Water Pollution Studies

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State: Colorado

Study No. F243R-10

Title: Water Pollution Studies

Period Covered: July 1, 2002 to June 30, 2003

Principal Investigator: Patrick H. Davies
Co-investigators: Stephen F. Brinkman and Daria Hansen

Objective: To develop quantitative chemical and toxicological data on the toxicity of pollutants to aquatic life, investigate water pollution problems in the field, and provide expertise in aquatic chemistry and aquatic toxicology.

STUDY PLAN A: REGULATORY AND LEGAL ACTIVITIES

Objective: To provide technical assistance to regulatory and legal entities toward the development, implementation, and enforcement of water quality standards needed to protect or enhance the aquatic resources of Colorado.

Job A1. Water quality standards for the protection of aquatic life in Colorado

Job Objectives:

A1.1 To apply research results and toxicological information from literature toward the development, enactment, and implementation of water quality standards and appropriate aquatic life use classifications.

A1.2 To provide technical information and/or expert testimony in aquatic toxicology and aquatic chemistry in agency meetings, regulatory hearings, and/or court litigations as needed to protect aquatic resources of Colorado.

A1.3 To develop or compile toxicological and chemical data on toxicants for which state or federal governments have not developed a standard.

Job A1.3a. Water quality standards to sustain and protect trout in the Arkansas River below Leadville, CO.

Job Objective: To conduct insitu toxicity tests on rainbow and/or brown trout in the Arkansas River to assess current conditions and toxic impacts associated with metals entering the Arkansas River from California Gulch and alluvial deposits downstream of Leadville.

ACCOMPLISHMENTS

Pat Davies continues to spend most of his time working under this study plan. He has continued working with the Attorney General's Office and the other state and federal Trustee's on negotiations toward the disposition of Natural Resource Damages (NRD) claims, and the restoration of natural resources in the Arkansas River. Approximately four days a month were spent participating in meetings with the state and federal trustees, the Arkansas River Stakeholders group, the Lake Fork Restoration Council, and meetings with the EPA and other federal and state trustees relating to resource injuries on the NPL Site (Superfund Site) and source control of metals and cleanup.

Numerous reports and papers on NRD issues have been reviewed or prepared. The final Site Characterization Report of some 1300 pages was reviewed and comments written. Pat worked on drafting Restoration Goals, Objectives, and the State's expectations on Restoration Alternatives for addressing injuries to Surface Water Quality, Aquatic life, and Habitat. He drafted a paper on the inadequacy of the Consulting Team's evaluation and prioritization of fluvial tailings deposited adjacent to the Arkansas River. Davies developed and recommenced an alternative clarification method stressing entrainment potential of tailings getting into the river and lack of vegetative cover due to high metal concentrations in tailings. He reviewed and commented on a report by Resurrection Mining Company entitled "Preliminary Report on the Biological Data for the Upper Arkansas River, 1994 - 2002", and coordinated review and comments by DOW fisheries biologists - Barry Nehring and Greg Policky whose assistance was greatly appreciated.

Pat also served on a BTAG committee for EPA relating to the development of a Sensitive Species Distribution (SSD) list of aquatic species in the Arkansas River. He has proposed site specific water quality standards needed to protect brown trout in the Arkansas River from DOW data on the toxicity of zinc to different life stages of brown trout in water with hardness ranging from 30 to 150 mg/liter. These criteria will be presented to EPA and the MOU Parties and recommended for implementation as site specific water quality standards. Also with Steve Brinkman's review of existing literature, recommendations were developed for cadmium standards to protect brown trout in the Arkansas River. Similar toxicity tests, as those conducted on zinc and brown trout, are planned for late fall.

Also under this Study Plan we reviewed and commented on a use attainability assessment of the Blue River and French Gulch. Negotiations ensued to develop hardness-based zinc and cadmium standards for the protection of brown trout in segment

2 of the Blue River. All parties agreed on a hardness-based zinc standard for the protection of brown trout in segment 2 of the Blue River which were developed based on recent toxicity experiments conducted under this project. Negotiations are ongoing in an attempt to develop cadmium standards.

Pat Davies, after almost 37 years as an Aquatic Toxicologist/Chemist with DOW, plans on retiring later this year.

STUDY PLAN B: LABORATORY STUDIES

Objective: To research and develop information on, or analytical tools in, aquatic chemistry and aquatic toxicology to better assess toxic responses of pollutants to aquatic life in laboratory and natural waters, such as the Arkansas River.

Job B1. Chemical Equilibria and Kinetic Effects on the Bioavailability and Toxicity of Metals to Aquatic Life

Job Objective: To develop analytical methods using Ion Chromatography, ion separation and/or ultrafiltration to measure toxic fractions and effects of chemical kinetics on toxicity of zinc, copper, lead, cadmium and/or silver to Ceriodaphnia dubia, rainbow trout, brown trout and/or fathead minnows in waters of different complexing capacity. Concurrently, investigate effects of chemical kinetics on results obtained from toxicity tests.

Job B2. Use of Biochemical Methods to Measure Disruption of Ion Regulation and Stress in Aquatic Organisms Exposed to Metals

Job Objective: To develop biochemical methods to measure effects on enzyme systems using electrophoresis or other methods to assess stress in rainbow and brown trout exposed to zinc, copper, lead and/or cadmium.

Job B3. Investigations on the Toxicity of Silver to Aquatic Organisms in Waters of Different Complexing Capacity

Job Objective: To develop acceptable toxicant concentrations of silver for cold- and warmwater fishes in hard high alkaline, and soft low alkaline waters and assess the toxicity of different silver compounds.

Job B4. Effects of Calcium Hardness, Inorganic and Organic Ligands and Sediments on Toxicity of Metals to Aquatic Organisms

Job Objective: To determine antagonistic effects of calcium hardness in low alkaline waters and the effects of specific inorganic and organic ligands and sediments on acute and long-term toxicity of zinc, copper, lead, cadmium, and/or silver to rainbow trout, brown trout and/or fathead minnows.

Job B5. Investigations on Enhanced Toxicity of Unionized Ammonia to Fish at Cold Water Temperatures

Job Objective: To determine effects of temperature on toxicity of unionized ammonia to rainbow trout and fathead minnows or other warmwater species at optimal and less than 5°C water temperatures.

Job B6. Effects of Episodic Exposure on Toxicity and Sensitivity of Aquatic Life to Intermittent Exposure to Metals

Job Objective: To determine toxic effects and organism sensitivity to intermittent exposure of zinc, copper, lead, and/or cadmium to rainbow trout, brown trout and/or fathead minnows, and their ability to acquire and/or lose tolerance.

Job B7. Investigations on Enhanced Toxicity of Water-Borne Metals to Aquatic Life Exposed to Dietary Sources of Metals

Job Objective: To determine effects of water-borne zinc, copper, cadmium, lead and/or manganese on their toxicity to rainbow and brown trout following and/or concurrent with exposure to dietary metals.

Job B8. Investigations on Effects and Interactions of Multiple Metal Exposure on Toxicity to Aquatic Life

Job Objective: To determine effects of exposure of rainbow trout and/or brown trout to zinc, copper, cadmium, lead, and manganese at different combinations found in Colorado's mining areas. Will require an ability to measure bioavailable forms on metals as outlined in Job B1.

ACCOMPLISHMENTS

Several experiments were initiated to study the effect of water hardness on the toxicity of zinc to different life stages of brown, rainbow and cutthroat trout. The tests with brown trout were completed during this segment and the results are reported below. The rainbow trout test was recently completed and data are currently being analyzed. The results from the rainbow trout exposures will be reported next segment. The investigation with cutthroat trout is ongoing and will also be reported next segment.

Toxicity tests were also conducted to evaluate the acute and chronic toxicity of silver chloride to rainbow trout. Results of those tests are reported below.

A 30 days toxicity test to evaluate the chronic toxicity of zinc to young mottled sculpin was also completed and results reported below.

Effect of Hardness on Zinc Toxicity to Brown Trout (*Salmo trutta*) Embryos and Fry.

ABSTRACT

The toxicity of zinc to brown trout at low and high water hardness (30 and 150 mg $CaCO_3$/L) was studied. Tests were conducted at each hardness using early life stage (ELS) and 30 day post swim up fry. Toxicity was negatively related to zinc toxicity for both life stages. Significant effects were observed on early life stage (ELS) time to hatch, survival and termination length and weight. Hatching of eggs was delayed in a dose-dependent manner and chronic values based on delay of hatch was the most sensitive endpoint for the ELS tests (162 and 720 µg Zn/L at 30 and 150 mg $CaCO_3$/L hardness, respectively). Median lethal concentrations (LC_{50}s) after 96 hours were 367 and 1104 µg Zn/L, at 27 and 131 mg $CaCO_3$/L hardness, respectively. Reduced survival was the primary effect of zinc exposure of swimup fry. Effects on growth were not observed. Chronic values based on reduced survival of fry at the low and high hardness were 148 and 598 µg Zn/L, respectively. Chronic values from the fry tests were lower than those from the ELS tests suggesting that acclimation occurred during the early stages of the ELS tests.

