inc.) continuously received the formulated water that was adjusted to the desired pH by automatic pH analyzer/controllers (Leeds and Northrup model 7084) using dilute H_2SO_4 and KOH. Temperature and pH were monitored and recorded continuously with a Hewlett Packard (model 3497A) data-acquisition and alarm system. Exposure and control waters were analyzed daily to ensure that the water quality parameters were within 10% of the desired levels for hardness, alkalinity, pH, dissolved oxygen, and temperature.

2.2. Test procedure

Rainbow trout (*Oncorhynchus mykiss*) eyed eggs were obtained from a hatchery source and were hatched at the laboratory in incubators using well water (hardness, 220 mg l^{-1} as CaCO_3 ; alkalinity, 180–210 mg l^{-1} as CaCO_3 ; pH, 7.2–7.8; temperature, 4°C). After hatching, the trout were transferred to circular holding tanks and acclimated for more than 14 days to the control water used in the bioassay (hardness, 25–30 mg l^{-1} as CaCO_3 ; alkalinity, 22–28 mg l^{-1} as CaCO_3 ; pH, 7.3–7.6; temperature, 10°C). Fish condition and health were monitored daily, and fish remained disease-free; there was no mortality during acclimation to soft water. At the swim-up stage, trout were fed frozen BioDiet trout food once daily at a ration in excess of 4.5% (wet wt. food/wet wt. body). Throughout the Cu exposures, the trout were maintained at 4.5% rations; this was determined daily for each exposure concentration based on the average biomass per replicate. Approximately 3 h after feeding, each aquarium was siphoned to remove debris. Mean (\pm SD) Cu content of the diet used in this study was $8.7 \pm 0.9 \text{ mg kg}^{-1}$, wet wt. ($n = 3$). A 12 h light/dark cycle was used throughout the study.

At the initiation of the bioassay, swim-up fry had a mean (\pm SD) wet weight of $0.120 \pm 0.031 \text{ g}$ ($n = 60$) and a total length of $25.7 \pm 2.2 \text{ mm}$ ($n = 60$). The fry were transferred from holding tanks to exposure aquaria and continuously exposed to Cu concentrations for 60 days. Fry were observed daily to evaluate mortality and abnormal locomotory and feeding behaviors. At 20-day intervals, ten live fish

from each replicate were collected and sacrificed for measurement of wet weight, total length, whole-body Cu concentration, and whole-body MT concentration.

After the weight and length measurements, the ten fish within each replicate were combined and ground under liquid nitrogen with a stainless steel mortar and pestle. Ground tissue samples were split into two aliquots and stored at -70°C for further processing. Aliquots for Cu concentration determinations were lyophilized to constant dry weights and digested in 30% HNO_3 (Instra-Analyzed). Digested tissues were brought to volume with deionized water and analyzed for Cu concentrations with atomic absorption spectrophotometry (AAS) using a Varian Spectra AA-600 equipped with graphite furnace and Zeeman background correction (method detection limit for Cu, $0.9 \mu\text{g l}^{-1}$). Aliquots for MT determinations were partially thawed, weighed, and homogenized in 50 mM Tris-HCl (pH 8.0, 1°C , at a minimum of 1:3 weight:volume ratio). The homogenate was centrifuged at $8800 \text{ rev min}^{-1}$, 4°C for 10 min. Then, $100 \mu\text{l}$ of each supernatant were extracted, frozen in liquid nitrogen, and stored at -70°C for MT analysis. MT concentrations were determined by a competitive double-antibody radioimmunoassay (RIA) (Hogstrand and Haux, 1990), as modified for rainbow trout MT by Hogstrand et al. (1994).

Water samples were collected every 3–6 days from randomly determined replicate tanks to determine water quality parameters, Cu concentrations, and concentrations of major cations and anions. Water samples (25 ml) for Cu and other cation analyses were preserved with $25 \mu\text{l}$ of 70% HNO_3 (Instra-Analyzed grade) and analyzed using either graphite furnace AAS or flame AAS (Perkin-Elmer model 2380). Water samples (25 ml) for anion analyses were stored at 4°C in the dark and analyzed using ion chromatography. Method detection limits for the cations and anions were as follows: Cu, $0.9 \mu\text{g l}^{-1}$; Ca, 0.09 mg l^{-1} ; K, 0.1 mg l^{-1} ; Mg, 0.01 mg l^{-1} ; Na, 0.06 mg l^{-1} ; Cl, 0.12 mg l^{-1} ; SO_4 , 0.34 mg l^{-1} . Table 1 shows a summary of the water chemistry data from the control and different exposure levels.

