Water Pollution Studies

Federal Aid Project F-243-R13

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Job Progress Report

Colorado Division of Wildlife

Fish Research Section

Fort Collins, Colorado

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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.

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State: Colorado

Study No. F243R-11

Title: Water Pollution Studies

Period Covered: July 1, 2005 to June 30, 2006

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: TOXICITY STUDIES

Job A.1. Feminization of Fish by Wastewater Treatment Plant Effluents

Job Objective:

Determine whether feminization of rainbow trout and/or fathead minnows occurs following exposure to wastewater treatment plant effluents and/or receiving waters. If feminization is found, tests will be conducted to measure the relative magnitude. Attempts will be made to identify possible compounds contributing to estrogenic activity and estimates made on the contribution of each compound. Feminized fathead minnows will be raised to sexual maturity and spawned to determine reproductive effects of exposure to estrogenic compounds.

Job A.2. 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 sensitive species. Results from these experiments will compare toxicity thresholds to USEPA metal criteria to ensure that these species are protected.

Job A.3. 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.4. Toxicity of Unionized Ammonia to Fish at Cold Water Temperatures

Job Objective:

Determine effects of temperature on toxicity of unionized ammonia to rainbow trout and fathead minnows or other warmwater species at optimal and very cold (less than 5ºC) water temperatures.

STUDY PLAN B: TECHNICAL ASSISTANCE

Job B.1. Development of a Field Test for Rotenone

Job Objective:

To develop a test for rotenone that can measure subpiscidal concentrations in water, can be completed in an hour, and can be used in the field.

Job B.2. Water Quality Assistance to Division of Wildlife Personnel and Other State and Federal Agencies

Job Objective:

To provide 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 when such data in the literature are lacking or inadequate.

Job B.3. Regulatory and Legal Assistance

Job Objective:

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

ACCOMPLISHMENTS

Job A.1.

Fathead minnows were exposed for 4 months to estrogen and effluent from Ft. Collins wastewater treatment plant in the previous segment. Surviving organisms were preserved and submitted for histological examination to detect intersex structures in the gonads. Whole-body homogenates are currently maintained at -80ºC for analysis of vitellogenin content. Histological examination and analysis of vitellogenin were not completed during this segment.

The project continues to provide equipment for an onsite bioassay conducted by the University of Colorado. The objective of the study is to detect and quantify estrogenic activity in the city of Boulder wastewater treatment plant effluent.

A graduate student at Colorado State University was funded by DOW Aquatic Research, with Dr. Nicole Vieira as point contact and graduate committee member, to study the effects of 17-β estradiol (E_2) on the reproduction of the red shiner (Cyprinella lutrensis). Michelle McGree developed a Masters Research proposal to explore direct endocrine disruption effects of E_2 on male red shiners at the histopathological and morphological levels, and determine indirect endocrine disruption effects at the behavioral and reproductive levels. Specifically, her project investigates whether exposure of males to E_2 affects female mate choice of these males. She also investigates whether E_2 exposure results in reduced reproductive output of males and females. This proposal was accepted by her committee, and the project is now in the logistics phase. The experimental set-up, including tank construction, water supply routing and chemical delivery, has been developed. Red shiners were collected by DOW (Jim Ramsey, Fishery Biologist) and fish culturing techniques have been perfected. A temperature exposure regime was developed to bring males into breeding color in preparation for the mate choice tests.

Job A.2.

Efforts to develop a reproducing laboratory culture of mottled sculpin are continuing. The objective is to evaluate chronic toxicity of zinc, copper, and/or cadmium to early life stages of mottled sculpin. Adult sculpin previously maintained at the CDOW Research Hatchery were brought to the Aquatic Toxicology laboratory where temperature and photoperiod have been manipulated to simulate winter conditions. Temperature and photoperiod will be increased in order to stimulate spawning activity. Additional efforts to collect sculpin eggs include the planned field collection of gravid females to be brought into the laboratory where it is hoped they can be induced to spawn on artificial substrates.

Job A.3.

No activities during this segment.

Job A.4.

A chronic early life stage ammonia toxicity test was conducted using Colorado River strain of rainbow trout. Freshly fertilized eggs were exposed to low levels of ammonia to determine whether recent ammonia criteria developed by the U.S. Environmental Protection agency adequately protect rainbow trout (USEPA 1999). Results of the toxicity test titled "Chronic toxicity of ammonia to Early Life-Stage Rainbow trout (*Onchorhynchus mykiss*)" are reported below.

Job B.1.

No activities during this segment

Job B.2.

Several toxicity tests were conducted to measure the toxicity of zinc, copper, and cadmium to the mayfly *Rhithrogena hageni*. Preparations were made for the collection of other metal-sensitive benthic macro-invertebrates. Laboratory toxicity tests will be conducted to evaluate toxicity of zinc, copper, and/or cadmium to collected organisms. The objectives of the tests are to evaluate the protectiveness of USEPA criteria to these taxa and to create data for use in developing site-specific water quality standards for protection of aquatic organisms. Results of studies will be reported next segment.