INTRODUCTION

An estimated 2080 km of streams in Colorado are impacted by metals (Water Quality Control Division 1988). Brown trout are an important component of Colorado ecosystems in many headwater streams, but their numbers are often reduced due to metal contamination in streams (Davies and Woodling 1980). Data on the toxicity of zinc to brown trout are limited and for the most part exist only for a water hardness of 40 mg/L (Davies and Brinkman 1994, Davies and Brinkman 1999, Davies et al. 2000, Davies et al. 2002). Additional data are needed to assess the effect of hardness on zinc toxicity to allow development of site specific water quality standards in zinc impacted areas such as the Arkansas River downstream from California Gulch and the Blue River below the confluence with French Gulch. The objective of this investigation was to determine the effect of hardness on the acute and chronic toxicity of zinc to different life stages of brown trout. The effect of water hardness was evaluated by conducting long term flow through toxicity tests at a water hardness of 30 and 150 mg $CaCO_3$/L. Effect of zinc exposure at the two hardnesses on traditional endpoints such as survival, growth and biomass were compared. These endpoints were also used to compare the zinc sensitivity of early life stages (ELS) to the sensitivity of 30 day post swimup fry. Acute toxicity to brown trout fry at a water hardness of 400 mg $CaCO_3$/L was also determined.

MATERIAL AND METHODS

Organisms

Brown trout embryos were obtained as newly eyed eggs from the Colorado Division of Wildlife Research Hatchery in Bellevue Colorado. The source of the eggs was a Colorado Division of Wildlife spawning operation using feral brown trout in the North

Delaney Butte Reservoir in Northern Colorado. Ten eggs were placed into each exposure chamber for the ELS tests. Remaining eggs were divided into two lots, placed into a five gallon glass aquaria supplied by the same waters utilized in the 30 and 150 hardness ELS tests and later used for the brown trout fry toxicity tests. Eggs began hatching 12 days after initiation of exposure. Brown trout embryos remained as sac fry for approximately 23 days before reaching swimup stage. The ELS tests continued for an additional 30 days post swimup for a total of 65 days of exposure. The fry toxicity tests were conducted using 34 days post swimup fry. Swimup fry were fed appropriately sized trout food (Silver Cup) four times daily (twice daily on weekends and holidays) at an estimated rate of 3% body weight /day. Swimup fry in the ELS test were fed the trout food diet supplemented with a concentrated suspension of brine shrimp naupalii (San Francisco brand).

Exposure Apparatus

The source water for the 30 mg/L hardness toxicity tests consisted of dechlorinated Fort Collins municipal tap water mixed with reverse osmosis water. The 150 mg/L hardness water was a mixture of an onsite well water and dechlorinated Fort Collins municipal tap water. These waters supplied two modified continuous-flow diluters (Benoit et al. 1982) constructed of teflon, polyethylene and polypropylene components. Chemical stock solutions were prepared by dissolving a calculated amount of reagent grade zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) (Mallinkrodt) in deionized water. The chemical stock solutions were delivered to the diluters via peristaltic pumps (Cole-Palmer model C/L) at a rate of approximately 2.0 mls/minute. New stock solutions were prepared as needed during the toxicity tests. The diluters delivered five exposures with a 50% dilution ratio, and an exposure control. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 30 mls/minute each. Exposure chambers consisted of polyethylene containers with a capacity of 2.8 liters. Loading during the ELS was less than 1.2 g/L of tank volume and less than 0.08 g/L of flow per 24 hrs. During the fry tests, loading never exceeded 2.9 g/L of tank volume and less than 0.19 g/L of flow per 24 hrs. These loading rates are well below suggested maximum rates (ASTM 1993). Test solutions overflowed from the exposure chambers into water baths which were maintained at 12°C using temperature-controlled recirculators (VWR Scientific Products). Dim fluorescent lighting provided a 12 hour day/night photoperiod. The diluters and toxicant flow rates were monitored daily to ensure proper operation.

ELS Test Methods

The target zinc exposure concentrations were 1600, 800, 400, 200, 100 and 0 µg Zn/L for the 30 hardness test. For the 150 hardness test, the target concentrations were 6400, 3200, 1600, 800, 400 and 0 µg Zn/L. The number of hatched eggs and mortality of eggs and fry were monitored and recorded daily. Dead fry were blotted dry with a paper towel and total length (to the nearest mm) and weight (to the nearest 0.001 g) measured and recorded. At the end of the tests, surviving fish from each exposure chamber were terminally anesthetized, blotted dry with a paper towel and total lengths and weights measured and recorded.

Water quality characteristics of exposure water were measured weekly in all treatment levels within a replicate. Replicates were alternated each week. Hardness and alkalinity were determined according to Standard Methods (APHA 1985). A Thermo Orion 635 meter measured pH and conductivity. The meter was calibrated with 4.00, 7.00 and 10.00 pH buffers and two conductivity standards prior to each use. Dissolved oxygen was measured using a YSI Model 58 or Orion 1230 dissolved oxygen meter.

Water samples for dissolved zinc analyses were collected weekly from each exposure level with surviving fry. Exposure water was passed through a 0.45 µm filter (Acrodisc), collected in disposable polystyrene tubes (Falcon), and immediately preserved with Ultrex triple distilled nitric acid to pH <2. Analysis of samples occurred within 24 hours of collection. Analyses were performed using an Instrumentation Laboratory Video 22 (Allied Analytical Systems, Franklin, MA) atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction. The spectrometer was calibrated prior to each use and the calibration verified using a NIST traceable QAQC standard from an outside source (High Purity Standards, Charleston SC). Sample splits and spikes were collected and analyzed to verify analytical reproducibility and recovery. The zinc detection limit was <10 µg/L.

Fry Test Methods

Brown trout fry experiments utilized the same exposure apparatus as the ELS tests. Test methods were identical with the following exceptions. The target zinc exposure concentrations were reduced to 800, 400, 200, 100, 50 and 0 µg Zn/L for the 30 hardness test. For the 150 hardness test, the target concentrations were reduced to 3200, 1600, 800, 400, 200 and 0 µg Zn/L. Samples for water quality characteristics and zinc analysis were collected daily during the initial 96 hours of exposure and weekly thereafter. Fry were not fed during the initial 96 hours of exposure but were fed twice daily thereafter (once on weekends and holidays). Zinc exposure lasted for a total of 30 days. An acute-only test was conducted at a target water hardness of 400 mg $CaCO_3$/L. Nominal zinc exposure concentrations for the 400 hardness test were 8000, 4000, 2000, 1000, 500, and 0 µg Zn/L.

Statistical Analyses

Statistical analyses were conducted using Toxstat version 3.5 software (West Inc. 1996). Analysis of variance (ANOVA) was used to test toxicity endpoints which included hatching success, fry and swimup survival, biomass at the end of the test, mean time to hatch, and lengths and weights of surviving at test termination. Hatching success and survival data were transformed using the arcsine square root prior to ANOVA (Snedecor and Cochran 1980). Normality and homogeneity of variances were tested using Shipiro-Wilk's test and Levene's test, respectively (Weber et al., 1989). Treatment means were compared to the control using William's one-tailed test (Williams 1971, Williams 1972) or Dunnett's one-tailed test (Dunnett 1955, Dunnett 1964), both at $p<0.05$. The highest zinc concentration not associated with a treatment effect (e.g. decreased survival, decreased body weight) was designated as the no-observed-effect

concentration (NOEC). The lowest concentration of zinc that was associated with a treatment effect was designated as the lowest-observed-effect concentration (LOEC). Chronic values were calculated as the geometric mean of the LOEC and NOEC. The inhibition concentration (IC_{20}), the concentration estimated to cause a 20% reduction in organism performance compared with the control (USEPA 1993), was calculated using the combined weight of surviving organisms from each treatment. Ninety six hour median lethal concentrations (LC_{50}) were estimated by the Trimmed Spearman-Karber technique (Hamilton et al. 1977, 1978) using log transformed zinc concentrations. The LC_{50} estimations from the 400 hardness fry acute test used 33% trim while all other estimates were obtained using 10% trim.

RESULTS

The average recovery of the external QAQC sample was 99.8% (range 94.8-102.8%). The average spiked sample recovery was 102.2 % (range 96.0-108.3%). The mean percent difference of split sample analyses was 1.2% (range 0.0-4.3%).

30 Hardness ELS

Standard deviations of water quality characteristics during the Early Life Stage test in the 30 hardness test were generally low were generally low and the ranges are narrow indicating the water quality characteristics were consistent over the course of the experiments (Table 1). The mean measured hardness of 26.8 mg/L was slightly lower than the target of 30 mg/L. Mean alkalinity was 19 mg/L and pH was 7.4. Temperatures were maintained in a narrow range around 12°C. Dissolved oxygen exceeded 6.9 mg/L. Mean conductivity was 46.8 µS/cm.

The time to hatch, hatching success, sac fry and swimup fry survival for the brown trout embryos and the associated zinc exposure concentrations in the 30 hardness ELS test are shown in Table 2. Time to hatch exhibited a generally increasing trend with zinc exposure concentration. The lowest observed effect concentration (LOEC) based on time to hatch was 221 µg/L. The no observed concentration (NOEC) based on time to hatch was 119 µg/L for a chronic value of 162µg/L. Hatching success exceeded 72 % in all exposure levels and was unaffected by the zinc concentrations used in this experiment. Substantial mortality occurred during the sac fry stage in the four highest zinc exposures. Sac fry survival in concentrations ≥ 424 µg/L were significantly reduced (LOEC). Sac fry survival at 221 µg/L was only 50% but was not significant at the 0.05 level (NOEC). The chronic value based on sac fry survival is 306 µg/L. The NOEC and LOEC based on survival through the swimup stage was 119 and 221 µg/L, respectively, for a chronic value of 162 µg/L.