Cu speciation was modeled using the computer program MINEQL⁺ (Schecher and McAvoy, 1991). Input data for water quality parameters and measured cations and anions were the mean values for each Cu exposure level (Table 1). Chemical equilibrium constants used in model calculations were adopted from the MINEQL⁺ database, or from Sunda and Hanson (1979) for Cu-hydroxide and Cu-carbonate species. In each of the Cu exposure levels, the percentage of total Cu for the Cu species was as follows: 21% for Cu^{2+} ; 19% for $\Sigma\text{Cu}(\text{OH})_x^{2-x}$; and 60% for $\Sigma\text{Cu}(\text{CO}_3)_x^{2-2x}$ species (Table 2).

2.3. Statistical analysis

Differences in fish growth, whole-body Cu, and whole-body MT in samples collected for each of the sample periods were tested by analysis of variance (ANOVA). In cases where the ANOVA showed significant effects caused by Cu concentration, Dunnett's multiple comparison procedure (Zar, 1984) was used to make comparisons to control measurements and to determine threshold effects concentrations (Gelber et al., 1985). Within each Cu exposure level, differences in tissue

concentration of Cu at successive time periods were compared using Kruskal–Wallis tests to evaluate changes in tissue accumulation of Cu over time.

Pearson's correlation coefficients were used to evaluate relationships between Cu exposure concentration, Cu tissue concentration, and growth responses on each sample day. Multiple regression analysis was used to examine relationships between exposure duration, tissue residues, and growth. Several candidate models were examined with respect to the quality of fit; subsequently fish weight was ln-transformed for models evaluating fish growth, because of a good fit to the data and conformance to the common assumption of exponential growth of biomass in fish fry (Warren, 1971).

Unless otherwise indicated, a type-I error rate (α level) of 0.05 was used to judge significance in statistical tests. All statistical analyses were performed using SAS (Statistical Analysis Systems Institute Inc., 1990) and S-PLUS (Statistical Sciences, 1993).

Table 1
Mean (SD; *n*) water chemistry values during the 60-day bioassay

	Nominal Cu exposure concentration ($\mu\text{g l}^{-1}$)					
	0.0 (control)	0.6	1.2	2.5	5.0	10.0
Cu ($\mu\text{g l}^{-1}$)	< 0.9 (0.36; 20)	< 0.9 (0.21; 26)	1.1 (0.36; 24)	2.2 (0.37; 25)	4.6 (0.84; 25)	9.0 (0.95; 23)
Ca (mg l^{-1})	5.52 (0.30; 18)	5.42 (0.50; 6)	5.37 (0.49; 6)	5.15 (0.48; 8)	5.20 (0.72; 6)	5.40 (0.40; 7)
K (mg l^{-1})	3.39 (3.95; 18)	3.00 (4.12; 6)	1.64 (3.27; 6)	4.37 (4.34; 8)	3.03 (4.20; 6)	3.83 (4.38; 7)
Mg (mg l^{-1})	2.55 (0.16; 18)	2.55 (0.26; 6)	2.56 (0.21; 6)	2.41 (0.18; 8)	2.44 (0.33; 6)	2.46 (0.15; 7)
Na (mg l^{-1})	0.90 (0.14; 18)	0.88 (0.10; 6)	0.86 (0.07; 6)	0.88 (0.06; 8)	0.86 (0.14; 6)	0.90 (0.10; 7)
Cl (mg l^{-1})	0.69 (0.17; 17)	0.66 (0.10; 6)	0.68 (0.19; 6)	0.80 (0.35; 7)	0.65 (0.10; 6)	0.65 (0.10; 7)
SO ₄ (mg l^{-1})	3.44 (0.77; 17)	3.65 (0.94; 6)	3.63 (0.92; 6)	3.46 (0.95; 7)	3.65 (0.94; 6)	3.48 (0.96; 7)
pH	7.41 (0.18; 29)	7.49 (0.14; 59)	7.47 (0.08; 24)	7.48 (0.09; 21)	7.48 (0.10; 22)	7.47 (0.09; 23)
Alkalinity (mg l^{-1} CaCO ₃)	27.8 (6.0; 21)	27.6 (5.8; 60)	27.7 (6.1; 21)	27.7 (6.1; 20)	27.7 (5.9; 21)	27.6 (5.9; 22)
Hardness (mg l^{-1} CaCO ₃)	24.6 (0.9; 22)	24.6 (0.9; 63)	25.0 (0.9; 22)	24.6 (0.9; 21)	24.5 (0.9; 22)	24.6 (0.9; 23)
Dissolved oxygen (mg l^{-1})	8.1 (0.2; 22)	8.2 (0.3; 54)	8.1 (0.2; 22)	8.1 (0.2; 20)	8.2 (0.2; 20)	8.3 (0.2; 20)
Temperature (°C)	9.9 (0.5; 24)	9.8 (0.4; 59)	9.8 (0.5; 24)	9.8 (0.5; 21)	9.8 (0.5; 22)	9.9 (0.5; 23)

