Water Pollution Studies

Federal Aid Project F-243R-17

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Job Progress Report

Colorado Division of Wildlife

Aquatic Wildlife Research Section

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The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Division of Wildlife policy by the Director or the Wildlife Commission.

Table of Contents

List of Tables

List of Figures

State: Colorado

Study No. F243R17

Title: Water Pollution Studies

Period Covered: July 1, 2009 to June 30, 2010

Project 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: LABORATORY TOXICITY STUDIES

Brief Description: Conduct laboratory-based experiments to test effects of contaminants on aquatic organisms.

Job A.1. Reproductive Toxicity of Endocrine Disrupting Compounds

Job Objective:

Determine whether exposure to hormonally active agents results in feminization of rainbow trout, fathead minnows and/or other aquatic organisms. Effects of feminization on reproduction and fecundity will be measured. Concentrations of endocrine disrupting compounds that result in significant feminization will be compared to concentrations observed in wastewater treatment plant effluents and in Colorado streams.

Job A.2. Reproductive Toxicity of Atrazine Herbicide

Job Objective:

Measure fecundity and biomarkers of feminization of red shiners exposed to a range of atrazine. Relate concentrations that result in impairment in the laboratory with concentrations observed in Colorado eastern plains streams.

Job A.3. Toxicity of Metals to Fish

Job Objective:

Measure acute (96 hour) and chronic (60 day) effects of zinc, copper and/or cadmium exposure on hatching, survival and growth of different life stages of mottled sculpin, longnose dace and/or other species. Results from these experiments will compare toxicity thresholds to USEPA metal criteria to ensure that these species are protected.

Job A.4. Effects of Dietary Exposure of Metals to Fish

Job Objective:

Measure the effect of zinc, copper, cadmium and/or selenium from dietary sources on survival and growth of fish in the laboratory. Evaluate the sensitivity of dietary-exposed organisms to waterborne exposure. Relate dietary levels that cause diminished performance in the laboratory with levels found in dietary sources in metal impacted areas such as the upper Arkansas River, Clear Creek and the Eagle River.

Job A.5. Testing and Validation of the Biotic Ligand Model

Job Objective:

Determine the ability of the Biotic Ligand Model to estimate acute and chronic toxicity effects of metals on aquatic organisms exposed under multiple water quality conditions.

STUDY PLAN B: TECHNICAL ASSISTANCE

Brief Description: Conducts toxicological experiments as requested from regulators to be incorporated into policy; conducts water chemistry analysis and training for CDOW and other agencies.

Job B.1. Water Quality Assistance to Division of Wildlife Personnel and Other State and Federal Agencies.

Job Objectives:

To provide technical assistance and expertise, consultation, evaluation and training in aquatic toxicology and aquatic chemistry to Division of Wildlife and other state and federal personnel as requested. Conduct short or long term experiments to produce toxicity data, or develop site-specific field studies, when such data in the literature are lacking or inadequate. Ultimately, these activities will assist regulatory agencies in the development, implementation, and enforcement of water quality standards needed to protect or enhance the aquatic resources of Colorado.

ACCOMPLISHMENTS

Job A.1. Reproductive Toxicity of Endocrine Disrupting Compounds

The project continued to provide equipment and support for onsite bioassays conducted by personnel at the University of Colorado and Unversity of Denver. The studies' objectives were to detect and quantify estrogenic activity in the city of Boulder wastewater treatment plant effluent.

Job A.2. Reproductive Toxicity of Atrazine Herbicide

No activities during this segment.

Job A.3. Toxicity of Metals to Fish

Six flow-through toxicity tests were conducted comparing toxicity of zinc to Greenback, Colorado River and Rio Grande cutthroat trout fry at two different water hardnesses. Results are reported below.

Job A.4. Effects of Dietary Exposure of Metals to Fish

Factors affecting bioaccumulation of mercury in sport fish in Colorado reservoirs.

This ongoing study is being conducted by Jesse Lepak (Post Doctoral Fellow) and Dr. Brett Johnson in the Department of Fish, Wildlife and Conservation Biology at Colorado State University. Several reports generated from this work are in currently in review at CDOW for final approval.

Mercury (Hg) testing by the Colorado Department of Public Health and Environment (CDPHE) has uncovered a growing number of Colorado waters that contain fish with Hg concentrations that exceed 0.3 ppm, the USEPA (2001) fish tissue residue criterion for the protection of human health. In 2008, Colorado Division of Wildlife (CDOW) funded a four-year investigation to address the issue of Hg contamination in Colorado reservoirs. The goal of this research is to characterize the relative importance of factors influencing Hg bioaccumulation in reservoirs and evaluate how fishery management strategies affect Hg concentrations in sport fish. We originally selected to study four Colorado reservoirs based on their attributes (e.g., similar size, fish assemblages and available data). Carter and Horsetooth reservoirs were selected to represent contaminated food webs, both having Hg consumption advisories for walleye (*Sander vitreus*). Chatfield and Union reservoirs also contain walleye and were elected to represent food webs without fish consumption advisories. We collected zooplankton (pelagic), chironomids (profundal), crayfish (littoral), prey fish and walleye from each reservoir to characterize Hg bioaccumulation in food webs. We also characterized abiotic factors thought to influence Hg dynamics in reservoirs including water level fluctuation, water temperature, secchi depth, conductivity and water chemistry (e.g., DO, P, Al).

Progress on this project includes the addition of Brush Hollow, a walleye reservoir in the SE region, to the study. Food web and walleye growth data, as described above, were collected as part of a CDPHE-funded grant. In addition, bioergetics modeling scenarios were constructed for all five reservoirs to determine which factors (walleye population or food web factors) have the strongest influence on Hg accumulation in walleye. Model results show that the stocking of prey fish with high

caloric value, but low mercury levels, would dilute mercury levels below the CDPHE action limit. By contrast, management scenarios aimed at increasing walleye growth rate, or size at age, would have limited influence on reducing mercury risk. Other factors, such as increasing reservoir nutrient productivity and reducing water level fluctuations may serve to reduce Hg in prey fish, thereby reducing Hg accumulation in walleye. An interesting result for Carter and Brush Hollow reservoirs, observed through isotope analysis, is that annually stocked rainbows trout are acting as a major prey item for larger/older walleye, and because they have low mercury content, are significantly reducing mercury burdens in walleye that take advantage of this resource. Unfortunately, this "subsidy" role has resulted in a return to creel at Carter that is less than desirable. At Brush Hollow, a balance of walleye and rainbow is more sustainable. Our findings provide a better understanding of Hg bioaccumulation that will be used to design fishery management strategies aimed at remediating Hg contamination in reservoirs.

Job A.5. Testing and Validation of the Biotic Ligand Model

A fundamental assumption of the biotic ligand model is that the binding affinity and capacity of metals to gills is similar among different taxa. Thus, different tolerances of different species to metals such as zinc are due to different abilities to withstand different amounts of zinc on the gills, measured by LA50. Brook trout and brown trout fingerlings were exposed to a range of concentrations of the stable zinc isotope ^{67}Zn. Accumulation of the stable isotope by the gills was measured in low water hardness over a range of time intervals between 45 minutes and 72 hours. An acute toxicity test was conducted concurrently so that a median lethal accumulation value (LA50) could be calculated. The gill-binding affinity and capacity of brook trout and brown trout will be determined and compared to rainbow trout. Tissue and water samples are currently awaiting analysis by United States Geological Survey (USGS) and will be reported next segment.

Job B.1. Water Quality Assistance to Division of Wildlife Personnel and Other State and Federal Agencies.

Pete Cadmus (MS student) and Dr. Will Clements from Department of Fish, Wildlife and Conservation Biology, Colorado State University continue to collaborate with CDOW to determine the dietary effects of metals on aquatic invertebrates. A method to measure subcellular compartmentalization of zinc was modified and adapted for mayfly nymphs. Although laboratory toxicity tests have shown that mayflies are highly tolerant to aqueous Zn exposure, field biomonitoring studies have shown marked decreases in mayfly abundance at relatively low concentrations of metals. To investigate possible causes of this discrepancy, we examined the role dietary exposure to Zn in a series of laboratory toxicity tests. Two species of grazing mayflies (*Ameletus* sp. and *Rhithrogena* sp.) were collected from unpolluted streams (Cache La Poudre at the Narrows) and exposed for seven days to sublethal levels of Zn. Experimental treatments included three levels of aqueous exposure and three levels of dietary exposure. We measured total accumulation of Zn as well as Zn associated with several sub-cellular fractions including exoskeleton, cell fragments, heat-labile cytosolic proteins and

metallothionein-like proteins. In general, dietary exposure increased total Zn concentration in mayflies compared to the aqueous only treatments. We compared these metal concentrations to those in organisms collected from the Arkansas River, a metal-contaminated stream in Colorado. Despite much greater aqueous concentrations of Zn in the laboratory experiments, Zn bound to heat-labile cytosolic proteins was consistently greater in mayflies collected from the field. The disproportionately large amount of Zn associated with heat-labile proteins in organisms collected from the Arkansas River may help explain the discrepancy between results of laboratory toxicity tests and field biomonitoring studies.

Water samples for metals analysis were collected from the Arkansas River upstream of Leadville downstream to Salida. Livers and kidneys were collected from brown trout at selected stations. Tissues were digested and analyzed for cadmium, copper and zinc. Results are reported below.

Days to Hatch: Evolutionary Plasticity in Native Cutthroat Trout

Cutthroat trout were once widely distributed in the Rocky Mountain streams of Colorado but have declined due to nonnative competition and hybridization. Management strategy has relied on segregation of genetically pure strains from nonnative salmonids via translocations above migration barriers, limiting cutthroat trout populations to higher elevations. Attempts to translocate cutthroat trout for the purpose of establishing new populations are frequently unsuccessful. Harig and Fausch (2002) identified low summer temperatures as an important factor for failure of translocations of greenback and Rio Grande cutthroat. Low summer temperatures delay spawning and prolong egg incubation. As a result, cutthroat fry may be unable to acquire sufficient energy reserves in the summer to survive the winter. Indeed, cutthroat fry reared at a low temperature regime experienced lower survival than fry in an intermediate and high temperature regime (Coleman and Fausch 2007). Cold summer temperatures in high elevation, low temperature streams may present a selection pressure for cutthroat trout that favors rapid embryonic development. Variable degree-days to hatch in cutthroat trout was noted in a population of greenback cutthroat trout that appeared to be adapted to life at high elevation and the cold water that comes with it (Behnke 2002). When eggs from this population in the South Fork Poudre River were cultured alongside greenback cutthroat trout from Cascade Creek, they hatched much sooner (in only 256 degree days rather than the 312 degree days for the Cascade Creek fish which is more typical of cutthroat trout and rainbow trout (Behnke 2002). The ability of trout eggs to develop quickly and hatch early would allow fry to elaborate enough tissue before winter conditions descend on the high country to persist where reintroduction efforts with greenback cutthroat trout that were not cold-adapted failed. An experiment was conducted to determine whether different cutthroat populations had adapted to cold temperatures and high elevation by reducing the amount of time required for eggs to hatch. Time to hatch was explored for five populations of cutthroat trout that represent a broad spectrum of elevations. Fertilized eggs were either obtained from hatchery broodstocks recently founded from wild populations (Lake Nanita, Carr Creek and Trapper Creek), or directly from spawning fish collected in the wild (Cunningham Creek,

and South Fork Poudre River). Results from the study will be analyzed and reported next segment.

Mountain Whitefish Temperature Experiments

Studies were conducted to measure effects of temperature and agitation on mountain whitefish egg hatch. An additional study was conducted to measure effect of temperature on growth and survival of mountain whitefish fry. Results of these studies are reported below.

This project assisted with fish kill investigations at Nee Noshe and Nee Gronde. Rotenone concentrations were determined in several water sample collected as part of a rotenone operation at Monument Reservoir. Considerable assistance and resources were provided to a CSU Masters graduate student studying effects of water quality parameters on *Didymosphenia*.

DOW participated as Party Status in several Water Quality Control Commission Rulemaking and Administrative Action Hearings, and provided input to WQCC and Stakeholders at regulatory workgroups (nutrient criteria and aquatic life classifications). We continue to serve on BTAG (Biological Technical Assistance Group) committees for the Arkansas River mine site and for the Standard Mine on Coal Creek near Crested Butte, where we provide expertise and data. We represent DOW on CDPHE's Technical Advisory Committee for mercury contamination in fish tissues. Mercury action limits are being set and protocols for notifying the public of potential health hazards are being developed. We assisted DOW biologists in coordinating their fish collection with CDPHE chemical analysts to assess risks to anglers at numerous reservoirs around the State.

DOW worked with the USFWS, BLM, CDPHE, EPA and the Attorney General's Office on inter-agency water quality restoration projects, including Natural Resource Damage Claims for the upper Arkansas River and the Rocky Mountain Arsenal superfund sites. DOW wrote several letters of support for academic researchers and agencies who are seeking nationally-sponsored funding to conduct experiments with metals (zinc, cadmium, mercury) and endocrine disrupting compounds.

Toxicity of Zinc to Colorado River, Greenback and Rio Grande Cutthroat Trout at Two Water Hardnesses

INTRODUCTION

Zinc is often present in high and toxic concentrations from acid mine drainage and affects an estimated 842 miles of streams in Colorado (CDPHE 2008). Many of the affected streams are headwaters impacted by historic mining activities. Water quality standards and criteria are based on laboratory toxicity tests conducted on numerous aquatic species. However, cutthroat trout are not included in calculations that determine ambient water quality criteria for zinc (USEPA 1987, USEPA 1995) due to limited data. Cutthroat trout are one of two native salmonids in Colorado. Colorado River, Greenback and Rio Grande cutthroat trout are three subspecies that occur in Colorado. The purpose of this study was to measure toxicity of zinc at different water hardnesses and to compare the sensitivity of the three subspecies.

METHODS and MATERIALS

Organisms

Freshly fertilized Colorado River and Greenback cutthroat trout eggs were obtained from Colorado Division of Wildlife hatchery broodstocks recently founded from wild populations (Lake Nanita and Carr Creek, respectively). The eggs of four Lake Nanita females were fertilized with the milt of eight males. Five females were fertilized with ten males for Carr Creek eggs. Fertilized eggs were transported in a cooler to the Colorado Division of Wildlife aquatic toxicology where they were treated with 1600 ppm formalin for 15 minutes upon arrival. Rio Grande cutthroat trout eggs were collected from wild spawning fish collected in Haypress Lake and incubated at the Colorado Division of Wildlife Pitkin Hatchery before being shipped to the toxicity lab as eyed eggs. Eggs were cultured in dechlorinated Ft. Collins municipal tap water. After swimup, fry were fed starter salmon chow. Two weeks after swimup, fry were randomly divided. Half of the fry were maintained in the 50 ppm hardness dechlorinated tap water while the other half of the fry were acclimated to a mixture of onsite well water and dechlorinated tap water. Conductivity controllers were used to maintain the tap –well water mixture at a water hardness near 150 ppm. Fry were maintained in their respective waters for two weeks prior to toxicity tests. Fry were not fed for 48 hours prior to initiation of toxicity tests.

Toxicity Tests

A continuous-flow serial diluter (Benoit et al. 1982) delivered exposure concentrations. The diluter was constructed of Teflon, polyethylene, and polypropylene components. Nalgene food-grade vinyl tubing delivered test solutions to exposure chambers. Test solutions overflowed from the exposure chambers into a water bath maintained at 12°C using a recirculating chiller (VWR model 1175MD). A stock solution

was prepared by dissolving a calculated amount of zinc sulfate salt in deionized water ($ZnSO_4 \cdot 7H_2O$ Mallincrodt). Stock solutions were delivered to the diluter via a peristaltic pump at a rate of 2.0 mls/min. Diluters delivered five concentrations with a 50% dilution ratio and a control. Target concentrations for the 50 mg/L test were 1000, 500, 250, 125, 62, and 0 µg/L. Target concentrations for the 150 mg/L test were 5000, 2500, 1250, 625, 312, and 0 µg/L. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 ml/min for each chamber. Exposure chambers consisted of 2.8 L polypropylene containers. Dim fluorescent lighting provided a 16-h/8-h light-dark photoperiod. Diluters and toxicant flow rates were monitored daily to ensure proper operation. At the start of exposure, 12 fry were randomly allocated to each exposure chamber.