Effects of zinc exposure on sublethal endpoints (biomass, mean lengths and weights of surviving fish) are presented in Table 3. Mean length of surviving fish was significantly reduced at zinc concentrations of 798 but not 424 µg/L (LOEC and NOEC, respectively) for a chronic value of 582 µg/L. The NOEC- LOEC values based on surviving weights were lower resulting in a chronic value of 306 µg/L. Mean biomass at

the end of the experiment was even more sensitive than surviving weight. The LOEC based on biomass was 221 µg/L and the NOEC was 119 µg/L for a chronic value of 162 µg/L. The 20% inhibitory concentration (IC_{20}) was 180 µg/L. Chronic values and IC20s are summarized in Table 15.

Table 1. Mean, standard deviation and range of water quality characteristics of exposure water used during 30 hardness ELS toxicity test.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	26.8	19.1	7.45	12.2	46.8	8.04
Std. Dev.	2.2	1.3	0.18	0.2	3.5	0.53
Range	23.4-31.8	17.0-21.4	7.20-7.80	11.9-12.6	42.2-53.2	6.91-8.70

Table 2. Mean dissolved zinc concentrations (µg/L) and associated time to hatch (hrs), hatching success, sac fry and swimup fry survival (%) of brown trout ELS exposed in 30 mg/L water hardness. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (8)	119 (14)	221 (19)	424 (16)	798 (7)	1734 (38)
Time to Hatch (hrs)	306 (8)	324 (13)	356* (4)	383* (13)	411* (19)	373* (11)
Hatching Success (%)	87.5 (9.6)	85.0 (5.8)	92.5 (9.6)	80.0 (14.1)	72.5 (12.6)	77.5 (12.6)
Sac Fry Survival (%)	70.0 (18.3)	75.0 (12.9)	50.0 (11.6)	32.5* (12.6)	27.5* (22.2)	15.0* (23.8)
Swimup Fry Survival (%)	65.5 (12.9)	72.5 (15.0)	47.5* (9.6)	30.0* (14.1)	20.0* (14.1)	5.0* (5.8)

*Significantly less than control ($p<0.05$)

Table 3. Mean measured dissolved zinc concentrations (µg/L) and associated mean lengths (mm) and weights (g) of brown trout surviving 30 hardness ELS test. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (8)	119 (14)	221 (19)	424 (16)	798 (7)	1734 (38)
Mean Length (mm)	34.2 (0.4)	35.4 (1.7)	34.2 (1.1)	32.8 (1.0)	28.3* (3.4)	27.5* (0.7)
Mean Weight (g)	0.326 (0.011)	0.344 (0.036)	0.324 (0.038)	0.260* (0.024)	0.185* (0.048)	0.192* (0.013)
Mean Biomass (g)	2.11 (0.36)	2.46 (0.32)	1.52* (0.27)	0.78* (0.37)	0.41* (0.39)	0.10* (0.11)

*Significantly less than control ($p<0.05$).

150 Hardness ELS

Water quality characteristics measured during the 150 hardness ELS test are presented in Table 4. Mean hardness was very near the 150 mg/L target. Alkalinity at 100 mg/L was about 70% of the hardness, a similar ratio as the 30 hardness ELS test. Conductivity was 256 µS/cm. Temperature, pH and dissolved oxygen were similar to the 30 hardness ELS test.

Time to hatch, hatching success, sac fry and swimup fry survival rates for the brown trout embryos and the associated zinc exposure concentrations in the 150 hardness ELS test are shown in Table 5. The LOECs for each of these endpoints are greater than those from the 30 hardness ELS test demonstrating the well established protective effect of hardness on zinc toxicity. As observed in the 30 hardness ELS test, time to hatch was increasingly delayed with increasing zinc exposure. This delay was significant at a concentration of 983 µg/L but not 1734 µg/L. Hatching success was 90% in the controls but significantly reduced at 6402 and 1734 µg/L, but not at 3477 µg/L. Most mortality occurred during the sac fry stage with little or none during the swimup stage. For both endpoints, the NOEC and LOEC were 983 and 1734 µg/L, respectively. The chronic value based on sac fry and swimup fry survival was 1306 µg/L. Surviving length, weights, biomass and associated zinc exposure concentrations are shown in Table 6. The NOEC and LOEC based on surviving lengths and weights was 1734 and 3477 µg/L, respectively. The chronic value for these endpoints is 2455 µg/L. For biomass, 983 µg/L was the NOEC and 1734 µg/L was the LOEC for a chronic value of 1306 µg/L. Chronic values and IC20s are summarized in Table 15 with those from the 30 hardness ELS test for comparison.

Table 4. Mean, standard deviation and range of water quality characteristics of exposure water used during 150 hardness ELS toxicity test.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	153	100	7.53	12.4	256	8.40
Std. Dev.	17	13	0.11	0.4	23.7	0.60
Range	137-201	88.4-133	7.36-7.78	11.7-13.0	235-321	7.25-9.03

Table 5. Mean dissolved zinc concentrations (µg/L) and associated time to hatch (hrs), hatching success, sac fry and swimup fry survival (%) of brown trout ELS exposed in 150 mg/L water hardness. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (3)	528 (44)	983 (88)	1734 (159)	3477 (306)	6402 (524)
Time to Hatch (hrs)	292 (10)	307 (18)	352* (32)	363* (31)	381* (21)	390* (46)
Hatching Success (%)	92.5 (9.6)	85.0 (5.8)	85.0 (10.0)	72.5 (9.6)	87.5 (9.6)	42.5* (12.6)
Sac Fry Survival (%)	90.0 (8.2)	77.5 (5.0)	77.5 (5.0)	45.0* (25.2)	62.5* (17.1)	7.5* (9.6)
Swimup Fry Survival (%)	90.0 (8.2)	75.0 (5.6)	77.5 (5.0)	45.0* (25.2)	60.0* (18.3)	7.5* (9.6)

*Significantly less than control ($p<0.05$)

Table 6. Mean measured dissolved zinc concentrations (µg/L) and associated mean lengths (mm) and weights (g) of brown trout surviving 150 hardness ELS test. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (3)	528 (44)	983 (88)	1734 (159)	3477 (306)	6402 (524)
Mean Length (mm)	35.1 (1.1)	34.7 (0.7)	34.2 (0.6)	33.9 (1.0)	33.2* (1.0)	26.8* (1.8)
Mean Weight (g)	0.335 (0.013)	0.322 (0.020)	0.309 (0.003)	0.307 (0.015)	0.302* (0.036)	0.168* (0.040)
Mean Biomass (g)	2.93 (0.31)	2.40 (0.11)	2.40 (0.16)	1.38* (0.81)	1.79* (0.51)	0.24* (0.06)

*Significantly less than control ($p<0.05$).

30 Hardness Fry

Water quality characteristics for the test conducted with brown trout fry in 30 hardness are presented in Table 7. All characteristics are similar to the test conducted with the brown trout ELS. Table 8 contains the acute (96 hour) and 30 day chronic survival of brown trout fry exposed to zinc in 30 mg/L water hardness. The 96 hour median lethal concentration was 367 µg Zn/L with a 95% confidence interval of 319-421 µg Zn/L. Because of nonzero variances in some treatments, 30 day transformed survival data failed normality tests, but passed Levene's test of homogeneity of variance ($p=0.26$). The results of the ANOVA for 30 day survival are considered reliable because ANOVA is generally considered to be robust with respect to nonnormal data. A single mortality in a control treatment occurred after the initial 96 hours as a result of cleaning operations. Inclusion of this mortality did not affect the results of Williams' means comparison. The LOEC based on 30 day survival was 206 µg Zn/L. The NOEC was 106 µg Zn/L and the chronic value was 148 µg Zn/L. Effects of zinc exposure on growth, as measured by length and weight of surviving fry, were not detected (Table 9). Biomass was significantly reduced for fry exposed to 407 µg Zn/L (LOEC), but not 206 µg Zn/L (NOEC). The chronic value based on biomass was 290 µg Zn/L.

Table 7. Mean, standard deviation and range of water quality characteristics of exposure water used during 30 hardness brown trout fry toxicity test.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	27.3	20.6	7.49	11.8	50.1	8.04
Std. Dev.	3.2	3.1	0.21	0.2	6.7	0.39
Range	24.0-32.4	17.2-26.6	7.12-7.89	11.6-12.3	43.4-60.8	7.23-8.74

Table 8. Mean dissolved zinc concentrations (µg/L) and associated acute and 30 day survival (%) of brown trout fry exposed in 30 mg/L water hardness. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (2)	56 (2)	106 (5)	206 (8)	407 (13)	879 (9)
96 hr Survival (%)	100 (0)	100 (0)	97.5 (5.0)	90.0 (8.2)	40.0 (8.2)	0 (0)
30 day Survival (%)	97.5 (5.0)	100 (0)	97.5 (5.0)	90.0* (8.2)	40.0* (8.2)	0* (0)

LC_{50} (95% C.I.)=367 µg Zn/L (319-421)
*Significantly less than control ($p<0.05$)

Table 9. Mean measured dissolved zinc concentrations (µg/L) and associated mean lengths (mm) and weights (g) of brown trout fry exposed in 30 mg/L hardness. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (2)	56 (2)	106 (5)	206 (8)	407 (13)	879 (9)
Mean Length (mm)	42.5 (1.0)	41.9 (0.6)	42.8 (1.1)	41.9 (1.9)	40.1 (2.8)	--
Mean Weight (g)	0.729 (0.046)	0.717 (0.041)	0.744 (0.058)	0.736 (0.094)	0.663 (0.145)	--
Mean Biomass (g)	7.11 (0.61)	7.17 (0.41)	7.24 (0.51)	6.63 (1.15)	2.68* (1.01)	0* (0)

*Significantly less than control ($p<0.05$).