Table 2

Chemical speciation of Cu, expressed as concentration ($\mu\text{g l}^{-1}$), as determined by the computer program MINEQL⁺

Analyzed Cu	Cu^{2-}	$\Sigma\text{Cu}(\text{CO}_3)_x^{2-2x}$	$\Sigma\text{Cu}(\text{OH})_x^{2-x}$
<0.9	0.10	0.29	0.09
1.1	0.23	0.65	0.20
2.2	0.47	1.32	0.41
4.6	0.97	2.74	0.86
9.0	1.92	5.40	1.70

3. Results

Throughout the 60-day bioassay, whole-body Cu concentrations in the 4.6 and 9.0 $\mu\text{g l}^{-1}$ Cu exposures differed significantly ($P < 0.05$) from the concentrations in the control fish (Table 3). Whole-body Cu concentrations in the control fish ranged from 3.45 to 3.68 $\mu\text{g g}^{-1}$ (dry wt.) over the 60-day exposure. Within the control and within each of Cu exposure concentrations less than 4.6 $\mu\text{g l}^{-1}$, the means of whole-body Cu concentrations were not significantly different between sample times. However, for both the 4.6 and 9.0 $\mu\text{g l}^{-1}$ Cu exposures, means of whole-body Cu concentrations increased significantly from 20 to 40 days ($P < 0.05$) but were not significantly different between 40 and 60 days (Table 3). Thus, for the fish exposed to Cu levels (4.6 and 9.0 $\mu\text{g l}^{-1}$) causing significantly elevated whole-body residue levels over the control, the rate of Cu accumulation in tissue appeared to decline steadily with exposure duration (Table 3).

Whole-body concentration of Cu prior to exposure, determined for the test fish sampled on day 0, was $5.51 \pm 0.40 \mu\text{g g}^{-1}$ (dry wt.; $n=6$) or $0.86 \mu\text{g g}^{-1}$ (wet wt.). For comparison, Shearer (1984) reported that the whole-body Cu concentration in rainbow trout ranged from approximately 2 to 7 $\mu\text{g g}^{-1}$, dry wt. Laurén and McDonald (1987) reported that whole-body Cu concentrations in rainbow trout averaged 1.23 $\mu\text{g g}^{-1}$, wet wt. These results indicate that initial Cu concentrations of

Table 3

Whole-body Cu concentrations for the control and test rainbow trout at 20-day intervals throughout the 60-day bioassay

Sample time (days)	Cu exposure concentration ($\mu\text{g l}^{-1}$)					
	<0.9 (control)	<0.9	1.1	2.2	4.6	9.0
20	3.45 (0.50)	4.02 (0.52)	4.34 (0.74)	4.72 (1.11)	5.82* (1.40)a	9.89* (2.44)a
40	3.68 (0.82)	3.77 (0.45)	4.24 (0.88)	6.19 (1.23)	8.90* (2.54)b	15.82* (2.65)b
60	3.60 (0.91)	4.20 (1.38)	4.85 (0.58)	5.85 (0.83)	8.57* (2.18)b	17.91* (3.23)b

Values are means ($\mu\text{g g}^{-1}$, dry wt.; SD in parentheses) for the replicates ($n=6$), where each replicate is a composite of ten individual fish. Asterisks indicate significant differences ($P < 0.05$) from corresponding control for each sample time. Within each Cu exposure concentration, values followed by different letters are significantly different ($P < 0.05$).

rainbow trout used in the study were similar to concentrations reported in other unexposed rainbow trout.