Water quality parameters were measured at 0, 48, and 96 hrs in all treatment levels within a replicate. Different replicates were selected each sampling event. Hardness and alkalinity were determined according to Standard Methods (APHA 1998). A Thermo Orion 635 meter was used to measure pH and conductivity. Dissolved oxygen was measured using an Orion 1230 dissolved oxygen meter. The conductivity, pH and dissolved oxygen meters were calibrated prior to each use.

Water samples for zinc and major cations and anions were collected at 0, 48, and 96 hrs. Samples for zinc and cation analysis were passed through a 0.45µm filter and immediately preserved with high purity nitric acid to pH <2. Chambers with no survivors remaining were not sampled. Zinc and major cations were measured 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). Calcium and magnesium were analyzed with 0.1% lanthanum as a modifier. Sodium and potassium were analyzed with 0.1% cesium to control ionization. Chloride and sulfate concentrations were measured with a flow-injection analyzer (QuikChem 8000; Lachat Instruments) using USEPA methods 325.1 and 375.4, respectively (USEPA 1983). Sample splits were collected and spikes prepared at each sampling event to verify reproducibility and analytical recovery. Ninety six hour median lethal concentrations (LC_{50}) were estimated using the Trimmed Spearman-Karber technique with automatic trim (Hamilton et al. 1977, 1978).

RESULTS and DISCUSSION

Water quality characteristic were consistent within each toxicity test as evidenced by relatively low standard deviations of measurements (Table 1). Characteristics were constant among the different cutthroat trout strains within the 50 and 150 hardness tests. Dissolved organic carbon was not measured but previous analyses of the water sources used in this test were 1-2 mg/L. In general, measured dissolved zinc concentrations were near target levels except for the two tests conducted with Rio Grande strain which was about 20% low (Tables 2-7). Zinc concentrations were constant during the 96 hour test. No mortalities occurred in the control exposures.

Median lethal zinc concentrations for Greenback and Rio Grande cutthroat trout were about five times higher at 150 mg/L hardness than at 50mg/L hardness (Table 8). Median lethal zinc concentrations for Colorado River cutthroat trout was almost eight times higher at 150 mg/L hardness than at 50mg/L hardness. Using the 95% confidence intervals as a test of significant difference, Greenback cutthroat trout were significantly more tolerant to zinc that Rio Grande cutthroat trout at both 50 and 150 mg/L hardness. Colorado River cutthroat trout were intermediate in sensitivity in that they were similar to Rio Grande cutthroats at 50 mg/L but similar to Greenbacks at 150 mg/L.

Median lethal concentrations plotted as a function of water hardness shows the effect of water hardness on zinc toxicity (Figure 1). Included in the figure are additional data from toxicity tests with Colorado River cutthroat trout (Davies et al. 2000, Brinkman and Hansen 2004) and a mixed strain of cutthroat (Brinkman and Vieira 2008). Median lethal concentrations from the present studies are in general agreement with previously determined values. A log-log regression of all data yields the following equation (r^2=0.94):

$$LC50 = e^{(1.535*\ln(Hardness)-0.4900)}$$

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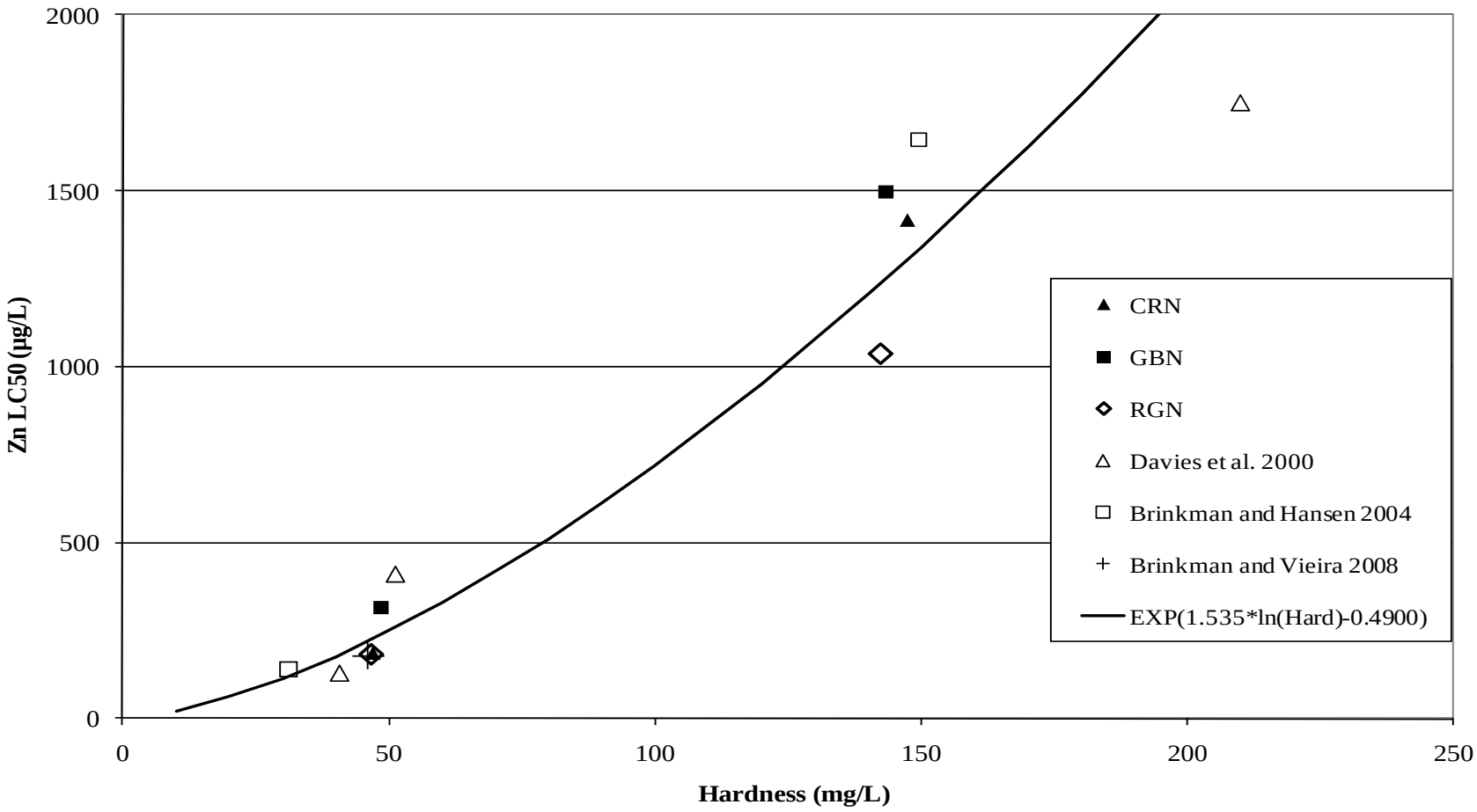

Figure 1. 96 hr median lethal concentrations of zinc to Colorado River (CRN), Greenback (GRN),and Rio Grande (RGN) cutthroat trout as a function of water hardness.

Table 1. Mean water quality measurements of zinc toxicity tests with Colorado River, Greenback, and Rio Grande cutthroat trout conducted in 50 and 150 mg/L hardness waters. Standard deviations are in parentheses.

Water	50 Hardness			150 Hardness		
Subspecies	Colorado River	Greenback	Rio Grande	Colorado River	Greenback	Rio Grande
Hardness* (mg/L)	46.9 (0.7)	48.6 (0.8)	46.8 (0.7)	147.4 (0.5)	143.3 (1.0)	142.4 (0.5)
Alkalinity (mg/L)	35.7 (0.5)	35.7 (0.4)	36.0 (0.6)	106.0 (1.6)	105.2 (1.5)	103.8 (1.2)
pH (S.U.)	7.42 (0.06)	7.49 (0.06)	7.66 (0.05)	7.57 (0.06)	7.62 (0.08)	7.74 (0.04)
Temperature (ºC)	11.9 (0.1)	12.0 (0.1)	12.7 (0.2)	11.7 (0.1)	11.6 (0.1)	11.9 (0.1)
Conductivity (µS/cm)	87.1 (1.7)	89.3 (0.8)	89.2 (3.1)	259.3 (6.0)	259.5 (5.5)	256.3 (7.4)
DO (mg/L)	9.29 (0.34)	9.55 (0.12)	9.46 (0.14)	9.11 (0.21)	9.30 (0.10)	9.13 (0.10)
Calcium (mg/L)	16.9 (0.3)	17.5 (0.3)	16.7 (0.2)	41.8 (0.2)	40.5 (0.4)	40.1 (0.2)
Magnesium (mg/L)	1.2 (0.0)	1.2 (0.0)	1.3 (0.0)	10.4 (0.0)	10.2 (0.0)	10.3 (0.0)
Sodium (mg/L)	2.0 (0.1)	2.2 (0.0)	2.4 (0.2)	9.5 (0.0)	9.6 (0.1)	9.8 (0.2)
Potassium (mg/L)	0.6 (0.0)	0.6 (0.0)	0.6 (0.0)	0.7 (0.0)	0.7 (0.0)	0.7 (0.0)
Chloride (mg/L)	2.4 (0.0)	2.5 (0.0)	2.8 (0.1)	11.2 (0.1)	10.8 (0.1)	10.9 (0.2)
Sulfate (mg/L)	12.3 (0.1)	12.0 (0.0)	12.1 (0.1)	43.6 (0.6)	42.4 (1.2)	43.5 (0.6)

*Calculated from calcium and magnesium ion concentrations.

Table 2. Mean measured dissolved zinc concentrations (µg/L) and associated 96 hr mortality (%) of Greenback cutthroat trout in 50 mg $CaCO_3$/L hardness water. Standard deviations are in parentheses.

Target	0	62	125	250	500	1000
Measured [Zn] (µg/L)	<10 (2)	65 (1)	124 (2)	239 (3)	484 (5)	994 (16)
96 hr Mortality (%)	0 (0)	0 (0)	0 (0)	29.2 (4.8)	83.3 (9.6)	100 (0)

Table 3. Mean measured dissolved zinc concentrations (µg/L) and associated 96 hr mortality (%) of Greenback cutthroat trout in 150 mg $CaCO_3$/L hardness water. Standard deviations are in parentheses.

Target	0	312	625	1250	2500	5000
Measured [Zn] (µg/L)	<10 (3)	289 (17)	597 (36)	1194 (75)	2297 (136)	4725 (7)
96 hr Mortality (%)	0 (0)	0 (0)	6.25 (8.0)	35.4 (8.0)	73.1 (17.4)	100 (0)

Table 4. Mean measured dissolved zinc concentrations (µg/L) and associated 96 hr mortality (%) of Colorado River cutthroat trout in 50 mg $CaCO_3$/L hardness water. Standard deviations are in parentheses.

Target	0	62	125	250	500	1000
Measured [Zn] (µg/L)	<10 (1)	44 (1)	95 (1)	211 (1)	506 (3)	1161 (1)
96 hr Mortality (%)	0 (0)	0 (0)	0 (0)	68.8 (10.5)	100 (0)	100 (0)

Table 5. Mean measured dissolved zinc concentrations (µg/L) and associated 96 hr mortality (%) of Colorado River cutthroat trout in 150 mg $CaCO_3$/L hardness water. Standard deviations are in parentheses.

Target	0	312	625	1250	2500	5000
Measured [Zn] (µg/L)	<10 (1)	297 (1)	615 (3)	1237 (6)	2320 (17)	4645 (35)
96 hr Mortality (%)	0 (0)	2.1 (4.2)	2.1 (4.2)	29.2 (4.8)	95.8 (8.3)	100 (0)

Table 6. Mean measured dissolved zinc concentrations (µg/L) and associated 96 hr mortality (%) of Rio Grande cutthroat trout in 50 mg $CaCO_3$/L hardness water. Standard deviations are in parentheses.

Target	0	62	125	250	500	1000
Measured [Zn] (µg/L)	<10 (3)	52 (6)	97 (11)	186 (17)	393 (6)	792 (6)
96 hr Mortality (%)	0 (0)	0 (0)	14.6 (10.5)	60.4 (23.3)	85.4 (12.5)	97.9 (4.2)

Table 7. Mean measured dissolved zinc concentrations (µg/L) and associated 96 hr mortality (%) of Rio Grande cutthroat trout in 150 mg $CaCO_3$/L hardness water. Standard deviations are in parentheses.

Target	0	312	625	1250	2500	5000
Measured [Zn] (µg/L)	12 (6)	260 (18)	523 (11)	1082 (10)	2020 (28)	4070 (85)
96 hr Mortality (%)	0 (0)	2.1 (4.2)	8.3 (11.8)	52.1 (28.4)	91.7 (9.6)	98.1 (3.8)

Table 8. 96-hr median lethal zinc concentrations (LC50) for Colorado River cutthroat, Greenback cutthroat and Rio Grande cutthroat trout in 50 and 150 mg/L water hardness. 95% confidence intervals are in parentheses.

	50 mg/l	150 mg/L
Colorado River Cutthroat	185 (165-207)	1418 (1286-1562)
Greenback Cutthroat	314 (279-353)	1498 (1307-1717)
Rio Grande Cutthroat	184 (159-214)	1036 (908-1181)

Effect of Temperature on Survival and Growth of Mountain Whitefish (*Prosopium williamsoni*) Fry

INTRODUCTION

Anecdotal evidence of declines in some mountain whitefish populations (*Prosopium williamsoni*) has spurred an interest in this native salmonid across the Rocky Mountain west. Managers alarmed by the local declines convened summits in both Colorado and Montana dedicated to summarizing what little is known about the status of these fish and develop priorities for future research. In Colorado, mountain whitefish are native to the White and Yampa Rivers and have been introduced to the Cache La Poudre River in 1956 and the Roaring Fork River in the Colorado River drainage. Catastrophic declines in the beginning of this decade on the Yampa River coincided with severe and widespread drought, invasion of a non-native predator (northern pike, *Esox lucius*), and the appearance of whirling disease (*Myxobolis cerebralis*). Drought-induced warming may adversely affect mountain whitefish due to sensitivity of eggs to elevated temperatures (Rajagopal, 1979). Our objective was to measure effects of temperature on mountain whitefish fry growth and survival. Specific goals were to determine the temperature for maximum growth and to determine the upper incipient lethal temperature (UUILT).

METHODS

Mature mountain whitefish were collected with backpack electrofishing gear in small tributary streams to larger rivers home to robust populations of mountain whitefish. Eggs were stripped and fertilized in the field, placed in 7.5 L water coolers and transported 160 miles (approximately 3 hr 20 min drive time) to the Colorado Division of Wildlife Aquatic Toxicology Laboratory in Fort Collins. Upon arrival in the lab, eggs were treated with 1600 ppm formalin for fifteen minutes (Piper et al. 1982). Eggs were placed in incubation trays and received dechlorinated Fort Collins municipal tap water at 5ºC until hatch.

Mountain whitefish fry growth was measured at temperatures spanning 4-25°C using eggs collected in two separate years. In 2009, growth was measured at target temperatures 4, 8, 12, 16°C and in 2010, growth was measured at 16, 19 22, and 25°C. Fry were nine days and 12 days post-hatch prior to the start of the 2009 and 2010 tests, respectively. Twenty fry were randomly distributed into 2 L glass tanks (18.5 x 9 x 12 cm). Each glass tank received 50 mls/min from aerated temperature-controlled stainless steel head tanks. The temperature of the head tanks were adjusted to target temperatures of at a rate of 1ºC/day. Five fry were subsampled after the tanks had attained the target temperature (day 0) and again at 11, 22, and 33 days. The number of fry subsampled from each tank was adjusted for mortality. Subsampled fry were terminally anesthetized with MS222, blotted dry with a paper towel and weighed to 0.001g. Fry were fed <24hr brine shrimp naupalii (Argent Chemical Laboratories, Redmond WA) to satiation three times a day (once a day on weekends and holidays) supplemented with a 50:50 mixture

of freeze-dried brine shrimp and bloodworms (Hikari, Hayward CA) sieved through a 500µm screen. Temperature of tanks and mortality were measured and recorded daily.