150 Hardness Fry

Water quality characteristics during the 150 hardness brown trout fry exposures are shown in Table 10. Mean hardness was 131, lower than the 150 hardness ELS test. Alkalinity was similarly reduced. Other characteristics were nearly identical to the 150 ELS test. Acute (96 hours) and chronic (30 day) survival are presented in Table 11. The LC_{50} was 1104 µg Zn/L with a 95% confidence interval of 951-1281. The NOEC and LOEC based on survival was 436 and 819 µg Zn/L, respectively, for a chronic value of 598 µg Zn/L. As in the 30 hardness fry test, there was no detected effect of zinc exposure on length or weight (Table 12). The NOEC and LOEC based on biomass was the same as survival with a chronic value of 598 µg Zn/L.

Table 10. Mean, standard deviation and range of water quality characteristics of exposure water used during 150 hardness brown trout fry toxicity test.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	131	90.8	7.57	12.3	243	8.25
Std. Dev.	4.1	4.1	0.10	0.5	7.6	0.55
Range	123-141	84.2-97.8	7.41-7.76	11.6-13.3	231-259	6.85-8.96

Table 11. Mean dissolved zinc concentrations (µg/L) and associated acute and 30 day survival (%) of brown trout fry exposed in 150 mg/L water hardness. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (3)	222 (21)	436 (38)	819 (62)	1501 (96)	3040 (141)
96 hr Survival (%)	100 (0)	100 (0)	95.0 (5.8)	72.5 (12.6)	27.5 (9.6)	0 (0)
30 day Survival (%)	95.0 (5.8)	100 (0)	92.5 (5.0)	65.0* (12.9)	22.5* (9.6)	0* (0)

96 hour LC_{50} (95% C.I.)=1104 µg Zn/L (951-1281)
*Significantly less than control ($p<0.05$)

Table 12. Mean measured dissolved zinc concentrations (µg/L) and associated mean lengths (mm) and weights (g) of brown trout fry exposed in 150 mg/L hardness. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (3)	222 (21)	436 (38)	819 (62)	1501 (96)	3040 (141)
Mean Length (mm)	40.1 (1.2)	40.3 (1.9)	40.7 (1.0)	38.8 (0.7)	38.2 (2.2)	--
Mean Weight (g)	0.628 (0.052)	0.659 (0.093)	0.663 (0.055)	0.610 (0.044)	0.548 (0.120)	--
Mean Biomass (g)	5.95 (0.40)	6.59 (0.93)	6.11 (0.29)	3.94* (0.68)	1.31* (0.72)	0* (0)

*Significantly less than control ($p<0.05$).

400 Hardness Fry

Water quality characteristics for the seven day acute test were consistent over the duration of the exposure (Table 13). Temperature, pH and dissolved oxygen were similar to previous tests. Table 14 shows zinc exposure concentrations as well as associated 96 hour and 7 day survival. The 96 hour LC_{50} concentration was 6259 µg Zn/L with a 95% confidence interval of 5073-7720). After 7 days, the LC_{50} decreased slightly to 6014 with a 95% confidence interval between 5022-7202). Mean length and weight of test organisms was 47.9 mm and 1.062 g, respectively.

Table 13. Mean, standard deviation and range of water quality characteristics of exposure water used during the brown trout fry toxicity test at 400 hardness.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	411.4	295.7	7.34	12.2	692	7.86
Std. Dev.	12.5	5.2	0.08	0.4	12.5	0.40
Range	392.8-434.6	284.8-302.6	7.60-7.88	11.8-13.0	678-713	7.35-8.39

Table 14. Mean dissolved zinc concentrations (µg/L) and associated 96 hour and 7 day survival (%) of brown trout fry exposed in 400 mg/L water hardness. Standard deviations are in parentheses.

Dissolved Zn (µg/L)	<10 (3)	540 (119)	1114 (226)	1629 (23)	3730 (340)	8107 (750)
96 hr Survival (%)	100 (0)	100 (0)	100 (0)	100 (0)	83.3 (13.6)	33.3 (13.6)
7 day Survival (%)	95.8 (5.8)	100 (0)	100 (0)	100 (0)	83.3 (13.6)	29.2 (21.0)

96 hour LC_{50} (95% C.I.)=6259 (5073-7720) µg Zn/L
7 day LC_{50} (95% C.I.)=6014 (5022-7202) µg Zn/L

Table 15. Chronic values (µg/L) and endpoints for zinc toxicity tests conducted with brown trout ELS and fry in 30 and 150 mg/L water hardness.

Endpoint	30 Hardness		150 Hardness	
	ELS	Fry	ELS	Fry
Time to Hatch	162	--	720	--
Hatch Success	>1734	--	4718	--
Sac Fry Survival	306	--	1306	--
Swimup Fry Survival	162	148	1306	598
Length	582	>407	2455	>1501
Weight	306	>407	2455	>1501
Biomass	162	290	1306	598
IC_{20}	180	251	1034	629
LC_{50}	--	367	--	1104

DISCUSSION

Early life stage tests at 30 and 150 hardness found a positive relationship between zinc exposure concentration and time to hatch. In fact, this was among the most sensitive endpoints in both ELS tests. This phenomenon has been previously reported for brown trout eggs exposed to zinc (Davies et al. 2002). Altered time to hatch is not a common endpoint for metal toxicity. Manganese accelerated hatching in brown, brook and rainbow trout eggs (Stubblefield et al. 1997, Davies et al. Brinkman 1998) and exposure to silver resulted in premature hatching of rainbow trout eggs (Davies et al. 1978). Changes of the timing of egg hatch could have important consequences in terms of survival of young of the year and their ability to recruit. The effect of a delay of 100 hours (such as we observed) is unknown and may be insignificant. Zinc exposures were initiated with eyed eggs and the temperature was maintained near 12°C. Brown trout spawn in fall and the eggs remain in the redds over the winter months before hatching in the spring. The relatively minor delay of hatching observed in this experiment could be expected to be much greater if exposure to zinc occurs starting at fertilization and at lower temperatures typical of streams in the winter time. The effect of zinc exposure on brown trout eggs starting with fertilization and using colder temperatures deserves further study.

Early life stage tests were more sensitive than fry tests at detecting effects of zinc exposure on growth. This finding may result from the longer duration of the ELS tests compared to the fry tests (65 versus 30 days). Variability of termination lengths and weights in the fry tests also reduced statistical power to detect effects on growth. In ELS tests, reduction of growth is often a more sensitive endpoint than survival. This was the case in the 30 but not the 150 hardness ELS test. In both tests, biomass at test termination was the most sensitive endpoint. Because biomass was a measure of both weight and survival, small effects on growth and survival became magnified. This compounded effects of each and led to a greater ability to detect effects of zinc exposure.

The results of this study confirmed the well established negative relationship between hardness and zinc toxicity. Previous toxicity tests with brown trout have been conducted over a narrow range of hardness precluding an analysis of the effect of hardness on zinc toxicity for this species. The LC_{50} for the three acute tests plotted against the corresponding hardness revealed that the relationship was approximately log linear (Figure 1). Combining these data with previous data from this project (Davies and Brinkman 1994, Davies and Brinkman 2000, Davies et al. 2002) allowed development of a relationship between hardness and brown trout LC_{50}. Data used for the regression are summarized in Appendix Table A1. The regression formula predicts the 96 hour LC_{50} for a given hardness with a correlation coefficient of 0.85. Dividing a predicted LC_{50} by a factor of 2 can be expected to protect brown trout from acute exposures to zinc which is often called the Criteria Maximum Concentration (CMC) (EPA 1985). The one hour average concentration should not exceed the CMC. The equation for the CMC that protects brown trout from zinc is

$$\text{Brown Trout Zn CMC} = e^{(1.029*(\ln(\text{hardness}))+1.625)}$$

Chronic toxicity of zinc decreased with increasing hardness for both life stages. Earlier studies investigating the chronic toxicity of zinc to ELS brown trout were limited to water hardnesses between 40 and 55 mg/L. Chronic values from ELS tests conducted by this project were 381 and 196 µg Zn/L at 48 and 54 mg $CaCO_3$/L, respectively (Davies and Brinkman 1999, Davies et al. 2002). Chronic values of chronic and subchronic tests with brown trout fry ranged between 147 and 457 µg Zn/L. Brown trout from those studies ranged between 39 and 92 mm and were conducted in water hardness between 37 and 55 mg $CaCO_3$/L (Davies and Brinkman 1994, Davies et al. 2002, Davies and Brinkman 1999). Chronic values of previous tests plotted against the corresponding water hardness displayed a roughly linear log relationship (Figure 2). The regression line shown in Figure 2 used chronic values from brown trout fry tests and did not include ELS data. These data are shown in Appendix Table A2. The regression equation is

$$\text{Brown Trout chronic Zn} = e^{(0.98059*(\ln(\text{hardness}))+1.402)}$$

Zinc values from this equation can be expected to protect unacclimated brown trout fry. While acclimated brown trout are capable of tolerating higher levels of zinc, it is important that water quality standards protect unacclimated organisms. Unacclimated fry from clean tributaries may wash into contaminated stream reaches. Protection of unacclimated individuals is also necessary because acclimation to metals can be quickly

lost once exposure to metals is removed (Gasser 1998, Davies and Brinkman 1999, Davies et al. 2002). Migration into a clean tributary could lead to a loss of acclimation followed by toxicity on return to a contaminated stream reach. Loss of acclimation can also occur during spring runoff when dilution from spring snowmelt substantially reduces metal concentrations in streams.