Exposure to Cu concentrations as low as $4.6 \mu\text{g l}^{-1}$ caused significant reductions in both weight and length compared with control fish (Table 4). At 20 days of exposure, the 4.6 and $9.0 \mu\text{g l}^{-1}$ concentrations caused, respectively, 17% and 27% reductions in mean weight compared with the controls. By 40 and 60 days, the $4.6 \mu\text{g l}^{-1}$ Cu exposure had caused approximately a 30% reduction in mean weight relative to controls. By 40 and 60 days of the $9.0 \mu\text{g l}^{-1}$ Cu exposure, the fish experienced a 45% reduction in mean weight relative to controls. At day 40, fish exposed to $2.2 \mu\text{g l}^{-1}$ Cu had significantly lower weights than the controls, but at 60 days this difference was no longer present.

The instantaneous growth rate (Ricker, 1979) was calculated using the mean weights of the control fish at 0 and 60 days of the bioassay to compare the rate of growth obtained in the laboratory with that of the hatchery. Growth records for fish of the same stock/lot number were obtained from the hatchery (unpublished data, Wyoming Game and Fish) and the instantaneous growth rate was calculated for these fry for the 88-day post swim-up stage; the hatchery reported a water temperature of 10°C (the same as our laboratory water). The instantaneous growth rate for the laboratory control fish was estimated to be 2.6% body wt. day^{-1} compared with an estimate of 2.2% body wt. day^{-1} for the hatchery fish. Although this comparison does not account for certain differences between the laboratory and hatchery conditions, such as environmental (e.g. water quality, biomass loading density) or nutritional (e.g. ration) differences, this comparison suggests that our

Table 4

Fish weight (g, wet wt.; measured to the nearest 0.001 g) and length (mm, total; measured to the nearest 0.1 mm) for the control and test rainbow trout at 20-day intervals throughout the 60-day bioassay

Parameter	Day	Cu exposure concentration ($\mu\text{g l}^{-1}$)					
		< 0.9 (control)	< 0.9	1.1	2.2	4.6	9.0
Weight	20	0.192 (0.054)	0.191 (0.060)	0.183 (0.049)	0.175 (0.057)	0.161* (0.040)	0.142* (0.035)
	40	0.369 (0.162)	0.327 (0.104)	0.325 (0.095)	0.303* (0.097)	0.258* (0.092)	0.203* (0.061)
	60	0.578 (0.234)	0.564 (0.227)	0.548 (0.220)	0.561 (0.244)	0.442* (0.181)	0.320* (0.134)
Length	20	29.7 (2.2)	29.7 (2.4)	29.3 (1.8)	29.2 (2.3)	28.7 (1.9)	28.4* (1.8)
	40	34.5 (4.1)	33.7 (3.0)	33.7 (2.8)	33.2 (3.3)	31.8* (3.0)	30.2* (2.6)
	60	39.3 (4.8)	39.2 (4.4)	38.9 (4.4)	39.2 (4.8)	36.7* (4.2)	33.6* (3.8)

Values are means (SD) for the replicates ($n=6$), where each replicate represents independent measurements for ten individual fish. Asterisks indicate significant differences from corresponding control value for the same sample time ($P < 0.05$).

laboratory control fish had growth rates that were adequate, exceeding those obtained under hatchery conditions.