Instantaneous growth rate (g/g/d) for each tank was determined from exponential regression of mean fry weight as a function of time. The instantaneous growth rate of each tank was plotted against mean measured temperature for that tank and a second order polynomial regression line fitted to the data. Mortality rates were corrected for subsampling using Kaplin-Meier adjustments. Fry mortality data was arc-sine transformed and analyzed by ANOVA. Mortality rates at the different temperatures were compared using Tukey's test (α=0.05). The ultimate upper incipient lethal temperature (UUILT) was calculated based on the estimated median lethal temperature (LT50). Median lethal temperatures were estimated using Trimmed Spearman-Karber technique with automatic trim (Hamilton et al. 1977, 1978).

RESULTS

Mean measured temperatures were near target temperatures except the 4ºC treatment which were about 1.5ºC warmer than the target (Table 9). Standard deviations of measured temperatures were between 0.2 and 0.5°C. Fry growth was exponential at lower temperatures. However, at higher temperatures, growth deviated from an exponential rate. The deviation from exponential fit was most pronounced at 22°C, the highest temperature with surviving fry. Fry growth at 22°C was initially rapid but declined at 22 and 33 days. Combining growth rates from 2009 and 2010 and plotting against temperature resulted in polynomial curve which fitted data reasonably well (coefficient of determination = 0.94) (Figure 2). Growth rates at 16°C were similar in 2009 and 2010. Temperature for maximum growth was 13.8°C.

Survival of fry was 100% at 8°C, 16°C (2008 and 2009), and 19°C. Survival was 98% and 93% at 4°C and 12°C, respectively. At 22°C, fry survival was 71%, significantly lower than survival of fry at temperatures ≤19°C (Figure 3). All fry died at 25°C, the highest temperature tested. The first mortality occurred during acclimation as the temperature was slowly ramped to 25°C. All remaining fry died within seven days after the 25°C target temperature was reached. The median lethal temperature was 23.6°C after seven days and 22.6°C after 33 days.

DISCUSSION

The regional decline of mountain whitefish in the Yampa River of Colorado has highlighted the general lack of data and poor understanding of the life-history of this native salmonid. This study was a continuation of work initiated in 2008-2009 to provide additional information on the thermal requirements of mountain whitefish fry (Brinkman and Vieira 2009).

The maximum growth temperature was derived from data collected over two years. Growth data over the 4°C-16°C were collected in 2009 and 16°C-22°C were collected in 2010. The consistency of growth rates at 16°C in both years and the fit of

the growth rate –temperature curve increased our confidence in the maximum growth temperature value. The temperature for maximum growth of mountain whitefish fry was 13.8 °C and is very similar to reports for other Rocky Mountain native salmonids. Maximum growth temperature for bull trout (*Salvelinus confluentus*) was 13.2°C (Selong et al. 2001) and 13.6°C for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) (Bear et al. 2007). Maximum growth temperatures for nonnative salmonids are 13-16°C for brook trout (*Salvelinus fontinalis*) (Dwyer et al. 1978) and 13.9°C for brown trout (*Salmo trutta*) (Elliott and Hurley 1999). Reported maximum growth temperatures of rainbow trout (*Oncorhynchus mykiss*) include 13.1°C (Bear et al. 2007) and 17.2°C (Hokanson et al. 1978).

Mountain whitefish fry grew rapidly on a diet of brine shrimp naupailii supplemented with freeze-dried brine shrimp and bloodworms. Growth rates were as high as 8% of body weight per day at 12°C. Casual observations indicated that fry clearly preferred live food but were observed consuming the dried food.

Survival of fry was very good and often 100% at temperatures ≤19°C suggesting that rearing conditions and diet were adequate. The UUILT after seven days was 23.6°C and decreased to 22.6°C after 33 days. Significant mortality continued to occur as late as 30 days suggesting that extending the duration of the test may have resulted in a further lowering of the UUILT. The maximum growth temperature and UUILT were determined using a modified acclimated chronic exposure (ACE) method (Zale 1984). The main modification to the method was that a 33 day exposure was used instead of 60 days as specified by ACE. Traditionally, UUILTs are based on survival after seven days. In nature, seasonally high temperatures typically last for much longer. Use of longer exposure times are more ecologically realistic and allow for delayed effects on growth and survival to become apparent (Selong et al. 2001, Bear et al. 2007).

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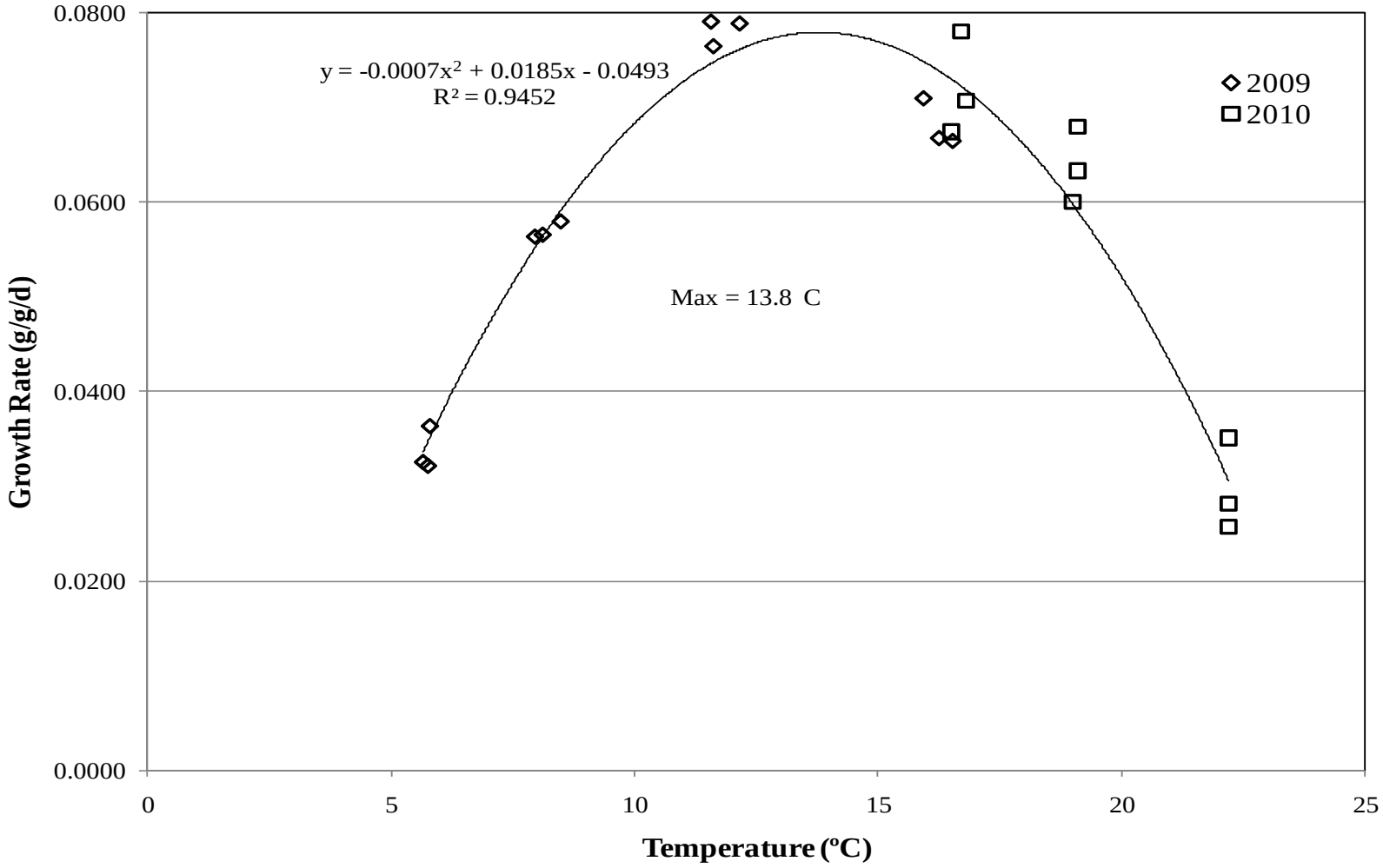

Figure 2. Effect of temperature on the growth rate of mountain whitefish fry.

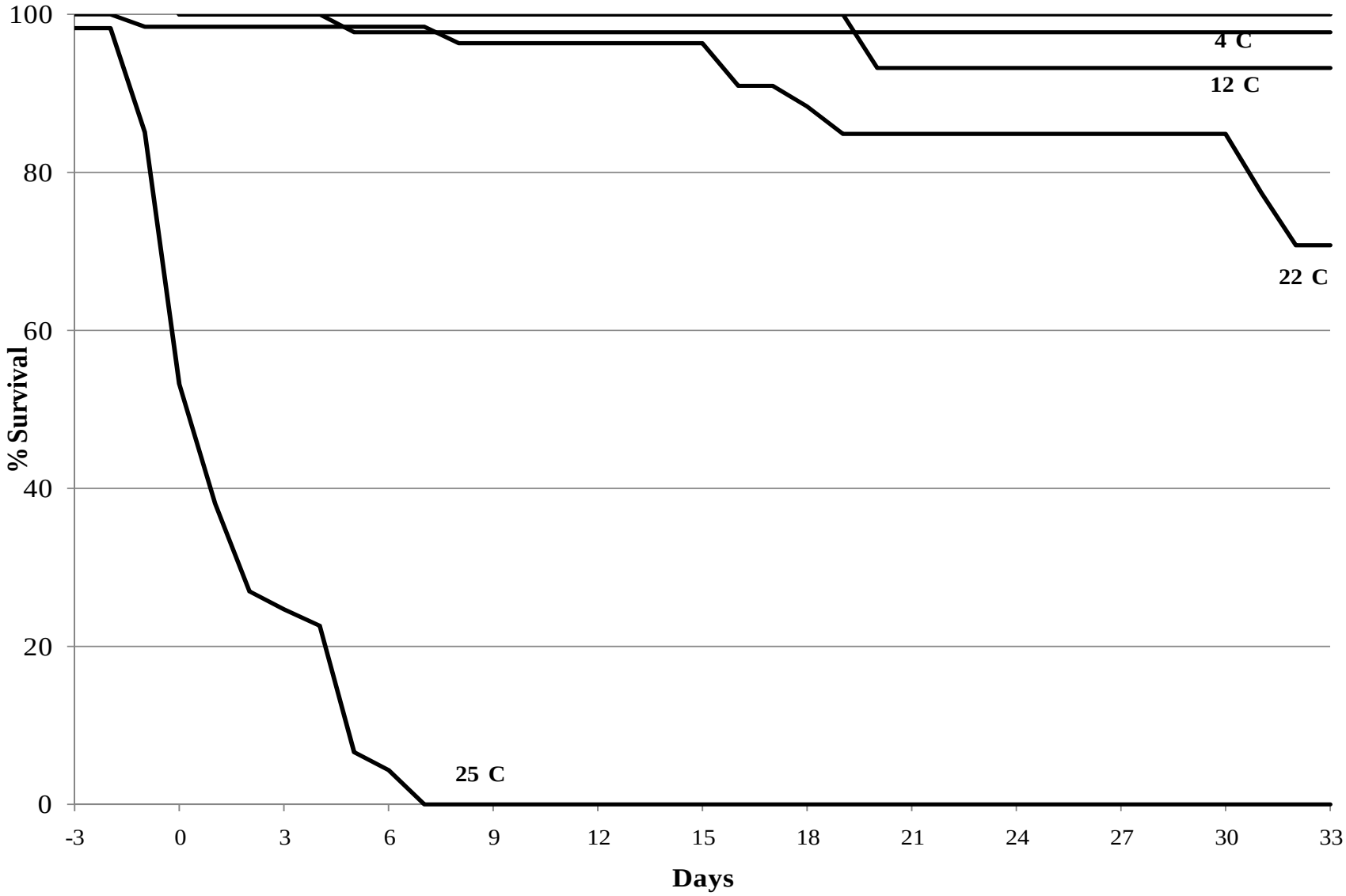

Figure 3. Survival of mountain whitefish fry at different temperatures. Survival was 100% at 8°C, 16°C (both years) and 19°C.

Table 9. Mean Measured Temperatures

Year	Target Temp. (°C)	Mean Temp. (°C)	Regression Equation	Coefficient of Determination	Instantaneous Growth Rate (g/g/d)	Survival (%)
2009	4	5.8 (0.2)	$0.0252*e^{(0.0364*t)}$	0.989	0.0364	100
2009	4	5.6 (0.2)	$0.0269*e^{(0.0326*t)}$	0.998	0.0326	93
2009	4	5.8 (0.2)	$0.0274*e^{(0.0322*t)}$	0.997	0.0322	100
2009	8	7.9 (0.2)	$0.0199*e^{(0.0564*t)}$	0.996	0.0564	100
2009	8	8.5 (0.2)	$0.0212*e^{(0.0580*t)}$	0.999	0.0580	100
2009	8	8.1 (0.2)	$0.0204*e^{(0.0566*t)}$	0.997	0.0566	100
2009	12	12.2 (0.5)	$0.0233*e^{(0.0798*t)}$	0.996	0.0798	100
2009	12	11.6 (0.5)	$0.0218*e^{(0.0791*t)}$	0.994	0.0791	80
2009	12	11.6 (0.5)	$0.0230*e^{(0.0765*t)}$	0.995	0.0765	100
2009	16	15.9 (0.2)	$0.0339*e^{(0.0710*t)}$	0.992	0.0710	100
2009	16	16.5 (0.2)	$0.0368*e^{(0.0791*t)}$	0.986	0.0791	100
2009	16	16.3 (0.2)	$0.0363*e^{(0.0668*t)}$	0.984	0.0668	100
2010	16	16.8 (0.3)	$0.0454*e^{(0.0707*t)}$	0.983	0.0707	100
2010	16	16.5 (0.3)	$0.0468*e^{(0.0674*t)}$	0.964	0.0674	100
2010	16	16.7 (0.3)	$0.0413*e^{(0.0780*t)}$	0.974	0.0780	100
2010	19	19.0 (0.2)	$0.0575*e^{(0.0600*t)}$	0.939	0.0600	100
2010	19	19.1 (0.2)	$0.0559*e^{(0.0663*t)}$	0.951	0.0663	100
2010	19	19.1 (0.2)	$0.0504*e^{(0.0780*t)}$	0.974	0.0780	100
2010	22	22.2 (0.2)	$0.0752*e^{(0.0257*t)}$	0.734	0.0257	80
2010	22	22.2 (0.2)	$0.0623*e^{(0.0351*t)}$	0.786	0.0351	65
2010	22	22.2 (0.2)	$0.0729*e^{(0.0282*t)}$	0.856	0.0282	67
2010	25	25.3 (0.2)	[1]	[1]	[1]	0
2010	25	24.9 (0.5)	[1]	[1]	[1]	0
2010	25	25.3 (0.2)	[1]	[1]	[1]	0

[1]Growth rates could not be calculated due to mortality

Agitation of Mountain Whitefish Eggs Induces Hatching

INTRODUCTION

In the course of raising mountain whitefish for research experiments, it was observed that physical agitation of the eggs would initiate the hatching of large numbers of eggs. This phenomenon was observed in separate years and in separate Colorado Division of Wildlife laboratories. European whitefish eggs (*Coregonus larvaretus*) hatch earlier with agitation (Naesje et al. 1988) and apparently use spring runoff-induced agitation as a cue for hatching (Naesje et al. 1986, Naesje et al 1995). To investigate the role of agitation on hatching, mountain whitefish eggs were allowed to develop under three conditions: constant agitation, delayed agitation and quiescence.