Figure 2 and Appendix Table A2 show that tests conducted using fry frequently resulted in lower chronic zinc values than ELS tests. Acclimation of ELS test organisms may account for this result. ELS tests are generally considered to encompass the most sensitive life stage of fish and were comparable to results from life cycle tests (Macek and Sleight 1997, McKim 1997). However, exposure initially occurs as eggs, a life stage that is relative tolerant to zinc. Exposure during a tolerant life stage provides an opportunity for the exposed organisms to become acclimated and more tolerant to lethal effects during a subsequent sensitive life stage (Sinley et al. 1974, Spehar 1976, Davies et al. 2002). Acclimation of rainbow trout to zinc is a well documented phenomena (Sinley et al. 1974, Bradley et al. 1985, Stubblefield 1988, Anadu et al. 1989). Brown trout also acclimate to zinc (Davies and Brinkman 1999) and a combination of zinc and cadmium (Gasser 1998). A comparison of ELS and fry dose-response curves for the 30 and 150 hardness tests show that acclimation by ELS brown trout may have occurred at the higher concentrations (Figures 3 and 4, respectively). Criteria that only allow ELS tests for chronic data can lead to acute-chronic ratios that are less than one in instances where embryonic exposure produces an acclimation response. Consideration should be given to tests conducted with the most sensitive life stage when calculating biological criteria. Failure to due so will result in the underestimation of chronic toxicity.

Appendix Table A1. Data and source used in regression of hardness and 96 hour LC50 of zinc to brown trout.

Hardness	Mean Length (mm)	96 hour LC50 (95% C.I.)	Reference
36.9	38.9	642 (523-789)	Davies and Brinkman 1994
51	39.6	871 (729-1041)	Davies and Brinkman 1999
52	52	392 (332-464)	Davies and Brinkman 1999
54.4	63	1033 (694-1372)	Davies and Brinkman 1999
52.6	92	484 (340-689)	Davies et al. 2000
54.6	63	603 (391-931)	Davies et al. 2000
45.3	35.4	382 (310-470)	Davies et al. 2002
49.5	35.4	508 (426-607)	Davies et al. 2002
27.3	35.0	367 (319-421)	This report
131	34.2	1104 (951-1281)	This report
411	47.9	6259 (5073-7720)	This report

Appendix Table A2. Data and source used in regression of hardness and chronic values of zinc to brown trout.

Hardness	Mean Length (mm)	Exposure Duration	Chronic Value	Life Stage	Reference
36.9	38.9	12 weeks	457	Fry	Davies and Brinkman 1994 [2]
51	39.6	31 days	146	Fry	Davies and Brinkman 1999*
52	52	18 days	194	Fry	Davies and Brinkman 1999*
54.4	63	30 days	187	Fry	Davies and Brinkman 1999*
54.1	28.3	68 days	381	ELS	Davies and Brinkman 1999 [1]
54.6	63	7 days	327	Fry	Davies et al. 2000 [3]
52.6	92	7 days	234	Fry	Davies et al. 2000*
45.3	35.4	7 days	151	Fry	Davies et al. 2002*
49.5	35.4	7 days	147	Fry	Davies et al. 2002*
48.1	31.9	58 days	196	ELS	Davies et al. 2002 [1]
26.8	33.8	65 days	162	ELS	This Report [1]
27.3	42.1	30 days	148	Fry	This Report*
153	34.1	65 days	1306	ELS	This Report [1]
131	39.9	30 days	598	Fry	This Report*

*Data used in hardness-chronic value regression

[1]ELS data not used in hardness-regression

[2]Eggs maintained in Arkansas River and froze prior to test. Data not used in hardness regression

[3]High mortality in control. Data not used in hardness regression

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Acute and Chronic Toxicity of Silver Chloride to Early Life Stage Rainbow Trout

ABSTRACT

Silver is extremely toxic to salmonids. However, most silver toxicity studies have used the soluble salt, silver nitrate. There is some question as to whether insoluble silver salts would result in the same toxicity as observed with silver nitrate. Water flowing through a column containing the relatively insoluble salt, silver chloride, was found to be acutely and chronically toxic to early life stage (ELS) rainbow trout and fry. Survival was 40% for rainbow trout ELS exposed to 0.738 µg Ag/L, but 87.5% or more for organisms exposed to 0.348 µg/L or less. Significant effects on growth, as measured by termination weight, occurred at 0.348 µg/L but not at 0.182 µg Ag/L. The 96 hr median lethal concentration (LC_{50}) was 5.51µg Ag/L. Chloride ions, the primary inorganic ligand affecting silver speciation the dilution water, was 2.3 mg/L. Based on speciation calculations, the seven day LC_{50} of free silver ion was 0.028µM, similar to a previously published value. Overall, silver chloride, once solublized, results in similar LC_{50}s and chronic values as silver nitrate.

INTRODUCTION

Silver is one of the most toxic metals to freshwater aquatic life (EPA 1980). Colorado was the first state to promulgate a silver chronic water quality standard for the protection of aquatic life, which has been adopted by 28 other states. There are little data, however, on the chronic toxicity of silver to fish. The vast majority of existing silver toxicity tests have been conducted using silver nitrate. Insoluble silver salts have been claimed to be less toxic than the soluble silver nitrate (EPA 1980, Hogstrand et al. 1996, Eisler 1996). Silver halides are generally considered to be insoluble. But because of the extreme toxicity of silver, these salts are capable of maintaining toxic levels of the free silver ion (Davies and Goettl 1978, Davies et al. 1998). Water passed through a PVC column containing solid silver iodide was lethal to 94% of rainbow trout early life stage organisms and led to a decreased rate of development and growth (Davies and Goettl 1978).

The objective of this study was to determine the acute and chronic toxicity of silver chloride to rainbow trout early life stages and fry. Toxicity thresholds of AgCl were compared to those obtained using silver nitrate to determine relative toxicity. Chronic toxicity endpoints evaluated were hatching success, sac fry and swimup fry survival, and growth as measured by length and weight at termination. Acute toxicity was evaluated using 96 hour and 7 day median lethal concentrations (LC_{50}).

MATERIAL AND METHODS

Early Life Stage Test

A modified continuous-flow diluter (Benoit et al. 1982) delivered the exposure solutions using dechlorinated Fort Collins municipal tap water as source water. The diluter was constructed of teflon, polyethylene and polypropylene components. Powdered

silver chloride (EM Science Gibbstown NJ) was sieved through 355 μm USA Standard Testing sieve (ASTM E-11 specification) and 75 g was dispersed onto polyester filter fiber (Blue Ribbon Pet Products, Inc., Commack NY) , as used in aquarium filtration. The filter fiber was lightly packed into a 3.0 cm diameter by 50 cm long PVC column. Fine nylon screen (100 μm) at each end of the column kept column contents in place. Dilution water was pumped through the column to the diluter using a peristaltic pump (Cole-Palmer model C/L). The flow rate through the column was adjusted so that when mixed with dilution flow would deliver a nominal high concentration of 3.2 μg Ag/L. The flow through the column containing AgCl ranged between 3.9 and 8.8 mls/minute and the concentration ranged between 160 and 281 μg Ag/L. The diluter delivered five exposures with a 50% dilution ratio, and an exposure control. Target concentrations were 3.2, 1.6, 0.8, 0.4, 0.2 and 0 μg Ag/L. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 mls/minute each. Operation of the diluter, toxicant flow and Ag concentration through the column were monitored daily to ensure proper operation. Exposure chambers consisted of polyethylene containers with a capacity of 2.8 liters. Test solutions overflowed from the exposure chambers into a water bath, which was maintained at 12 °C using temperature-controlled recirculator (VWR Scientific Products Recirculator). Dim fluorescent lighting provided illumination with a 12 hour light-dark photoperiod.

Eyed Tasmanian strain rainbow trout eggs were obtained from the Colorado Division of Wildlife Research Hatchery in Bellevue, Colorado. Ten eggs were randomly placed into each exposure chamber. The number of hatched eggs and mortality of eggs and fry were monitored and recorded daily. The eggs started hatching approximately two days after initiation of silver exposure. The embryos remained as sac fry in the exposure controls for approximately 16 days prior to swimup stage. Exposure of swimup fry continued for 30 days post swimup for a total of 48 days of exposure. Swimup fry were fed appropriately sized Silver Cup trout food four times daily (twice daily on weekends and holidays) at an estimated rate of 5% body weight /day. The trout food diet was supplemented with a concentrated suspension of brine shrimp naupalii (San Francisco brand). Brine shrimp naupalii were collected in a brine shrimp net, rinsed with deionized water and reconstituted in dilution water immediately prior to feeding. At the end of the ELS test, surviving fish from each exposure chamber were terminally anesthetized, blotted dry with a paper towel and the total lengths (mm) and weights (g) measured and recorded.