At each of the sample periods, whole-body Cu accumulation was positively and significantly correlated with Cu exposure concentration, whereas fish weight was negatively and significantly correlated with Cu exposure concentration. For example, Cu exposure concentration was highly correlated with whole-body Cu ($r = 0.86-0.94$, $P < 0.001$). Whole-body Cu and fish weight were significantly and negatively correlated, even on day 20 of the exposure ($r = -0.69$, $P < 0.05$) when relationships were expected to be less evident; this relationship was much more pronounced on days 40 and 60 ($r = -0.80$ and -0.82 , respectively; $P < 0.001$). Thus, a consistent pattern of Cu accumulation with increased Cu exposure and reduced fish weight was observed in the rainbow trout fry exposed to sublethal Cu.

Linear regression models were used to relate fish length/fish weight, exposure duration, and whole-body Cu. Results are presented for weight because length and weight were determined to be strongly correlated (i.e. analysis of one of these variables can serve as a reasonable surrogate for the other). Several alternative model formulations were considered, including use of an interaction term relating exposure duration and Cu residues. The interaction term was determined to be nonsignificant and was omitted from further consideration. The following model (Eq. 1) was found to relate exposure duration, Cu accumulation, and fish weight

$$\ln(W) = 4.799 + 0.028T - 0.0384Cu_f \quad (1)$$

where W is fish weight (μg , wet wt.); T is exposure duration (days); and Cu_f is whole-body Cu in fish tissue ($\mu\text{g g}^{-1}$, dry wt.). All estimated coefficients were significant ($P < 0.001$), the multiple R^2 was 0.94, and the residual standard error was 0.1142 (or 0.0011 at original scale). As expected, weight was an increasing function of exposure duration (i.e. fish weight increased with time) and a decreasing function of whole-body Cu burden. Modelled results were back-transformed to the original scale for graphical interpretation (Fig. 1).

Whole-body MT concentrations were measured in the trout sampled at 20, 40, and 60 days from selected Cu exposures. MT concentrations did not differ significantly among fish exposed to the various Cu concentrations during the 60 days of exposure. However, the mean measured concentrations of MT in control fish ranged from 13.10 to 19.26 $\mu\text{g g}^{-1}$ (wet wt.) and in fish exposed to 4.6 and 9.0 $\mu\text{g l}^{-1}$ Cu the MT ranged from 18.05 to 25.19 $\mu\text{g g}^{-1}$ (Table 5).

4. Discussion

We observed significant accumulation of Cu in rainbow trout fry at sublethal exposures. This accumulation was both dose- and time-dependent. Throughout the 60-day exposures, trout exposed to higher waterborne Cu concentrations accumulated more Cu (Table 3). In addition, whole-body Cu appeared to reach steady-state in the exposed trout, with apparent times to steady-state longer in the trout exposed

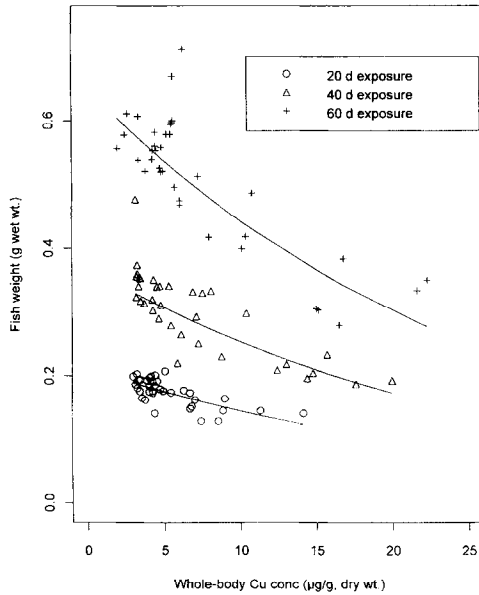


Fig. 1. Weight of rainbow trout fry and whole-body Cu concentration for replicates sampled at 20, 40, and 60 days of exposure. Fitted lines for the different sample days are derived from the multiple regression of $\ln(W)$ vs. T and Cu_f (Eq. 1).