METHODS and MATERIALS

Mature mountain whitefish were collected either with backpack electrofishing gear in small tributary streams to larger rivers home to robust populations of mountain whitefish or through boat electrofishing where tributary spawning aggregations were not obvious. Eggs were stripped and fertilized in the field, placed in 7.5 L water coolers and transported 160 miles (approximately 3 hr 20 min drive time) to the Colorado Division of Wildlife Aquatic Toxicology Laboratory in Fort Collins. Upon arrival in the lab, eggs were treated with 1600 ppm formalin for fifteen minutes (Piper et al. 1982). Photoperiod was 12h:12h light:dark. Within 24 hrs of eye-up (132 degree-days), forty eggs were randomly distributed to incubation cups. Incubation cups were constructed by cutting the bottoms off of 12oz PETE soda bottles, covering the opening with 1000 micron nylon mesh, inverted and suspended in a water bath. Each incubation cup received 60 mls/min flow from a head box containing aerated dechlorinated Ft Collins municipal tap water at controlled at 6-7°C. Each incubation cup was subjected to one of three treatments (each replicated four times). Quiescent eggs received no agitation. Constant Agitation was started immediately after allocation of eggs to the incubation cups. Eggs in the Delayed Agitation treatment were quiescent for 33 days (approximately 240 degree-days post eye-up) before agitation. Agitation was provided using sufficient aeration from a glass pipet to continually move and maintain the eggs in the water column. Temperature of each incubation cup was measured and recorded daily. Dead or fungused eggs were carefully removed using a glass pipette. Hatching was measured four times daily (twice on weekends and holidays). Hatched fry were removed from the incubation cup to facilitate counting. Average degree-days to hatch were compared using ANOVA and treatment means compared using Tukey's HSD.

RESULTS and DISCUSSION

Temperatures were constant during egg incubation and were consistent among treatments (Table 10). Hatch rate exceeded 95% in all treatments and was not affected by Constant Agitation or Delayed Agitation (Table 10). Mean hatch for eggs in the Quiescent Control occurred near 460 degree-days (Figure 4). Eggs subjected to Constant Agitation hatched near 410 degree-days, significantly earlier than the quiescent control. Delayed Agitation led to hatching at approximately 387 degree-days, significantly sooner than both

Constant Agitation and Quiescent Control. The reason for earlier hatching of mountain whitefish eggs after Delayed Agitation compared to Constant Agitation is unknown.

Agitation has been reported to induce earlier hatching of European whitefish (*Coregonus lavaretus*) (Naesje et al 1988) which is consistent with the observations that spring runoff provides a cue for hatching of European vendace (*Coregonus albula*) and whitefish (*Coregonus lavaretus*) (Naesje at al. 1986, Naesje at al. 1995). Agitation of mountain whitefish eggs resulted in significantly earlier hatching suggesting that mountain whitefish also use spring runoff as an environmental cue to hatch. Newly hatched mountain whitefish fry have fully developed mouth parts (Stalnecker et al. 1974) and we have observed them consuming food immediately after hatch. It seems reasonable to expect that mountain whitefish have evolved to hatch during spring runoff because of availability of food or other favorable conditions. If true, then alteration of flow regimes via dams or diversions may alter the timing of mountain whitefish hatch by affecting temperature and flow which could negatively affect growth and/or survival of fry.

Table 10. Mean temperature (°C) and hatch success (%) of mountain whitefish eggs subjected to Constant Agitation, Delayed Agitation and the Quiescent Control. Standard deviations are in parentheses.

	Constant Agitation	Delayed Agitation	Quiescent Control
Temperature (°C)	7.00 (0.27)	7.00 (0.22)	7.04 (0.06)
Hatch Success (%)	99 (1)	99 (1)	96 (4)

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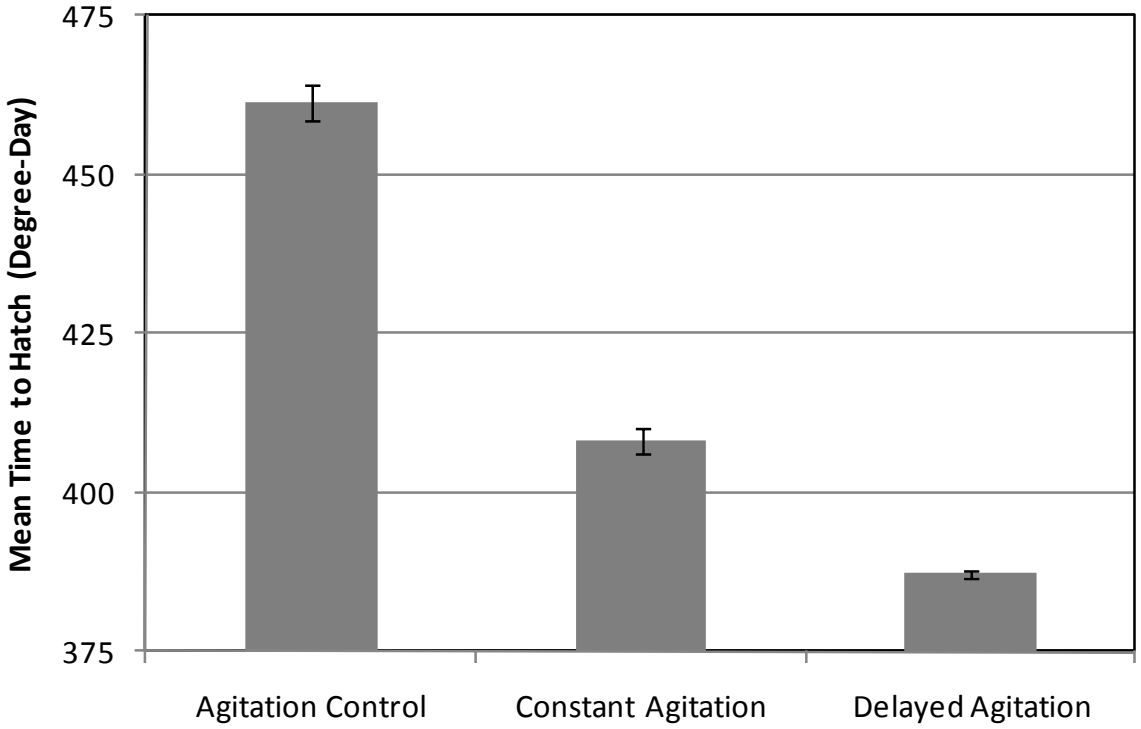

Figure 4. Mean time to hatch (degree-days) of mountain whitefish eggs subjected to Constant Agitation, Delayed Agitation and a Quiescent Control. Error bars represent standard deviation. Each treatment was significantly different from the others (Tukey's HSD $p<0.05$).

Metal Concentrations in Water Samples and Brown Trout Tissues of the Upper Arkansas River in 2008

Objective: Measure water quality, brown trout density and biomass, and accumulation of zinc, copper, cadmium, and lead in the livers and kidneys of brown trout in the upper Arkansas River.

METHODS and MATERIALS

Sampling Sites

Sampling sites for 2008 were chosen based on significant sources of metal, dilution from major tributaries, and availability of historical water quality and brown trout density data. Water samples and tissue samples for metals analyses were collected at several sites though some sites were sampled for water only or tissues only. Water sampling efforts were focused on the months during spring runoff. Previous investigations have shown that metal concentrations in the upper Arkansas River were greatest during periods of high discharge (Brinkman et al. 2006, EPA 2004). For the purposes of this report, spring runoff period was defined between April and July. Peak runoff in the Upper Arkansas River can occur between the end of April and end of June, depending on snowpack and spring temperatures. The sites are briefly described below (also see Figure 5).

EF1 is located on the East Fork upstream of Hwy 91 north of Leadville. Brown trout tissues but not water samples were collected at this site.

Station EF3 is located on the East Fork immediately downstream from Highway 24, at the USGS gage 07079300. This is the most downstream sampling station on the East Fork before the confluence with Tennessee Creek forming the mainstem of the Arkansas River. In 2008, construction on Highway 24 prevented the collection of water samples from the historic location. Consequently, starting 04/11/08, water samples were collected downstream of the USGS gage at county road 99D approximately 100 meters above the confluence of the East Fork and the Arkansas River. There are no known sources of metals or dilution between EF3 and EF4 so metal concentration data are combined for convenience. In the past, Lower East Fork and EF3 received metal loading from the Leadville Mine Drainage Tunnel. A treatment plant installed by the Bureau of Reclamation 1991 has greatly reduced metal loading into the East Fork. Water samples and brown trout tissues were collected from this site.

AR1 is the uppermost station on the mainstem of the Arkansas River at the USGS gauging station 07081200 located 150 meters downstream of the confluence of the East Fork and Tennessee Creek. This station is upstream of California Gulch and is considered a reference station although cadmium and zinc concentrations are elevated due to sources in Tennessee Creek. Water samples and brown trout tissues were collected from this site.

CG4 is California Gulch at USGS gage 07081800 a short distance from the confluence with the Arkansas River. California Gulch is the major source of metals to the upper Arkansas River and in the past significantly reduced brown trout density in the Arkansas River. A water treatment plant on the Yak tunnel installed in 1992 and other remediation activities have reduced the loading of metals into the Arkansas River resulting in improved density of brown trout downstream. However numerous sources continue to leach metals into California Gulch. Water samples but not brown trout tissues were collected from this site.

AR3a is on the Arkansas River downstream of California Gulch and is the site with the highest concentrations of metals in the upper Arkansas River. Water samples but not brown trout tissues were collected from this site.

AR5 is located between the U.S. Highway 24 bridge and Empire Gulch just upstream of USGS gage 07083710. AR5 receives significant dilution from the Lake Fork and to a lesser extent Iowa gulch. Brown trout tissues but not water samples were collected from this site.

AR6 is located immediately downstream of County Rd. 55. This station is downstream of several sources of dilution including the Lake Fork, Iowa Gulch and Empire Gulch. Water samples and brown trout tissues were collected from this site.

AR6a is located approximately a half mile upstream of County Rd. 55 at Kobe. Brown trout tissues but not water samples were collected from this site.

BV Water samples but not brown trout tissues were collected from this site.

Salida. Water samples were collected from this site.

Water Samples

Water samples were collected twice a week between 03/23/08 – 06/30/08 and again during electroshocking in August at sites AR1, CG4, AR3a, BV, and Salida. EF3/EF4 and AR6 were sampled weekly during the same interval. Alkalinity and pH were measured on site. Alkalinity was determined according to Standard Methods (APHA 1998). Site water for metals analyses was filtered on site using a 0.45 µm syringe filter (Acrodisc), collected in 60 ml high density polyethylene bottles (Nalgene), and immediately preserved with Ultrex triple distilled nitric acid (JT Baker) to pH <2. Field splits and blanks were collected during each sampling event. Metal concentrations were measured using an axial inductively coupled plasma spectrometer (Thermo Jarrell Ash), equipped with an ultrasonic nebulizer (CETAC). Each water sample was analyzed for aluminum (Al), cadmium (Cd), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), lead (Pb), selenium (Se), and zinc (Zn).

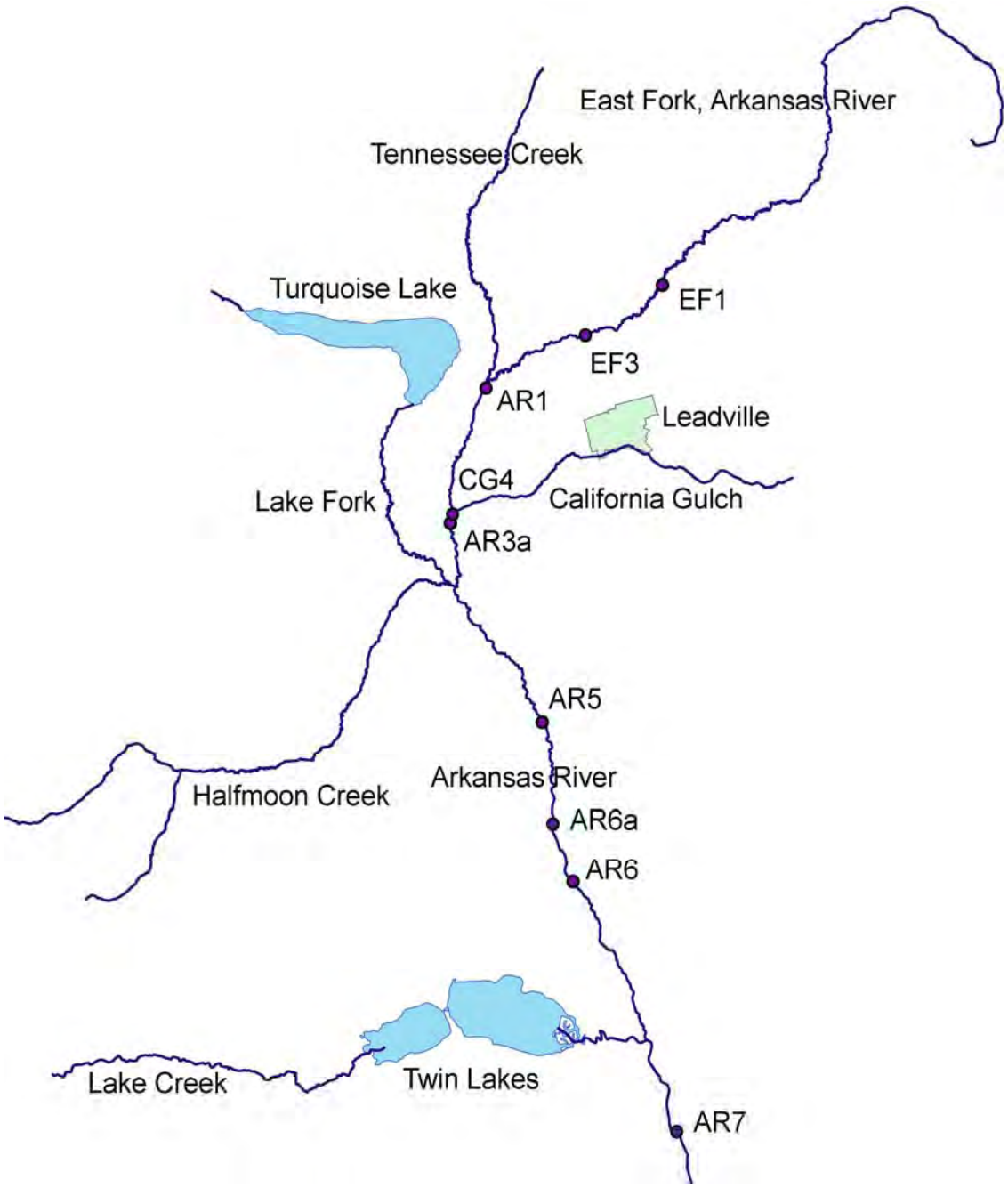

Figure 5. Water and brown trout tissue sampling sites of upper Arkansas River in 2008. Water sampling sites at Buena Vista (BV) and Salida not shown.

Tissue Analysis

Accumulation of Cd, Cu, and Zn in brown trout livers and kidneys was measured at sites EF1, EF3, AR1, AR5, AR6, and AR6a. Twenty brown trout representing a range of lengths were terminally anesthetized with MS222 and dissected in the field. Scale samples were collected from each fish and later aged. Livers and kidneys were removed and placed in preweighed polypropylene centrifuge tubes. Tubes were later placed in a drying oven at 80ºC and dried to constant weight. One half ml of trace metal grade nitric acid (JT Baker) was added to the tubes and allowed to predigest overnight. Tubes were then heated at 100ºC using a block digester for four hours. One half ml of 30% hydrogen peroxide (JT Baker trace metal grade) was added and the tubes heated for an additional four hours. Digests were diluted to 15 mls with Nanopure deionized water. Digests were analyzed using an Instrumentation Laboratory Video 22 (Allied Analytical Systems, Franklin, MA) atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction. Blanks and certified reference materials (NIST Bovine Liver Standard 1577a and Oyster tissue 1566b) were similarly digested and analyzed to assess introduction of metal contamination during digestion and accuracy of digestions and analysis.

RESULTS and DISCUSSION

Water Samples

Zinc is the primary metal of concern to aquatic life in the upper Arkansas River followed by cadmium (Brinkman et. al 2006, USEPA 2004). Spatial and temporal trends are similar for both metals and the two metals are highly correlated. The most significant source of zinc and cadmium in the upper Arkansas River is California Gulch which flows into the Arkansas River near Leadville. Historically, fish densities were significantly reduced in the Arkansas River downstream from California Gulch. However, treatment and cleanup of metal sources have reduced metal concentrations leading to a recovery of fish densities and biomass in previously impacted areas (Brinkman et. al 2006).