Water quality characteristics of exposure water were measured weekly in all treatment levels within a replicate. Replicates were alternated each week. Hardness and alkalinity were determined according to Standard Methods (APHA 1985). pH was measured using an Orion Research pH meter 811 which was calibrated prior to each use with pH 7.00 and pH 4.00 buffers. Conductivity was determined using a YSI Model 35 conductance meter. Dissolved oxygen was measured using a YSI Model 58 dissolved oxygen meter. Three additional water samples were collected during the test and analyzed by the Colorado State University Soil Testing Laboratory for major anions. A single sample was collected and analyzed for dissolved organic matter (Shimadzu Model TOC-5050A, Total Organic Carbon Analyzer).

A water sample for silver analysis was collected daily at the outlet of the column containing the silver chloride and immediately preserved with Ultrex nitric acid to pH <2. Water samples from the column were analyzed using an Instrumentation Laboratory Video 22 (Allied Analytical Systems, Franklin, MA) atomic absorption spectrometer with air-acetylene flame and using Smith-Hieftje background correction. The spectrometer was calibrated prior to each use and the calibration verified using a NIST traceable QAQC standard from an outside source (High Purity Standards, Charleston SC). Flow of water through the column was adjusted as necessary to delivery a nominal high concentration of 3.2 µg Ag/L by the diluter. Water samples from exposure chambers were collected weekly from each exposure level with surviving fry. Water samples were collected from a single replicate that was alternated each week. Samples were passed through a 0.45 µm filter (Acrodisc), collected in a polystyrene tubes (Falcon 2027) and immediately preserved with Ultrex® nitric acid to pH <2. Samples were immediately analyzed for silver using a Thermo Jarrell Ash SH4000 atomic absorption spectrometer and CTF 188 graphite furnace and using Smith-Hieftje background correction. The spectrometer was calibrated prior to each use and the calibration verified using a NIST traceable QAQC standard from an outside source (High Purity Standards, Charleston SC).

Acute Test

The silver chloride acute test was conducted using the same diluter and setup described for the ELS test. A higher capacity peristaltic pump (Cole-Palmer Model 77200-12) was used to increase the flow through the silver chloride column to enable the diluter to deliver a nominal high concentration of 20 µg Ag/L. The flow through the AgCl column ranged between 75 and 81 ml/minute and the concentration ranged between 96 and 100 µg Ag/L. Ten 40-day post swimup rainbow trout were randomly assigned to each exposure chamber. Fish were not fed during the initial 96 hours of exposure. The acute test was terminated after 7 days. Samples for water quality and silver analysis were collected daily during the initial 96 hours and analyzed as described above.

Statistical Analyses

Statistical analyses were conducted using Toxstat version 3.5 software (West Inc. 1996). Analysis of variance (ANOVA) was used to test toxicity endpoints that included hatching success, fry and swimup survival, mean lengths and weights of surviving organisms at test termination. Hatching success and survival data were transformed by arcsine square root prior to ANOVA (Snedecor and Cochran 1980). Normality and homogeneity of variances were tested using Shipiro-Wilk's and Bartlett's tests, respectively (Weber et al., 1989). Treatment means were compared to the control using William's one-tailed test ($p<0.05$) (Williams 1971, Williams 1972). The highest silver concentration not associated with a treatment effect (e.g. decreased survival, decreased body weight) was designated as the no-observed-effect concentration (NOEC). The lowest concentration of zinc that was associated with a treatment effect was designated as the lowest-observed-effect concentration (LOEC). Chronic values were calculated as the geometric mean of the LOEC and NOEC. The inhibition concentration (IC_{20} value), the

concentration estimated to cause a 20% reduction in organism performance compared with the control, was also calculated (USEPA 1993) using the combined weight of surviving organisms from each treatment. Median lethal concentrations (LC_{50}) were estimated by the Trimmed Spearman-Karber technique (Hamilton et al. 1977, 1978) using log transformed silver concentrations and 10% trim.

RESULTS

The results of water quality characteristics measured during the Early Life Stage test are shown in Table 16. Water quality characteristics during the ELS test were consistent throughout the duration of the test as evidenced by narrow ranges and relatively low standard deviations. Temperature was slightly greater than the 12°C target. Hardness and alkalinity were 46.6 and 32.7 mg $CaCO_3$/L, respectively. Dissolved oxygen was near saturation for the elevation of Ft Collins, CO (4780 ft) and pH was slightly greater than neutral at 7.2. Major anions and dissolved organic matter are shown in Table 17.

Measured Ag exposure concentrations and associated hatching success and survival for sac and swimup fry are shown in Table 18. Hatching success exceeded 97.5% in all exposures and was unaffected by exposure to the silver concentrations used in this test. There was no effect of silver exposure on time to hatch. Within five to seven days after hatching, silver related mortality began to occur. Near complete mortality occurred in exposures ≥1.67µg Ag/L and survival was significantly reduced at 0.738 µg Ag/L. At test termination 30 days after swimup, mean survival of trout exposed to 0.738 µg Ag/L was only 40% compared to 90% mean control survival. Survival was unaffected at concentrations of 0.348µg Ag/L and lower. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) based on survival was 0.738 and 0.348 µg Ag/L, respectively. The chronic value, calculated as the geometric mean of the LOEC and NOEC based on decreased survival, is 0.507µg Ag/L.

Table 16. Mean, standard deviation and range of water quality characteristics during rainbow trout early life stage exposure to silver chloride.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	47.4	31.9	7.19	12.4	85.1	8.30
Std. Dev.	2.7	2.4	0.22	0.4	3.3	0.34
Range	41.6-50.8	27.2-35.2	6.81-7.49	11.7-13.1	78.5-88.9	7.72-8.94

Table 17. Major anions and dissolved organic carbon (mg/L) of exposure water during rainbow trout early life stage exposure to silver chloride. Standard deviations are in parentheses.

Chloride (mg/L)	Fluoride (mg/L)	Nitrate (mg/L as N)	Sulfate (mg/L)	Bromide (mg/L)	Phosphate (mg/L as P)	Dissolved Organic Carbon (mg/L)
2.3 (0.2)	0.9 (0.05)	0.07 (0.008)	13.7 (0.8)	<0.1 (0)	0.002 (0.0008)	1.3 (–)

Table 18. Mean measured dissolved silver concentrations (µg/L) and associated hatching success, sac fry and swimup fry survival (%) of rainbow trout. Standard deviations are in parentheses.

Mean Dissolved Ag (µg/L)	<0.05 (0.01)	0.182 (0.048)	0.348 (0.096)	0.738 (0.134)	1.67 (0.18)	3.22 (0.049)
Hatching Success (%)	100 (0)	100 (0)	100 (0)	97.5 (5.0)	100 (0)	97.5 (5.0)
Sac Fry Survival (%)	97.5 (5.0)	97.5 (5.0)	95.0 (5.8)	77.5* (17.1)	2.5* (5.0)	0* (0)
Swimup Fry Survival (%)	90.0 (11.6)	92.5 (9.6)	87.5 (12.6)	40.0* (8.2)	0* (0)	0* (0)

*Significantly less than control ($p<0.05$)

Growth, as measured by lengths and weights of surviving rainbow trout, decreased as silver concentration increased (Table 19). Mean length was significantly reduced at 0.738 (LOEC) but not at 0.348 µg Ag/L (NOEC). Based on mean weight, the LOEC was 0.348 and the NOEC was 0.182 µg Ag/L. Using biomass (combined weight of surviving organisms) as an endpoint, the NOEC and LOEC was also 0.182 and 0.348 µg Ag/L, respectively. The chronic value based on length was 0.507 µg Ag/L and based on weight and/or biomass was 0.252 µg Ag/L. The 20% inhibitory concentration (IC_{20}) based on biomass (combined weight of surviving organisms) at test termination was 0.322 µg Ag/L.

Table 19. Mean measured dissolved silver concentrations (µg/L) and associated mean length (mm) and weight (g) of surviving rainbow trout. Standard deviations are in parentheses.

Mean Dissolved Ag (µg/L)	<0.05 (0.01)	0.182 (0.048)	0.348 (0.096)	0.738 (0.134)	1.67 (0.18)	3.22 (0.049)
Mean Length (mm)	38.9 (0.9)	37.6 (1.6)	36.3 (0.6)	26.0* (3.5)	--	--
Mean Weight (g)	0.550 (0.036)	0.489 (0.052)	0.438* (0.034)	0.160* (0.082)	--	--
Biomass (g)	4.927 (0.407)	4.500 (0.471)	3.839* (0.649)	0.678* (0.466)	--	--

*Significantly less than control ($p<0.05$)

Water quality characteristics during the acute test were consistent throughout the test (Table 20). These characteristics did not differ significantly from those in the ELS test (compare to Table 16). Mean silver exposure concentrations and associated survival of rainbow trout mortality after 96 hours and 7 days are shown in Table 21. Complete mortality occurred at 19.6 µg Ag/L within 96 hours and at 9.64 µg Ag/L by 7 days. All fish in the exposure control survived. The median lethal concentration (LC_{50}) after 96 hours was 5.81 µg Ag/L with a 95% confidence interval of 4.80-7.02. After 7 days, the LC_{50} was reduced to 3.48 µg Ag/L with a 95% confidence interval of 3.08-3.94.