to higher Cu concentrations than in trout exposed to lower Cu concentrations (Table 3). Times to Cu steady-state were on the order of 40–60 days for the trout exposed to 4.6 and 9.0 $\mu\text{g l}^{-1}$ Cu. The trend of whole-body Cu to reach steady-state has been observed previously by other authors. For example, juvenile rainbow trout exposed to 94–194 $\mu\text{g l}^{-1}$ Cu appeared to reach steady-state of whole-body Cu within 14–21 days (Dixon and Sprague, 1981a), and juvenile coho salmon exposed to 70 and 140 $\mu\text{g l}^{-1}$ Cu appeared to reach steady-state of Cu in the liver within 30–60 days (Buckley et al., 1982). The relatively slow time to reach steady-state that we observed (i.e. on the order of weeks) may suggest that Cu accumulates in slowly

Table 5
Whole-body MT concentrations for the control and test rainbow trout in selected exposure concentrations at 20-day intervals throughout the 60-day bioassay

Sample time (days)	Cu exposure concentration ($\mu\text{g l}^{-1}$)			
	< 0.9 (control)	< 0.9	4.6	9.0
20	19.26 (3.28)	17.37 (5.74)	20.57 (4.18)	20.95 (6.79)
40	18.59 (1.56)	19.00 (4.84)	20.46 (2.75)	25.91 (12.66)
60	13.10 (3.67)	13.97 (4.18)	18.12 (7.19)	18.05 (3.88)

Values are means ($\mu\text{g g}^{-1}$, wet wt.; SD in parentheses) for the replicates ($n=6$), where each replicate is a composite of ten individual fish.

exchangeable metal pools, as described by Carbonell and Tarazona (1994). If Cu accumulation is dose-dependent, as indicated by our data, this suggests that the fish's effective accumulation capacity is increased by exposure concentration. If this capacity is regulated by depuration from a slowly exchangeable pool, the time to steady state for Cu accumulation will increase as the dose-dependent accumulation capacity increases. Moreover, our observation that steady-states were reached across a range of accumulation capacities suggests that Cu depuration rates in the slowly exchangeable pool are concentration-dependent — this conclusion is consistent with the dose-dependent growth reductions we observed.

The physiological changes permitting metal detoxification and homeostasis cost energy (Hogstrand et al., 1995; Marr et al., 1995) and reduced growth caused by exposure to Cu has been attributed to metabolic demands associated with metal detoxification. Such increased metabolic demands divert resources from normal growth processes (Hogstrand et al., 1995). Waiwood and Beamish (1978) presented evidence to suggest that exposure to Cu influences the basal metabolic rate of salmonids, which could limit growth through decreased efficiency of energy utilization coupled with increased metabolic maintenance costs. As we suggest above, the observation of apparently dose-dependent accumulation steady-states is consistent with a metabolic demand explanation of the dose-dependent growth reductions we observed.

Reduced growth caused by waterborne copper exposures has also been associated with suppressed feeding, such as depressed appetite observed in rainbow trout (Lett et al., 1976) and reduced feeding activity in perch (Collvin, 1984). Waiwood and Beamish (1978) observed reduced growth rates in response to Cu in rainbow trout juveniles exhibiting depressed appetite and decreased food consumption; however, these authors observed reduced growth even at Cu concentrations that did not affect appetites, suggesting that factors other than food consumption were contributing to reduced growth. In our study, qualitative observations of fish feeding behavior indicated that fish in all groups fed actively, although a slight reduction in feeding activity level was observed in the highest Cu exposure. The extent to which reduced growth caused by Cu exposure is associated with suppressed feeding may be related to inter-specific differences in Cu sensitivity (as observed by Collvin, 1985) and differential effects of exposure levels.

Reduced growth has also been associated with the induction of metal-specific detoxifying proteins (i.e. metallothioneins) (Dixon and Sprague, 1981a; Marr et al., 1995), further indicating that exposure to sublethal Cu can entail a metabolic cost for metal detoxification mechanisms. Our current results could not confirm this hypothesis. Although we observed increased whole-body MT in the trout exposed to the highest Cu concentrations, we did not observe clear, dose-dependent, statistically significant increases in whole-body MT across all sample periods. However, this result may be related to limitations in detecting differences in whole-body MT in fry. Although we observed increased whole-body Cu, accumulation occurs predominantly in liver tissue (e.g. Buckley et al., 1982; Hogstrand and Haux, 1991), and induction of MT caused by Cu exposure would be expected to be most pronounced in liver tissue. Previous studies have demonstrated strong relationships