Brown trout dominate the fish species composition in the upper Arkansas River. Hardness-based metal concentrations for protection of brown trout can be derived for zinc and cadmium based on numerous acute and chronic toxicity tests conducted by this project. The hardness-based equation for the chronic protection of brown trout from zinc is:

$$\text{Brown trout chronic zinc value} = e^{(0.9634*\ln(\text{hardness})+1.763)}$$

The hardness-based equation for the chronic protection of brown trout from cadmium is:

$$\text{Brown trout chronic cadmium value} = e^{(1.258*\ln(\text{hardness})-4.692)}$$

Details on the derivation of the brown trout equations for zinc and cadmium can found in Appendix B in Brinkman et al. 2006 and Brinkman and Hansen 2007, respectively.

Measured concentrations of zinc and cadmium at each site were compared to their respective chronic brown trout value and also Colorado's hardness-based Table Value Standard (TVS) for protection of aquatic life (November 30, 2009) (http://www.cdphe.state.co.us/regulations/wqccregs/100231wqccbasicstandardsforsurfacewater.pdf?wwparam=1260994362).

All measured dissolved cadmium concentrations at East Fork sites EF3 and EF4 were less than the cadmium reporting limit of 0.15 µg/L (Figure 6a). Cadmium concentrations at this site were less than brown trout values and Colorado chronic cadmium TVS. Measured zinc concentrations were likewise low (Figure 6b). Most values were less than the reporting limit of 10 µg/L. Zinc was detected in only half of samples collected but all concentrations were ≤24 µg/L. Zinc concentrations at this site in 2008 were all less than TVS and brown trout values. The TVS and brown trout values decreased from April through the end of June, followed by an increase in August. This trend closely follows the trend in water hardness which decreases with the onset of spring runoff and increases as water levels return to base flow. The shape of the TVS and brown trout values at this site are characteristic of Rocky Mountain streams and are observed at other sites on the Arkansas River.

Site AR1 is just downstream from the confluence of Tennessee Creek and the East Fork. Tennessee Creek influence the water quality at this site in two ways. First, the water hardness of Tennessee Creek is very low which lowers the TVS and brown trout values compared to the East Fork. Second, Tennessee Creek has several sources of cadmium and zinc resulting in elevated levels at AR1. Cadmium and zinc concentrations were low during early runoff but were elevated in May and June (Figures 7a and 7b). Concentrations of both metals were below brown trout values but occasionally exceeded TVS in May and June.

Cadmium and zinc concentrations at CG4 are very high (Figures 8a and 8b). Cadmium concentrations approached 70 µg/L and zinc concentrations were near 14,000 µg/L. In 2008, there are two peaks; the first in late April and the second in late May-early June. All measured cadmium and zinc concentrations exceeded TVS. The water hardness in California Gulch is usually over 500 mg/L. TVS and brown trout toxicity values are capped at a hardness of 400 mg/L. Cadmium and zinc concentrations greatly exceed brown trout values from the middle of April through the end of June. California Gulch is devoid of aquatic life except for a few metal-tolerant invertebrates.

Metal concentrations at AR3 are elevated due to inputs from California Gulch. Cadmium concentrations exceeded Colorado chronic table value standards for all samples except in August (Figure 9a). However, Cd concentrations exceeded chronic brown trout toxicity thresholds only between 05/23/08 – 06/09/08. The magnitudes of the exceedences were small.

Zinc concentrations peaked on 04/25/08 at 1000 ppb (Figure 9b.). All of the measured zinc concentrations in samples collected during 2008 exceeded Colorado chronic

Table Value Standards except for a single sample collected in August. Zinc concentrations exceeded brown trout toxicity thresholds for about one-third of the samples. The magnitudes of the exceedences were small.

Cadmium and zinc concentrations are lower at AR6 than AR3a reflecting dilution from the Lake Fork of the Arkansas River (Figures 10a and 10b). Measured Cd and Zn concentrations nearly always exceeded Colorado TVS but were less than brown trout toxicity thresholds.

Buena Vista (BV) is downstream of AR6 and receives clean dilution flows from Lake Creek resulting in lower cadmium and zinc concentrations than AR6. All cadmium and zinc concentrations were well below brown trout values (Figures 11a and 11b). Cadmium concentrations exceed TVS in about 75% of samples but in all cases, the magnitude of the exceedences was small. Zinc concentrations exceeded TVS in 50% of samples but again, exceedences were very small.

Salida receives additional clean dilution flows that further reduce cadmium and zinc concentrations compared to upstream sites closer to California Gulch. Cadmium and zinc concentrations were below brown trout values (Figures 12a and 12b). Cadmium concentrations were below TVS except for a single small exceedence. All zinc concentrations were less than TVS.

To gain a better spatial perspective, zinc concentration data from all sites are combined into a single box and whisker plot (Figure 13). EF3, the uppermost site has the lowest concentrations of zinc. Moving downstream to AR1, zinc concentrations increase due to metal inputs from Tennessee Creek. The highest concentrations of zinc can be found at AR3 just downstream from California Gulch. Zinc concentrations decrease progressively downstream from AR6 to BV to Salida reflecting dilution from clean tributaries. While not shown, cadmium concentrations exhibit identical trends.

Tissue Samples

Analyses of digest blanks were less than the detection limit of the atomic absorption instrumentation. Results of analyses of Standard Reference Materials were within acceptable parameters. Mean cadmium recovery of NIST oyster tissue was 105% (range 97-110%). Mean recovery and range (in parentheses) of copper and zinc in NIST Bovine Liver tissue was 100 % (93-109%) and 99% (93-102%), respectively. Metal content in the livers of brown trout from the upper Arkansas River exhibit a few general trends (Figure 14). The livers have high levels of the essential elements copper and zinc. Concentrations of copper and zinc in the livers are similar between the uncontaminated sites (EF1, EF3, AR7) and the contaminated sites (AR5, AR6, AR6a). Cadmium content in the liver is low relative to copper and zinc. Cadmium accumulation increases with age and is slightly greater at contaminated site than clean sites. In the kidneys, copper concentrations were lower than in the livers while zinc concentrations were similar (Figure 15). Cadmium concentrations in the kidneys were lower than copper and zinc but much higher than in the liver. Cadmium concentrations in the kidney increased with increasing age and were highest at contaminated

sites. The general pattern of metal accumulation by brown trout in the upper Arkansas River was consistent with laboratory exposures using rainbow trout (McGeer et al. 2000, De Smet et al. 2001, Hollis et al.2001). These studies found a high degree of regulation of zinc with relatively low accumulation in livers or kidneys (McGeer et al. 2000). Copper was similarly well regulated with the liver and kidney rapidly achieving steady state with low accumulation relative to basal levels. Zinc and copper in the brown trout tissues would not be expected to increase greatly with exposure levels or with duration of exposure. In contrast, cadmium did not reach steady state and continued to accumulate, especially in the kidney. Cadmium elimination in fish kidneys is very slow (Wicklund et al. 1988). Consequently, cadmium accumulation in liver and kidney are a good time-integrated measure of time-integrated metal exposure of brown trout in the Arkansas River.

Comparison of contemporary Cd concentrations with historic values illustrates how the improvement in water quality has reduced metal exposure and accumulation. Accumulation of cadmium was measured in brown trout tissues collected 1985 (Nehring 1986), prior to operation of a water treatment plant on the Leadville Mine Drainage Tunnel, which discharges to the East Fork, and one at the Yak Tunnel, which discharges to California Gulch. The four sites from 1985 that are directly comparable to sites collected in 2008 are EF1, EF3, AR5, and AR6. Cadmium concentrations in livers of brown trout were much lower in 2008 compared to 1985 at sites EF3, AR5, and AR6 (Figure 16). Cadmium concentrations were similar for both years at EF1 which is upstream of metal sources. In general, cadmium concentrations increased with age at all sites. In 2008, brown trout from EF1 and EF3 accumulated similar levels of cadmium in livers demonstrating the effectiveness of the removal of metals from the Leadville Mine Drainage Tunnel. At AR5 and AR6, current cadmium concentrations in the liver are lower than in 1985 but continue to be elevated compared to control sites reflecting the loading of metals from California Gulch. Similar spatial and temporal trends are found in kidneys (Figure 17). Kidney cadmium concentrations at the clean site EF1 are low in both 1985 and 2008. At EF3, cadmium accumulation decreased substantially in 2008 to similar levels as EF1. At the contaminated sites AR5 and AR6, cadmium declined significantly from 1985 to 2008 but continue to be elevated relative to clean sites.

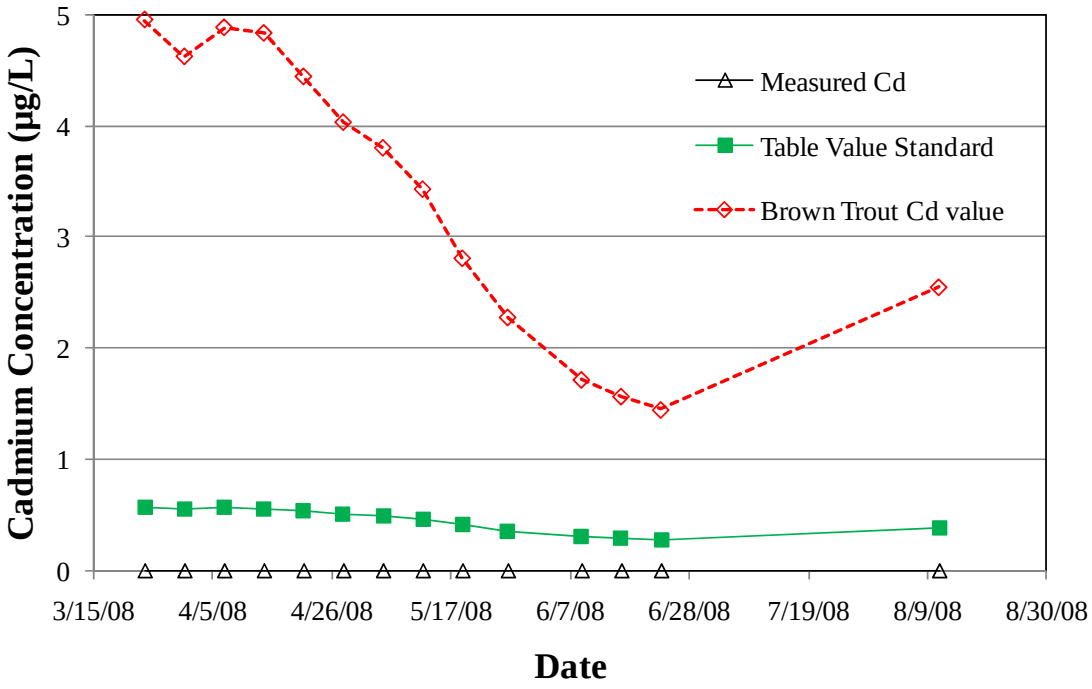

Figure 6a. Measured dissolved cadmium concentrations, Colorado chronic cadmium Table Value Standards and brown trout cadmium values at EF3 and EF4 in 2008.

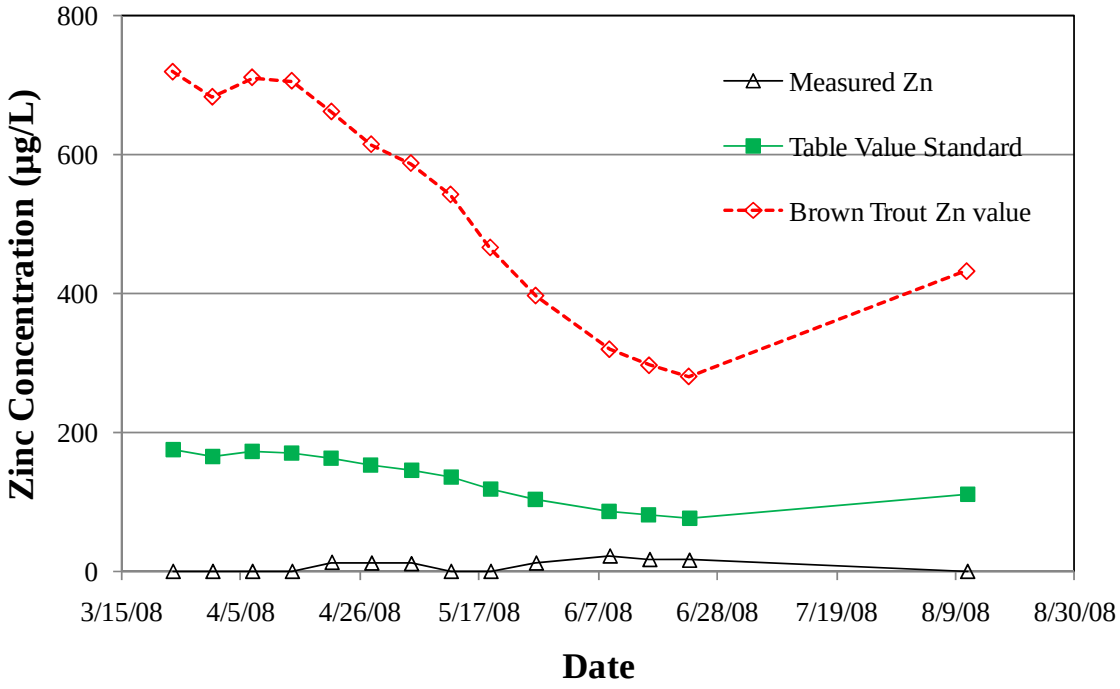

Figure 6b. Measured dissolved zinc concentrations, Colorado chronic zinc Table Value Standards and brown trout zinc values at EF3 and EF4 in 2008.

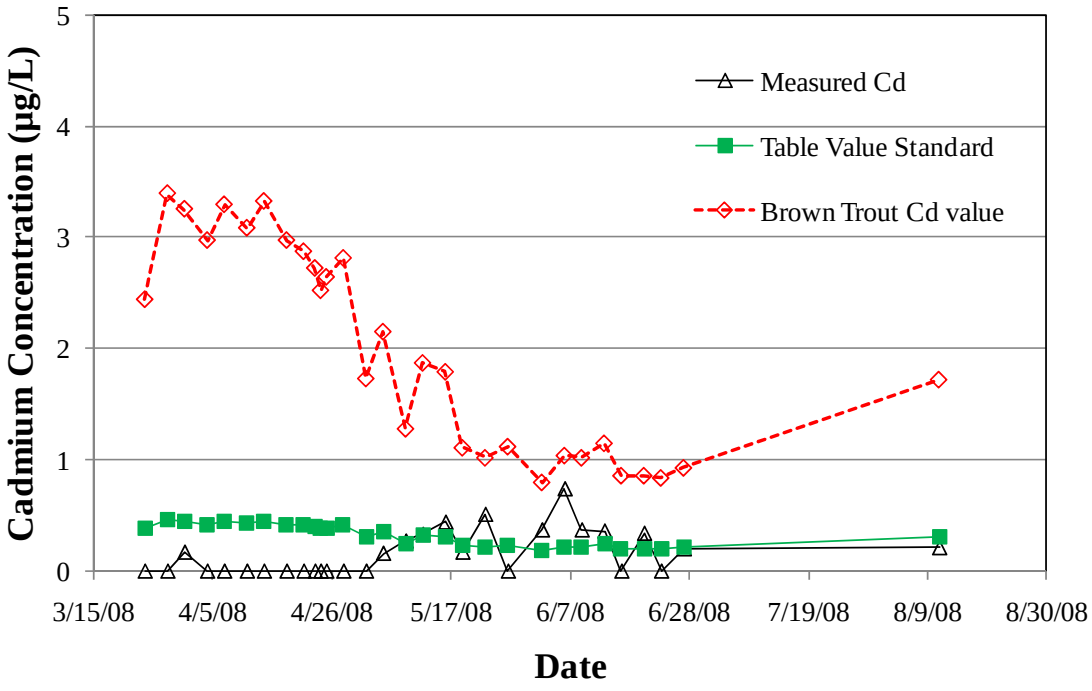

Figure 7a. Measured dissolved cadmium concentrations, Colorado chronic cadmium Table Value Standards and brown trout cadmium values at AR1 in 2008.