Table 20. Mean, standard deviation and range of water quality characteristics during acute 96 hour silver chloride toxicity test.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	46.6	32.7	7.20	12.4	83.2	8.97
Std. Dev.	1.1	0.8	0.19	0.2	1.3	0.26
Range	45.6-48.6	31.4-33.6	6.93-7.43	12.1-12.8	81.6-85.1	8.64-9.39

Table 21. Mean measured dissolved silver concentrations (µg/L) and 96 hour and 7 day survival (%) of rainbow trout during the acute test. Standard deviations are in parentheses.

Mean Dissolved Ag (µg/L)	<0.05 (0.03)	0.930 (0.13)	2.09 (0.18)	4.51 (0.19)	9.64 (0.51)	19.6 (0.71)
96 Hour Survival (%)	100 (0)	97.5 (5)	97.5 (5)	57.5 (17)	30.0 (12)	0.0 (0)
7 day Survival (%)	100 (0)	95.0 (6)	90.0 (8)	25.0 (10)	0.0 (0)	0.0 (0)

96 hour LC50 (95%C.I.) = 5.51µg Ag/L (4.80-7.02)
7 day LC50 (95%C.I.) = 3.48 µg Ag/L (3.08-3.94)

DISCUSSION

Silver is one of the most toxic metals to freshwater organisms (Eisler 1996). In water, silver exists in many forms (Hogstrand and Wood 1998). The free silver ion, [Ag+], is regarded as the primary toxic form of silver to aquatic life (LeBlanc et al. 1984). Toxicity of silver to rainbow trout is modulated primarily by chloride ions (Hogstrand et al. 1996, Galvez and Wood 1997, Hogstrand and Wood 1998, Bury et al. 1999) and not by hardness, as is the case with many other metals. Attenuation of silver toxicity by chloride ions reflects the complexing capability of the chloride ion. Binding of silver by chloride to form soluble silver chloride complexes ($AgCl_n$) reduces the concentration of [Ag+], thus reducing toxicity. The resulting soluble silver chloride complexes are considerably less toxic than the free silver ion (LeBlanc et al. 1984, Hogstrand and Wood 1998). Despite the protective effects of chloride ions, silver toxicity will result if silver concentrations are sufficiently high, or chloride concentrations sufficiently low.

The chronic value derived from this study was 0.51 and 0.18 µg Ag/L using solid silver chloride as a source and based on survival and growth, respectively. These values are in the range of previously published chronic toxicity values of 0.12-12 µg Ag/L for rainbow trout (Davies et al. 1978, EPA 1980). Davies et al. (1998) derived chronic values of 0.18, 0.58, and 1.07µg Ag/L in waters of hardness 25, 195, and 466 mg $CaCO_3$, respectively. The corresponding chloride concentrations in these waters were 2.9, 13, and 25 mg/L. The chloride concentration in this study was 2.3 mg/L. The chronic value (based on growth) is in close agreement with the chronic value of the 2.9 mg/L chloride study conducted by Davies et al. (1998) although the chronic value based on survival was higher. One possible explanation for the higher survival-based chronic value in this study is the duration of exposure. Exposures of Davies et al. (1998) was a total of 28 weeks whereas this study was 30 days post swimup (48 days total).

Chronic silver exposure can result in sublethal effects including premature hatching (Davies et al. 1978) and retarded development and growth (Davies et al. 1978, Davies and Goettl 1978, Davies et al. 1998). Chronic ELS exposure to silver in this test had a severely negative effect on growth. At test termination, mean weight of fry exposed to 0.738 µg Ag/L was a third of the weight of the control organisms. Effects of silver on time to hatch were not detected although development of sac fry embryos was slightly retarded. Failure to detect premature hatching may have been due to the short exposure time (2-3 days) of the eyed eggs prior to hatching.

The 96 hour median lethal concentration (LC_{50}) to rainbow trout was 5.51µg Ag/L (added as "so called" insoluble silver chloride) in water with chloride concentrations of 2.3 mg/L. This was slightly lower than the 6.77µg Ag/L LC_{50} at a chloride concentration of 2.1 mg/L (Davies et al. 1998) although the 95% confidence intervals overlapped considerably. Davies et al. (1978) reported a mean 96 hr LC50 of 6.5 µg Ag/L (unknown chloride concentration). Those studies used silver nitrate as the source of silver. The 7 day LC_{50} was 3.48 µg Ag/L, a significant decrease from 96 hours reflecting the mortality that continued to occur with exposure duration. MINTEQ (USEPA 1991) was used to speciate major inorganic components. The free silver ion consisted of 87.8% of the total silver present with AgCl (aq) making up 12.0%. This corresponds to 0.028µM [Ag+] free silver as a 7 day LC_{50}. Galvez and Wood (1997) estimated the 7 day LC_{50} as 0.0285 and 0.0294 µM using two different methods. The actual LC_{50} based on [Ag+] may be somewhat lower as our calculations (and presumably Galvez and Wood, as well) did not incorporate dissolved organic carbon in the speciation modeling.

Most noteworthy, solid silver chloride was sufficiently soluble to cause acute and chronic toxicity. Furthermore, toxicity using silver chloride occurred in the same concentration ranges as silver nitrate. This is not surprising since the speciation of silver is nearly identical regardless of whether the silver comes from solid silver chloride or silver nitrate. The amount of additional chloride ion (the predominant inorganic ligand in this test) from the dissolution of silver chloride is negligible relative to the concentration in the dilution water. At equilibrium, AgCl(s) will maintain a free silver ion concentration of 296 µg Ag/L (2.74 µM) in water with a chloride concentration of 2.3 mg/L (based on a log K_{sp} = -9.75, Lindsay 1977). Published acute and chronic thresholds for rainbow trout are well below the solubility of solid silver chloride. During the ELS test, measured total silver concentrations from the column containing AgCl(s) approached the calculated solubility. Flow through the AgCl(s) column was increased for the acute test to deliver higher concentrations through the diluter. Measured silver concentrations from the column decreased, suggesting that dissolution was kinetically limited at the higher flow rates.

The findings from this study contrast with those of Hogstrand et al. (1996) who found no acute toxicity with solid silver chloride. Their study did not involve chronic exposure. Differences in experimental design most likely account for the conflicting results. The experiment conducted by Hogstrand et al. (1996) was a static renewal test which added solid silver chloride as a suspension to aquaria containing fish. The solid silver chloride did not dissolve, since the authors found no silver in solution after filtration. In the

present experiment, solid silver chloride was allowed to dissolve prior to delivery to a diluter.

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ACUTE AND CHRONIC TOXICITY OF ZINC TO THE MOTTLED SCULPIN COTTUS BAIRDI AT 150 mg/L HARDNESS

ABSTRACT

The acute and chronic toxicity of zinc to young of the year mottled sculpin (*Cottus bairdi*) was studied using water with a hardness of 150 mg/L. The 96 hour LC50 was 439 µg Zn/L, but decreased considerably as duration of exposure increased, suggesting that acute mortality was still occurring. No effect on mortality was observed in concentrations as high as 172 but was significantly increased at concentrations of 379 µg Zn/L and greater. The resulting chronic value was 255 µg Zn/L. Effects of zinc exposure on sculpin growth were not detected. Using data from another sculpin toxicity test conducted at a lower hardness demonstrates that zinc toxicity is mitigated by water hardness at a level consistent with other aquatic species.

INTRODUCTION

Mottled sculpin (*Cottus bairdi*) were absent in a 19.3 km segment of the Eagle River directly downstream from an inactive mining operation dating to the 1800s near Minturn, Colorado, U.S.A. (unpublished Colorado Division of Wildlife fish monitoring data). At the same time, sculpin were present in the mainstem Eagle River immediately upstream of the mine operation, in areas downstream of the stream reach impacted by the mine operation, and in the mouths of three tributaries that entered the mainstem in the 19.3 km metal contaminated reach. Zinc was the primary metal of concern in mine impacted reaches. Similarly, shorthead sculpin (*Cottus confusus*) were absent in the Coeur d'Alene river system in Idaho where zinc exceeded ambient water quality standards, but were the most abundant species in reference stream reaches (Maret and MacCoy 2002).

A laboratory toxicity test found mottled sculpin (*Cottus bairdi*) were very sensitive to the effects of zinc in soft water (approximately 50 mg/L hardness) (Woodling et al. 2002). The toxic threshold derived from laboratory data was collaborated by field observations that showed an absence of sculpin in stream reaches where zinc concentrations were elevated. A similar study was conducted to evaluate the effect of water hardness on the toxicity of zinc to mottled sculpin (Davies et al. 2002). However, test organisms in the higher hardness test developed an *Ichthyophthirius multifilis* infection during the later stages of the experiment. The object of the current test is to repeat the higher hardness test with uninfected sculpin. Both acute (96 hour) and chronic (30 day) toxicity of zinc to mottled sculpin at a water hardness of 150 mg $CaCO_3$/L were investigated. Effects of zinc on survival and growth were evaluated.

METHODS AND MATERIALS

Recently emerged C. bairdi were collected from the White River approximately 5 km east of Meeker, Colorado on August 22, 2002, using a Smith Root backpack electrofisher unit. Hardness and conductivity at the site of collection was 240 mg/L $CaCO_3$ and 454 µS/cm, respectively. A water sample at the time of collection contained less than 10 µg Zn/L. The fish were immediately transported in an aerated, iced cooler to the Colorado Division of Wildlife Aquatic Toxicology Laboratory in Fort Collins, Colorado. Sculpin were maintained in a glass aquarium supplied with dechlorinated Ft Collins municipal tap water (water hardness approximately 50 mg $CaCO_3$/L). After 26 days, well water from an onsite well was added to dechlorinated Ft Collins municipal tap water to deliver a water hardness of 150 mg/L $CaCO_3$. Sculpin were acclimated to this mixture for 18 days prior to the start of the toxicity test. Treatment and care of test organisms were identical to Woodling et al. 2002. Toxicant diluter, test methods, water quality analyses, zinc analyses and statistical methodology were identical to Woodling et al. 2002 except that dilution water consisted of Ft Collins municipal tap and onsite well water mixed to achieve a nominal hardness of 150 µg/L $CaCO_3$ and photoperiod was 12h day-12 h night.