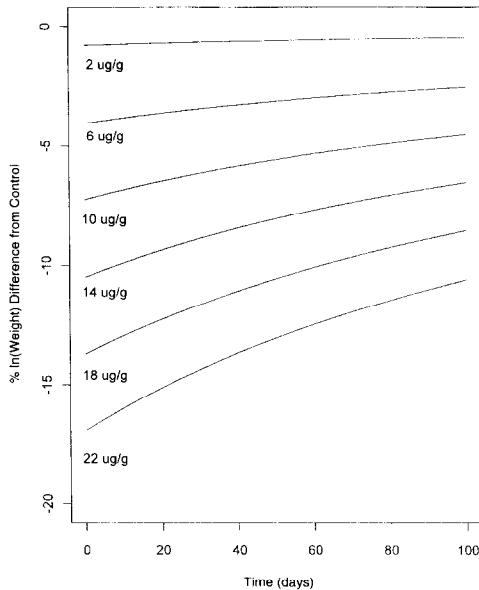


Fig. 2. Predicted differences in percent ln weight between exposure and control fish across exposure time. Plotted lines represent different whole-body residue levels of Cu ($\mu\text{g g}^{-1}$, dry wt.) and are based on the derived model (Eq. 1).

between liver MT and Cu residues (McCarter and Roch, 1983; Hogstrand, 1991; Marr et al., 1995). However, in our samples of whole fish, the liver constituted less than 2% (wet wt.) of the fish. Thus, hepatic MT concentrations most likely were obscured by the constituent MT present in other tissues. Data on whole-body MT in chinook salmon exposed to metals support the explanation of weak association between whole-body MT concentrations and metal exposures (Roch and McCarter, 1984). After 28 days of exposure to a metals mixture, whole-body MT did not increase (Roch and McCarter, 1984); in contrast, however, after 147 days of exposure, hepatic MT in exposed fish was 6.5 times greater than control values. In conclusion, our ability to detect differences in MT was likely a function of low experimental sensitivity in studies using whole-body samples instead of livers.

Our experiments demonstrated a clear relationship between Cu residues and growth responses. This relationship is consistent with previous studies. For example, Cu accumulation has been correlated with growth reductions in Cu-exposed coho salmon fry (Buckley et al., 1982), developing steelhead trout (Seim et al., 1984), and brown trout juveniles (Marr et al., 1995). The relationship between Cu residues and growth reductions offers the potential for the use of residues as an indicator of adverse effects (e.g. in field-collected fish), including in regulatory settings. Several authors (e.g. McGeachy and Dixon, 1992; McCarty and Mackay, 1993; Shutes et al., 1993) have indicated that certain regulatory and environmental impact thresholds for protecting aquatic biota could be based on residues rather than exposure concentrations. This approach offers the potential advantages of

integrating biological exposure conditions over time, exposure routes (e.g. water exposure vs. dietary exposure), and accumulation kinetics (Rand et al., 1995).

Despite the potential advantages of a residue-based approach, our data indicate that such an approach may not be appropriate for Cu exposures. For example, our regression model (Eq. 1) demonstrates that growth reduction (% ln weight) is a function of both exposure duration and Cu residues (Fig. 2). Exposure to low levels of Cu, with subsequent accumulation in tissues, results in substantial growth reductions. However, our modelling suggests that quantitative interpretation of adverse biological effects depends on information on both Cu residues and exposure times — an unlikely situation in many field settings. However, Cu residues may be a useful parameter to interpret potential adverse biological effects in conjunction with other biological/chemical information. For example, our data would support the conclusion that residue analysis could be used to evaluate the overall energetic status of different trout populations by comparing Cu residues at similar life stages (or by comparing hatch dates in fry). Similarly, our observations that Cu residues are related to growth, and that steady-state concentrations are dose-dependent, support the conclusion that fish with higher Cu residues are, on average, more likely to be subject to growth reductions than fish with lower Cu residues. Therefore, although waterborne Cu concentrations may be the most effective means of evaluating, directly, the likelihood of adverse biological effects of Cu on fish, Cu residues as integrators of different exposures over time, space, and exposure route appears to be a useful interpretative tool to evaluate sublethal effects when comprehensive data on water exposures are not available.

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