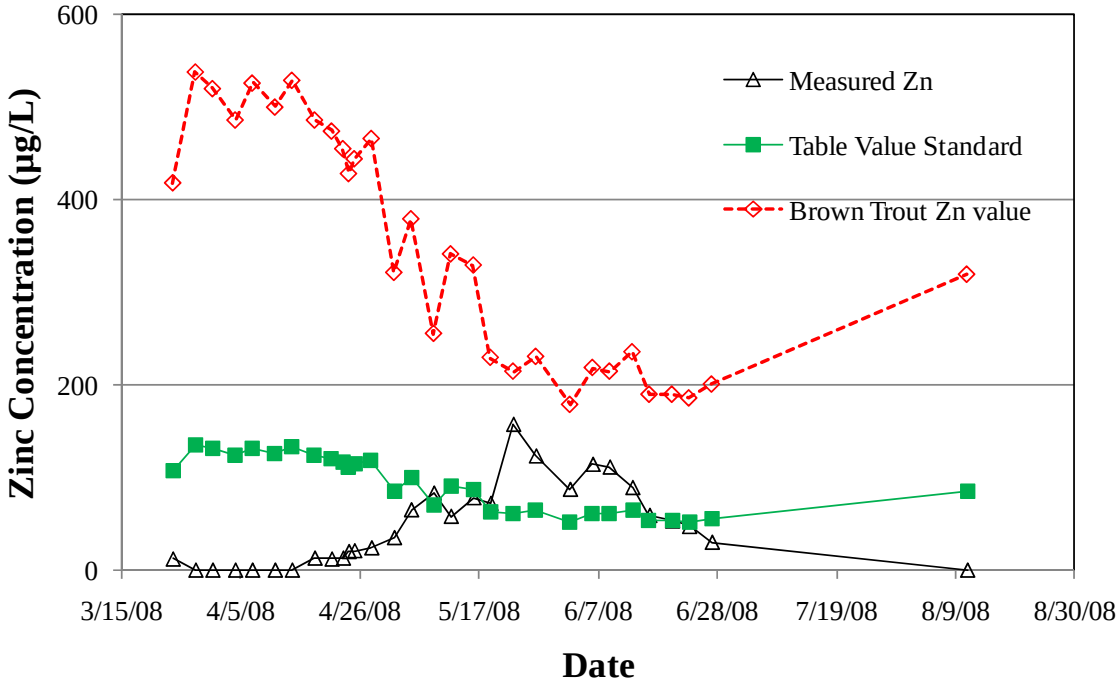

Figure 7b. Measured dissolved zinc concentrations, Colorado chronic zinc Table Value Standards and brown trout zinc values at AR1 in 2008.

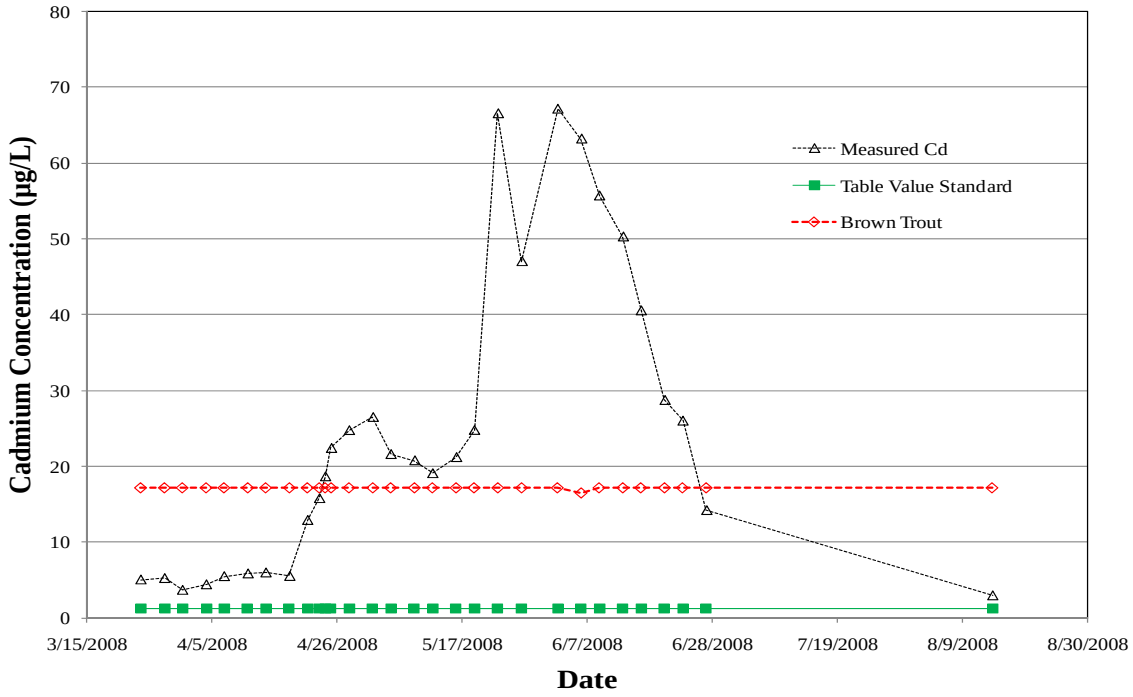

Figure 8a. Measured dissolved cadmium concentrations, Colorado chronic cadmium Table Value Standards and brown trout cadmium values at CG4 in 2008.

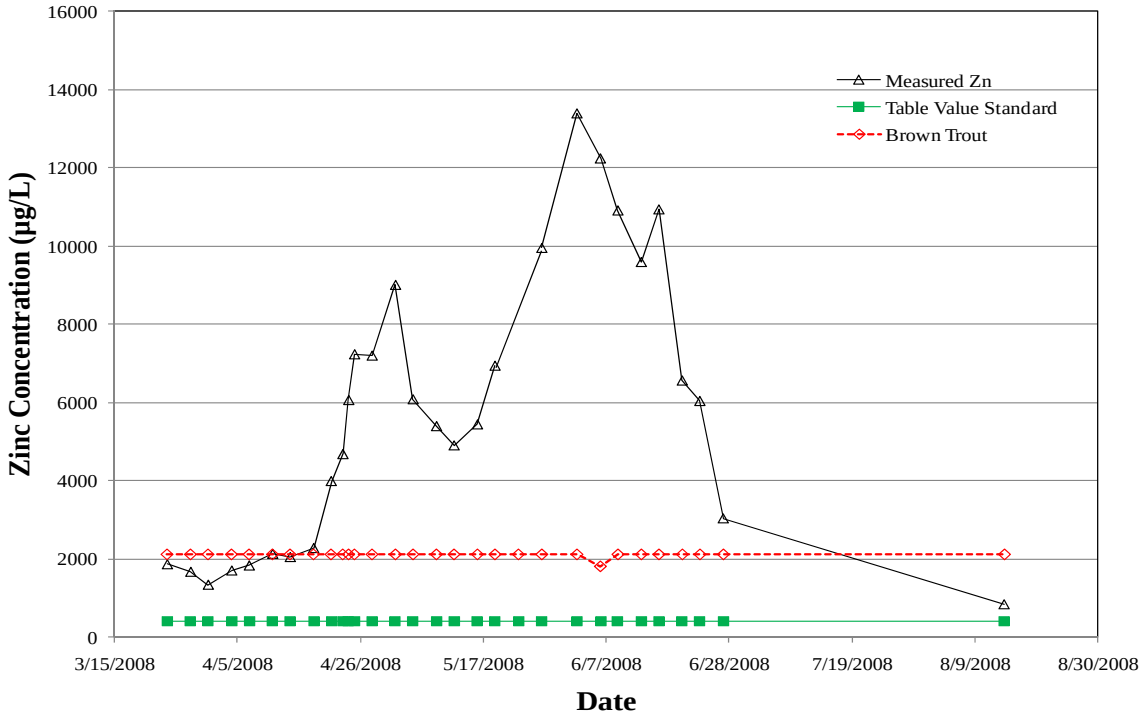

Figure 8b. Measured dissolved zinc concentrations, Colorado chronic zinc Table Value Standards and brown trout zinc values at CG4 in 2008.

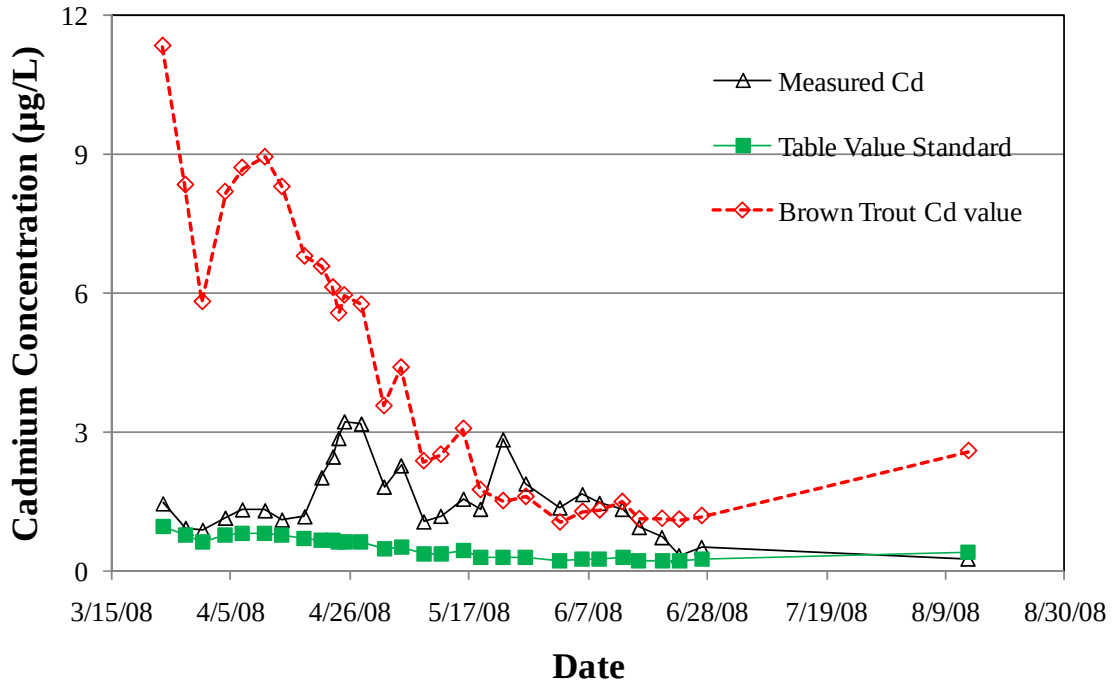

Figure 9a. Measured dissolved cadmium concentrations, Colorado chronic cadmium Table Value Standards and brown trout cadmium values at AR3 in 2008.

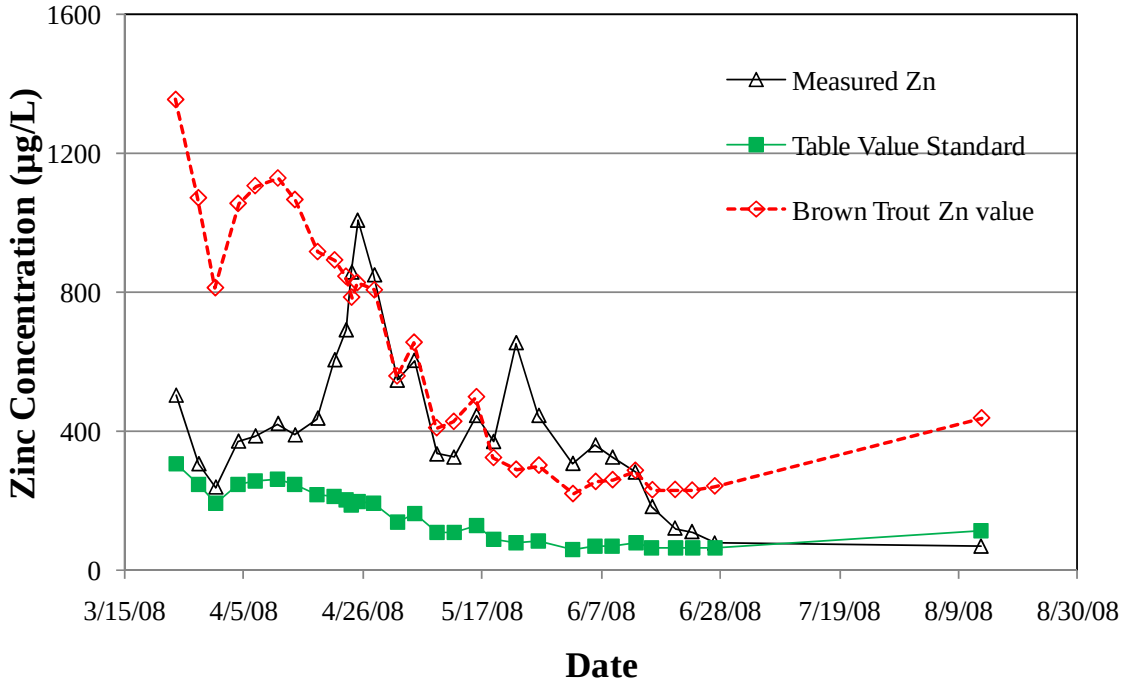

Figure 9b. Measured dissolved zinc concentrations, Colorado chronic zinc Table Value Standards and brown trout zinc values at AR3 in 2008.

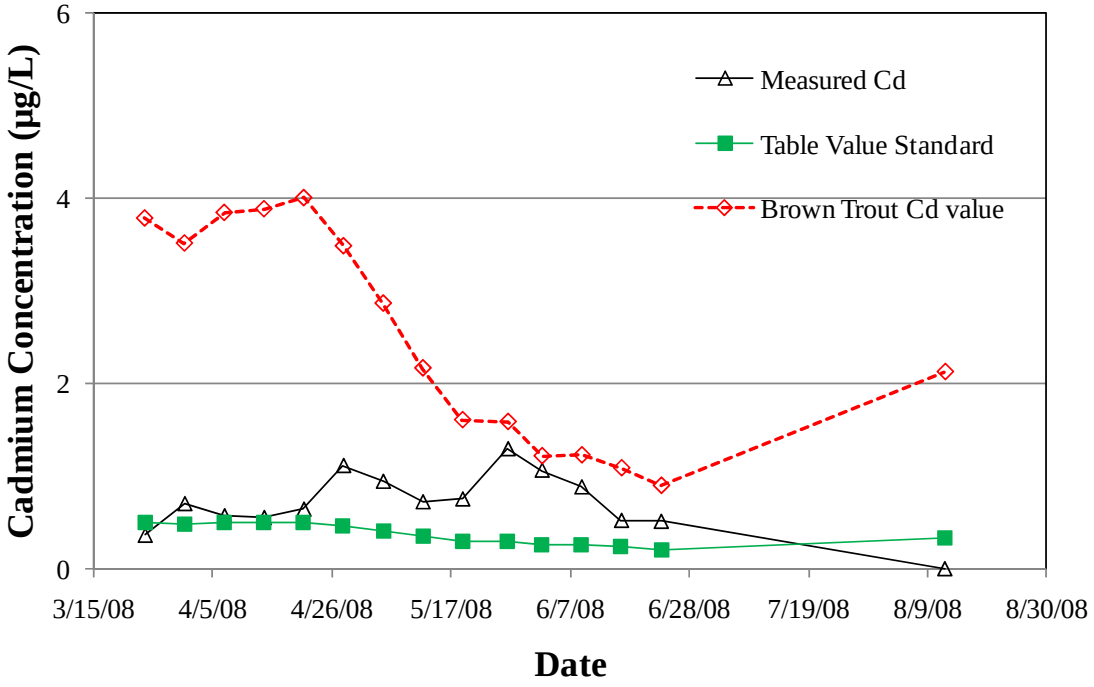

Figure 10a. Measured dissolved cadmium concentrations, Colorado chronic cadmium Table Value Standards and brown trout cadmium values at AR6 in 2008.