Conductivity of the dilution water was montored daily to assure that hardness of the mixed water was consistant and near target levels. Nominal exposure concentrations were increased to 800, 400, 200, 100, 50 and 0 µg Zn/L. Two fish escaped from their exposure chambers during the initial 96 hours of exposure; one each from a 172 and 94 µg/L treatment. For those two treatments, mortality rates were based on six fish per treatment.

RESULTS

Temperature and pH of exposure water was similar to Woodling et al. 2002 (Table 22). Mean water hardness was 154, near the target value of 150 mg $CaCO_3$ /L. Alkalinity and conductivity of exposure water was higher than Woodling et al. 2002 reflecting the influence of the well water in the mixture.

Measured dissolved zinc concentrations and associated mortality of sculpin are shown in Table 23. Complete mortality occurred at 778 µg Zn/L by the ninth day. Mortality of sculpin exposed to 279 µg Zn/L increased from 46% after 96 h to 86% after 13 days. Mortality was low in the other exposures. There was no mortality at exposure concentrations of 50 µg Zn/L or the control. No additional mortality occurred after 15 days through the end of test at 30 days. The estimated median lethal concentration (LC50) declined with duration of exposure from 439 µg Zn/L after 96 h to 266 µg Zn/L after 13 d (Table 24). The average length and weight of all sculpin that died within the initial 96 h of exposure were 34.6 mm and 0.429 g, respectively. Mean lengths and weights of sculpin surviving the 30 day test were 37.9 mm and 0.497 g. Length and weight of surviving sculpin was unaffected by exposure to zinc (Table 25). Mortality was unaffected by zinc exposure concentrations as high as 172 µg Zn/L (no observed effect concentration) but was significantly greater than the control at 379 µg Zn/L or

more (lowest observed effect concentration). The chronic value for juvenile sculpin exposed to zinc in water hardness for 30 days is 255 µg Zn/L.

Table 22. Mean, standard deviation and range of water quality characteristics of exposure water used for 30 day zinc toxicity test conducted with mottled sculpin.

	pH (S.U.)	Temp. (°C)	Hardness (ppm)	Alkalinity (ppm)	Conductivity (µS/cm)	Oxygen (mg O_2/L)
Mean	7.54	12.4	153.9	105.5	254	8.16
Std. Dev.	0.09	0.4	8.9	7.1	20	0.32
Range	7.38-7.70	11.7-13.1	138-167	95.4-118	229-284	7.59-8.82

Table 23. Mean dissolved zinc concentrations (µg/L) and associated mortality (%) of mottled sculpin at different durations of exposure. Standard deviations are in parentheses.

Nominal Zn (µg/L)	0	50	100	200	400	800
Dissolved Zn (µg/L)	<5 (3)	50 (6)	94 (9)	172 (17)	379 (16)	778 (21)
96 Hr (%)	0 (0)	0 (0)	0 (0)	0 (0)	46 (21)	71 (12)
5 Day (%)	0 (0)	0 (0)	0 (0)	0 (0)	75 (24)	82 (14)
6 Day (%)	0 (0)	0 (0)	0 (0)	4 (7)	82 (18)	89 (14)
7 Day (%)	0 (0)	0 (0)	4 (7)	7 (8)	82 (18)	89 (14)
8 Day (%)	0 (0)	0 (0)	4 (7)	7 (8)	82 (18)	96 (7)
9 Day (%)	0 (0)	0 (0)	4 (7)	7 (8)	82 (18)	100 (0)
13 Day (%)	0 (0)	0 (0)	4 (7)	7 (8)	86 (20)	100 (0)
15 Day (%)	0 (0)	0 (0)	7 (8)	7 (8)	86 (20)	100 (0)
30 Day (%)	0 (0)	0 (0)	7 (8)	7 (8)	86 (20)	100 (0)

Table 24. Median lethal concentrations of zinc and 95% confidence intervals (µg/L) to mottled sculpin at different durations of exposure.

Duration of exposure	LC50 Estimate (µg/L)
96 Hours	439 (290-664)
5 Days	302 (245-372)
6 Days	283 (243-328)
7 Days	278 (239-324)
8 Days	279 (243-321)
9 Days	273 (242-309)
13 Days	266 (240-295)
15 Days	266 (240-295)
30 Days	266 (240-295)

Table 25. Mean dissolved zinc concentrations (µg/L), mortality (%), total lengths (mm), and weight (g) of mottled sculpin after 30 days exposure. Standard deviations are in parentheses.

Nominal Zn (µg/L)	0	50	100	200	400	800
Dissolved Zn (µg/L)	<5 (3)	50 (6)	94 (9)	172 (17)	379 (16)	778 (21)
Mortality (%)	0 (0)	0 (0)	7 (8)	8 (9)	86 (20)*	100 (0)*
Length (mm)	38.2 (1.0)	38.1 (1.5)	37.1 (1.9)	38.6 (2.1)	40.4 (0.5)	--
Weight (g)	0.471 (0.042)	0.502 (0.060)	0.490 (0.044)	0.508 (0.078)	0.650 (0.060)	--

*Significantly greater than control ($p<0.05$)

DISCUSSION

The results of this and other studies (Woodling et al. 2002, Davies et al. 2002) find mottled sculpin to be very sensitive to the toxic effect of zinc. Those studies reported a 96 h LC50 of 156 and 590 µg/L and at a hardness of 48.6 and 156 mg/L $CaCO_3$,

respectively. The 96h LC50 of this study was 439 µg/L at a hardness of 154 mg/L $CaCO_3$, comparable to 590 µg/L found by Davies et al. 2002 using similar water quality. Combining all three results yields an acute toxicity-hardness (log-log) slope of 1.022 and coefficient of variation (r-squared) of 0.95. The slope for the hardness-toxicity relationship for sculpin falls within the range of 0.5603 for bluegill and 1.644 for guppy and is near the pooled mean acute slope of 0.8473 used in the EPA acute zinc criteria (USEPA 1987). The chronic value derived from this test is 255 µg/L, similar to the chronic value of 302 µg/L reported by Davies et al. (2002), which had similar water quality. The chronic values at 150 mg/L $CaCO_3$ hardness are greater than EPA's hardness-based chronic zinc criteria, indicating that sculpin are protected at this hardness. However, the chronic values of sculpin at 46 mg/L $CaCO_3$ hardness was 21 µg Zn/L, well below criteria (Woodling et al. 2002). Additional experiments are necessary to more fully investigate the role of hardness on zinc toxicity to sculpin.

This study did not detect an effect of zinc exposure on the growth of sculpin. Earlier studies also failed to detect effects of zinc on sculpin growth (Woodling et al. 2002, Davies et al. 2002). Growth is often a more sensitive endpoint than mortality in chronic experiments. However, detection of growth effects usually require that tests are started using eggs or early life stages. Additional experiments should be attempted using sculpin eggs and early life stages to evaluate potential effects of zinc on growth or other sublethal endpoints.

Acute mortality of mottled sculpin was delayed compared to trout, a phenomenon that was also observed by Woodling et al. (2002). Sculpin mortality did not occur until after 72 hours of exposure and continued until about the ninth day of exposure. As a comparison, in a brown trout zinc toxicity test conducted with similar water quality characteristics, all acute toxicity occurred in the initial 48 hours of exposure. Mortality of brown trout was minimal between 96 hours and the end of the test at 30 days (See Table 11, this report). On the other hand, mortality of sculpin increased from 46 to 86% at 379 µg Zn/L and from 71 to 100% at 778 µg/L. Much of this mortality occurred between 96 hours and the ninth day indicating that sculpin were still experiencing an acute response to zinc exposure.

Overall, the results from the present study are in close agreement with Davies et al. (2002), which was conducted using near identical methodology. In that test, an *Ichthyophthirius multifilis* infection occurred in control and low exposure treatments. However, the onset of nonmetal-related mortality did not occur until the last week of the thirty day exposure. The agreement of results from these two studies suggest the infection did not greatly affect endpoints from test by Davies et al.

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STUDY PLAN C: TECHNICAL ASSISTANCE

Objective:

To provide expertise, consultation, evaluation and training in aquatic toxicology and aquatic chemistry to Division of Wildlife personnel, and other state and federal agencies.

Job C1. Water Quality Assistance to Other Personnel

Objectives:

1. To oversee the training and evaluation of metal analysis by laboratory technicians.

2. To assist Division and other state and federal personnel in the analysis and toxicological assessment of water quality data.

3. To develop and maintain a quality assurance program to evaluate the quality of analytical results for metals.

4. To collect and analyze metals concentrations in samples from the Arkansas River.

ACCOMPLISHMENTS:

Water quality characteristics and or metal analyses were performed for the following persons and agencies:

Blair Prusha, Colorado State University
Donna Kashian, Colorado State University
Kevin Meyer, University of Southern Colorado
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Rotenone analyses were performed for :

Gary Dowler, CDOW
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