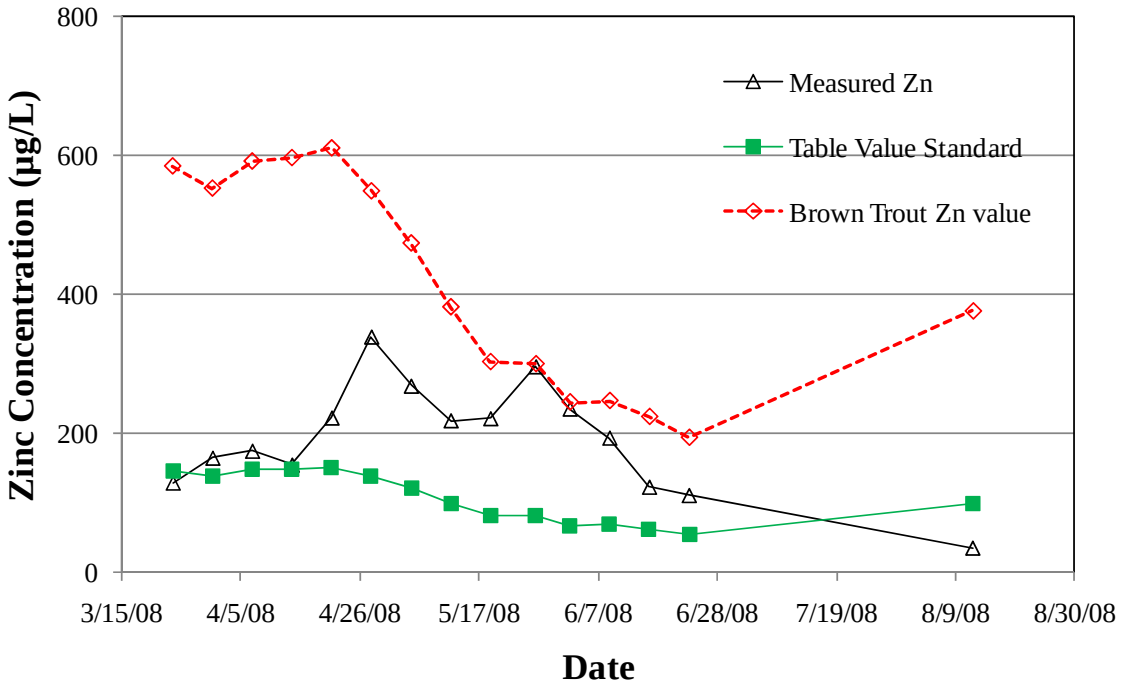

Figure 10b. Measured dissolved zinc concentrations, Colorado chronic zinc Table Value Standards and brown trout zinc values at AR6 in 2008.

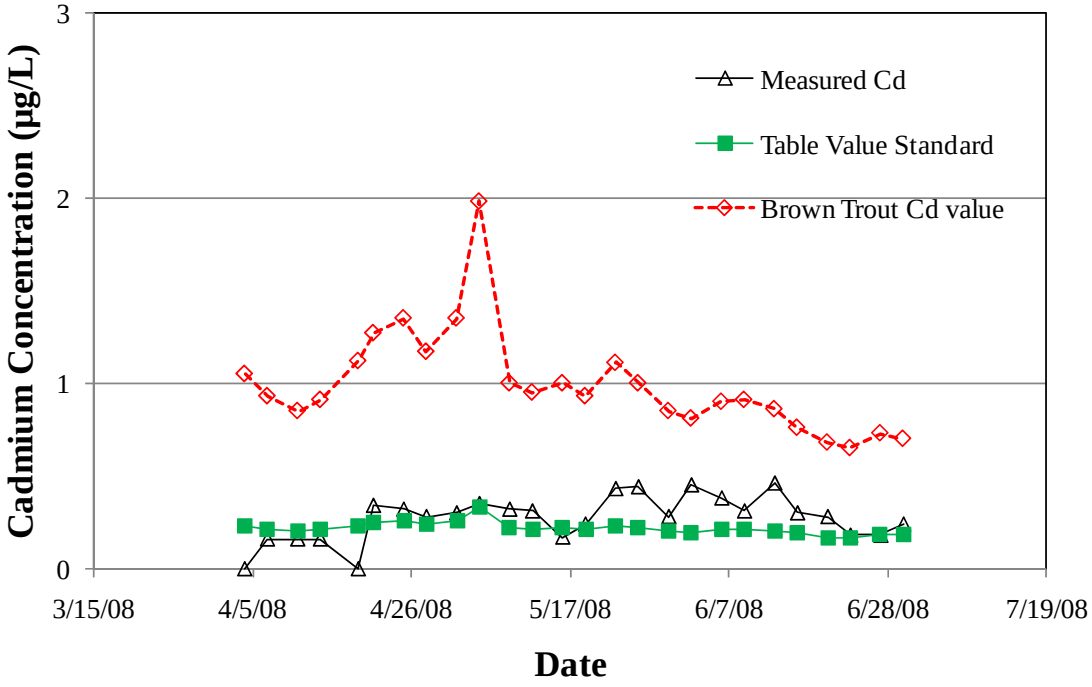

Figure 11a. Measured dissolved cadmium concentrations, Colorado chronic cadmium Table Value Standards and brown trout cadmium values at BV in 2008.

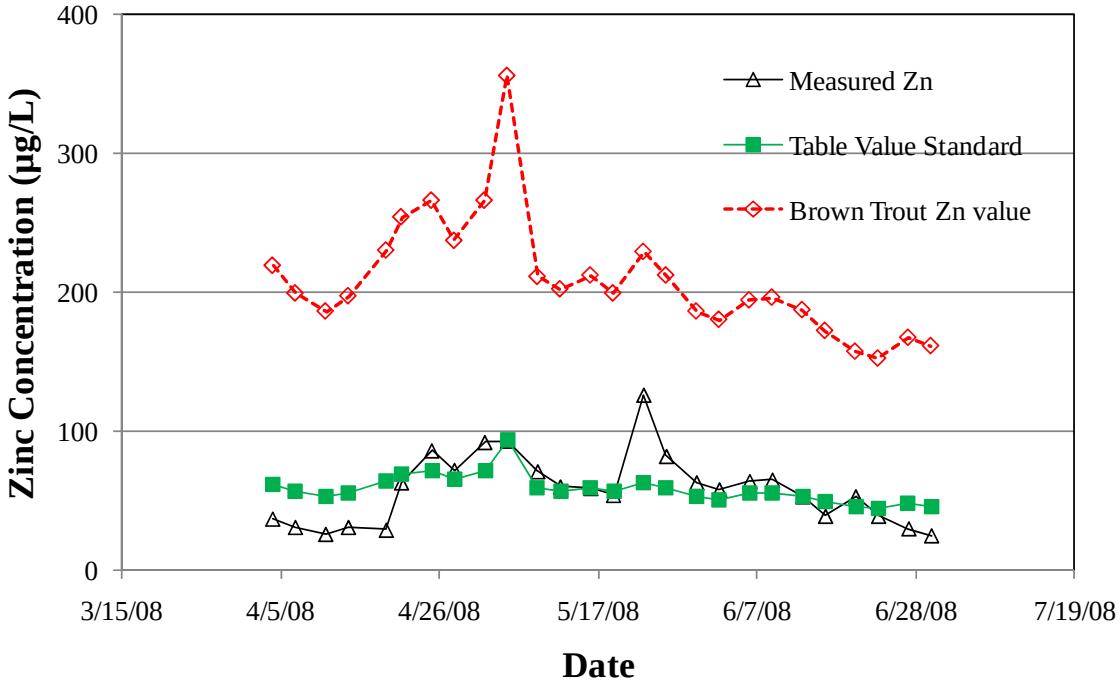

Figure 11b. Measured dissolved zinc concentrations, Colorado chronic zinc Table Value Standards and brown trout zinc values at BV in 2008.

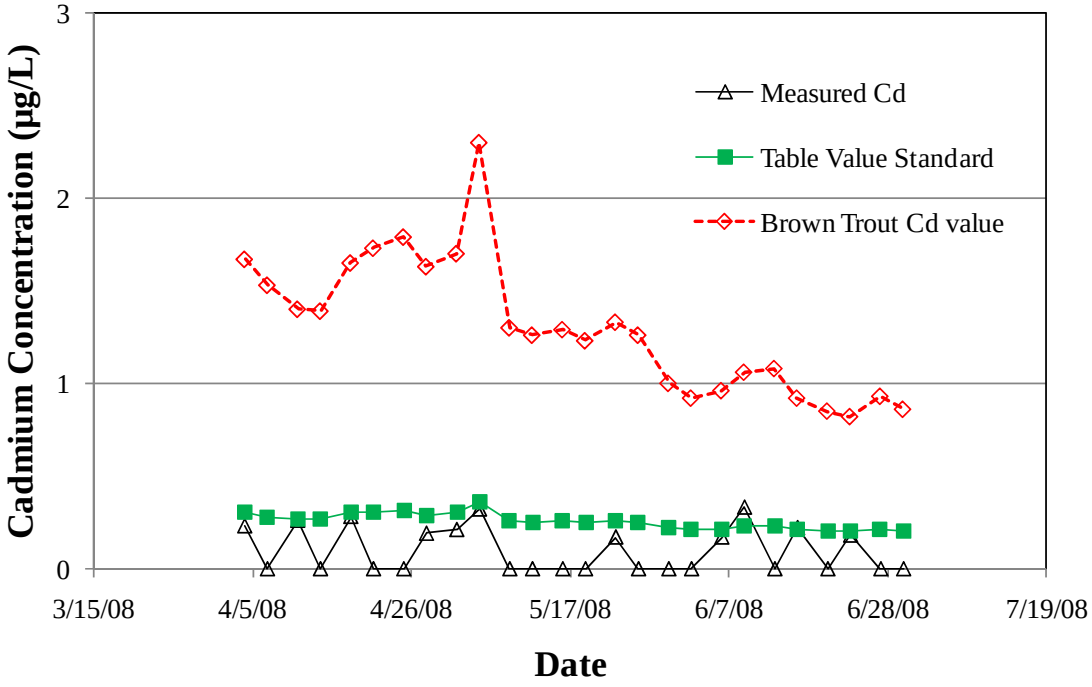

Figure 12a. Measured dissolved cadmium concentrations, Colorado chronic cadmium Table Value Standards and brown trout cadmium values at Salida in 2008.

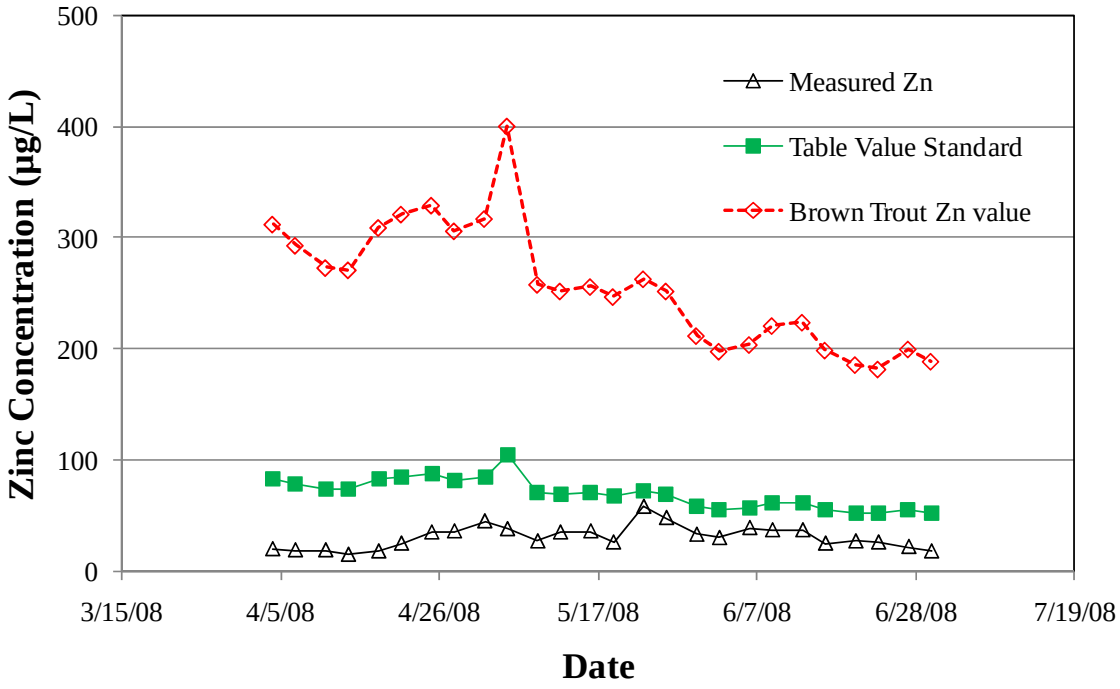

Figure 12b. Measured dissolved zinc concentrations, Colorado chronic zinc Table Value Standards and brown trout zinc values at Salida in 2008.

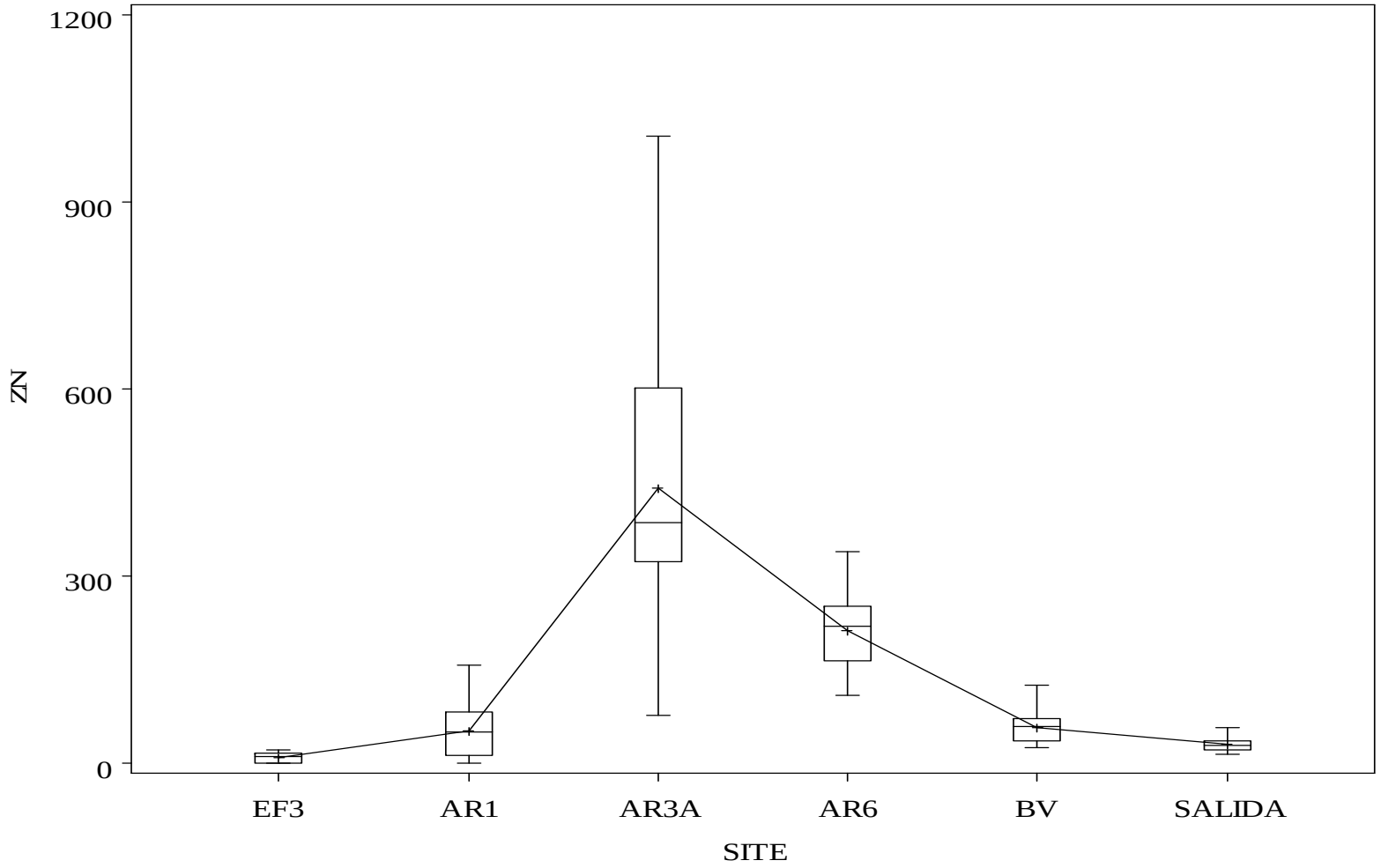

Figure 13. Box plot displaying mean, quartiles, minimum and maximum zinc concentrations (µg/L) in the upper Arkansas River from 03/24/08 – 06/28/08.

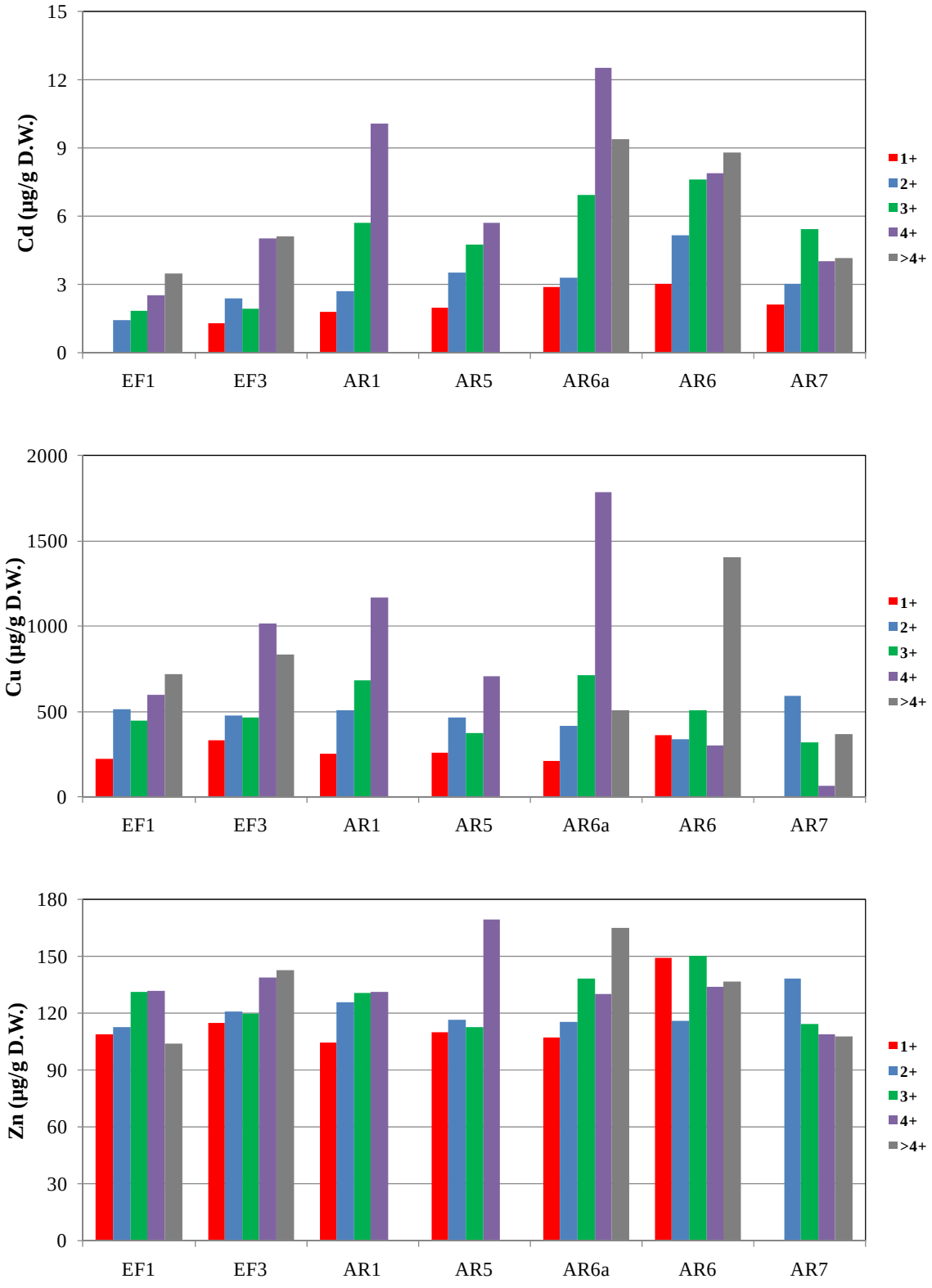

Figure 14. Concentrations of cadmium (top), copper (middle), and zinc (bottom) in the livers of brown trout collected from selected sites of the upper Arkansas River in 2008.

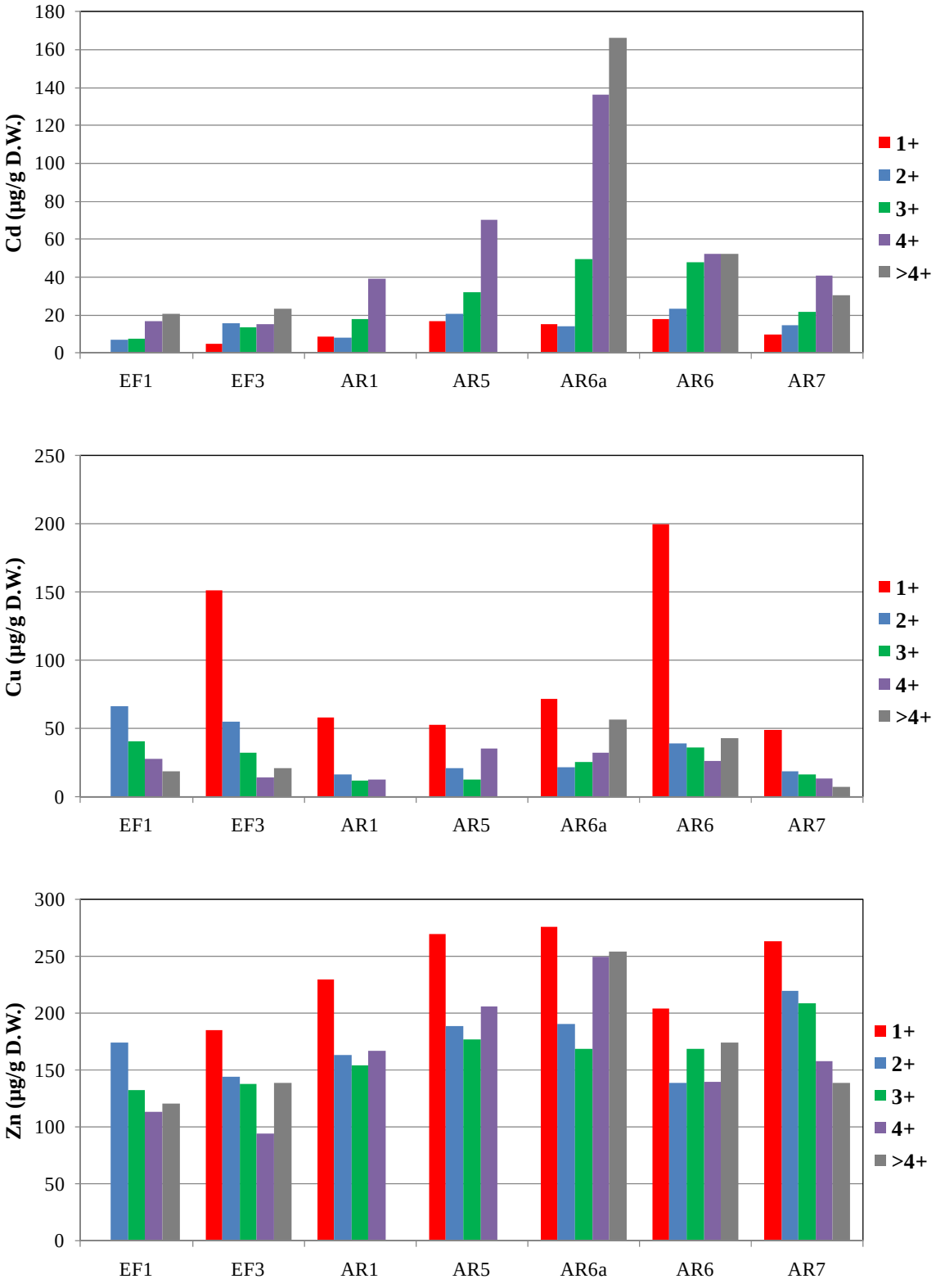

Figure 15. Concentrations of cadmium (top), copper (middle), and zinc (bottom) in the kidneys of brown trout collected from selected sites of the upper Arkansas River in 2008.

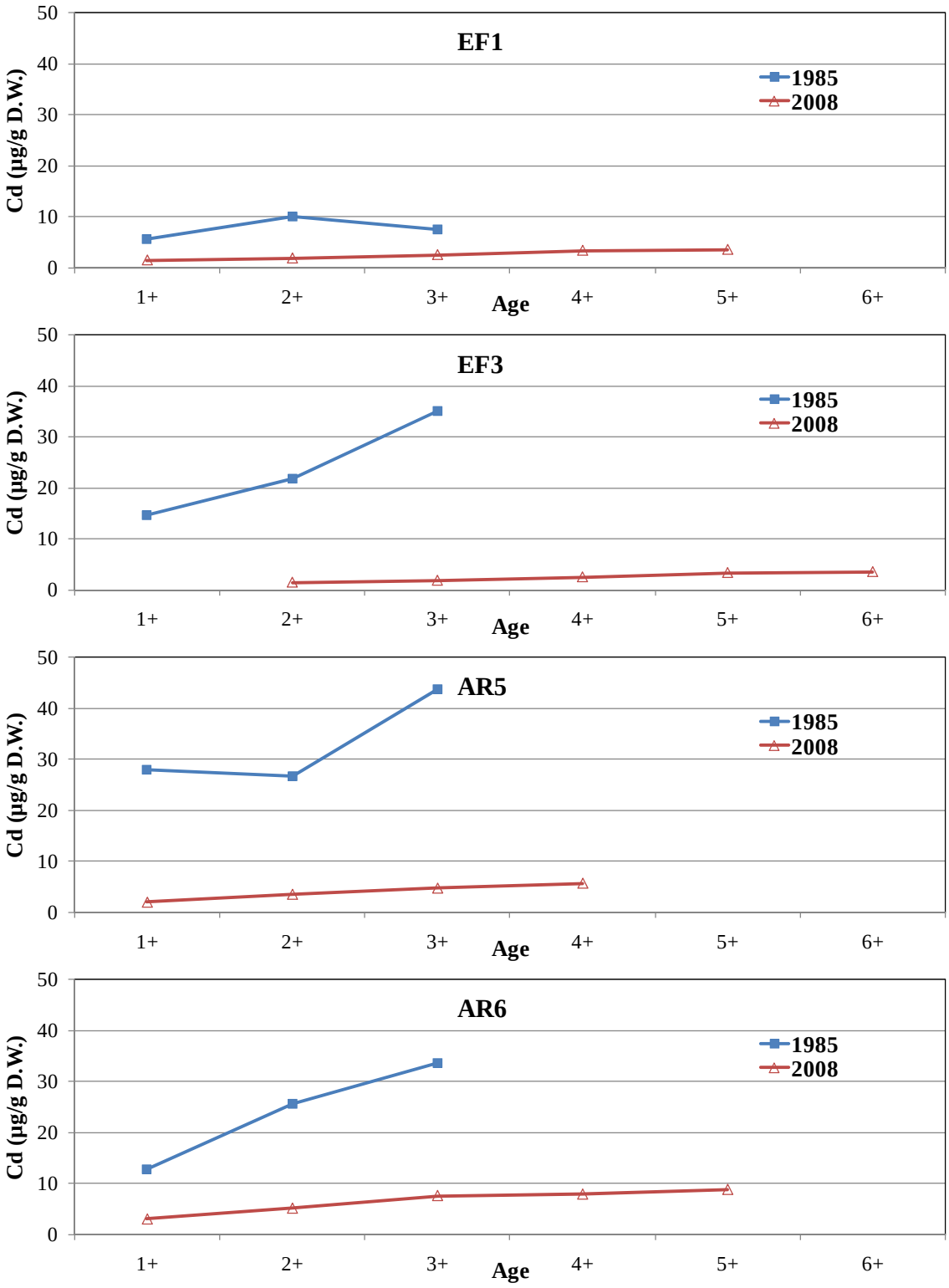

Figure 16. Cadmium concentrations in livers of brown trout collected at sites EF1, EF3, AR5, and AR6 in 1985 and 2008.

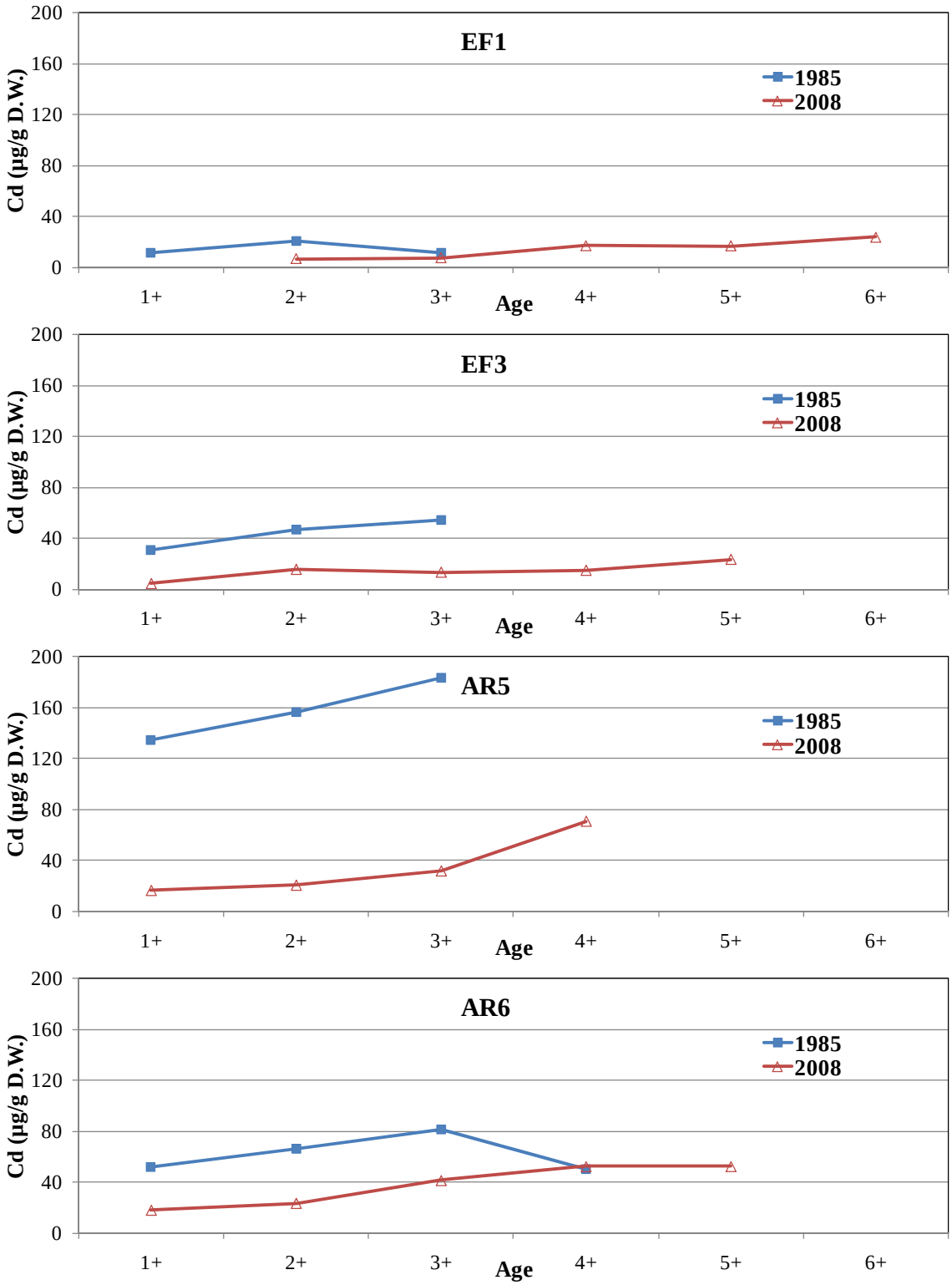

Figure 17. Cadmium concentrations in kidneys of brown trout collected at sites EF1, EF3, AR5, and AR6 in 1985 and 2008.

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