A study to investigate the effect of incubation temperature on toxicity of zinc to brown trout was completed during this segment. Results of the study titled "Effect of Embryo-Larval Incubation Temperature on the Toxicity of zinc to Brown Trout (*Salmo trutta*)" are reported below.

A toxicity test was initiated to evaluate potential differences in the sensitivity of different strains of rainbow trout to zinc. Results of the study titled "A Comparision of the Acute Sensitivity of two strains of Rainbow Trout (*Oncorhynchus mykiss*) to Zinc" are reported below.

Job B.3.

DOW has participated as Party Status in several Water Quality Control Commission Rulemaking and Administrative Action Hearings, including those for the Eagle River (December 2005), the 303(d) listings (February 2006), the Temperature Standards Hearing and Update (April 2006) and the San Juan/Gunnison/Dolores Triennial Review (June 2006). We have also participated in workgroups associated with these and other water quality issues, including the Water Quality Forum, the Aquatic Life Workgroup, The Consortium for Research and Education on Emerging Contaminants (CREEC), and the Temperature Advisory Committee (TAC). We served 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 continue to provide expertise and data. We represented 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.

We have also worked informally with the CDPHE, EPA and the Attorney General's Office on a number of other water quality issues, including approaches for site-specific standards at mine-impacted CERCLA sites. Appendix A reports the assessment of the "biocriteria approach" proposed for metals standards in the Eagle River (Vieira and Albeke, 2005).

Chronic Toxicity of Ammonia to Early Life-Stage Rainbow Trout (Onchorhynchus mykiss)

ABSTRACT

A 90 day Early Life-Stage ammonia toxicity test was conducted using freshly fertilized eggs from a wild strain of rainbow trout (*Onchorhynchus mykiss*). The toxicity test was conducted at a pH = 7.75 and temperature of 11.4ºC. Survival, growth and biomass were unaffected by ammonia exposures ≤ 7.44 mg NH_3-N/L but significantly reduced at 16.8 mg NH_3-N/L. The chronic value was 11.2 mg NH_3-N/L and the IC_{20}, based on biomass at test termination, was 7.72 mg NH_3-N/L. Lethality occurred shortly after swim-up stage. Hatch success and sac fry survival were not affected. Development of sac fry to the swim-up stage was affected by exposure to ammonia but the effect was relatively small at concentrations ≤ 7.44 mg NH_3-N/L, as fry apparently recovered by the end of the test.

INTRODUCTION

Ammonia discharged from domestic and industrial treatment facilities is regulated throughout the United States based on periodically updated criteria developed by the United States Environmental Protection Agency (USEPA) (USEPA 1985, USEPA 1998, USEPA 1999). The current criteria document was most recently updated in 1999. Acute toxicity of ammonia to rainbow trout has been extensively studied and is largely consistent among studies (see USEPA 1999 Appendix 4). In contrast, results of limited chronic studies with rainbow trout exhibit a wide range of toxicity thresholds. The most ambitious chronic test exposed multiple generations over five years (Thurston et al. 1984). No effects on survival or growth were detected at the highest concentration tested, 8.0 mg NH_3-N/L at pH 7.7 (5.4 mg NH_3-N/L at pH = 8.0). Other chronic thresholds considered in the USEPA criteria document range include <1.34 mg NH_3-N/L, 1.44 mg NH_3-N/L, and <18.7 mg NH_3-N/L (all normalized to pH 8.0 and temperature 25ºC). Because several thresholds were expressed as less than or greater than values, and the wide range of results, a species mean chronic value was not developed for rainbow trout. The current Criterion Continuous Concentrations results in ammonia levels that may be toxic to rainbow trout based on some reported results, but safe based on other test results. The first objective of the following study was to determine the chronic toxicity of ammonia to Early Life-Stage rainbow trout. The second objective was to compare toxicity thresholds to ammonia criteria to evaluate the protectiveness of criteria for rainbow trout. The strain selected for testing was Colorado River Rainbow Trout. A wild strain is expected to be more sensitive to ammonia than a domesticated strain and also better represent environmental exposures.

METHODS and MATERIALS

Organisms

Freshly fertilized Colorado River rainbow trout (Onchorhynchus mykiss) strain eggs were obtained from the Colorado Division of Wildlife Glenwood Springs Hatchery. Eggs from six females were stripped, fertilized, then allowed to water harden for one hour. Fertilized green eggs were immediately transported the Colorado Division of Wildlife Toxicology Laboratory in Fort Collins, Colorado, USA in a cooler. Temperature in the cooler was maintained using a 12-V chiller (Coolworks IceProbe). Aeration was provided with a small air pump. Water temperature was 3°C upon departure from the hatchery and 4.6°C upon arrival in Fort Collins four hours later. Water quality and temperature were slowly adjusted over the next 90 minutes to test conditions prior to random allocation to incubation cups and exposure. Eggs started hatching 23 days after initiation of exposure. Swim-up of organisms in the exposure control occurred approximately 15 days later. Upon swim-up, fry were fed a concentrated suspension of brine shrimp naupulii supplemented with starter trout chow (Silver Cup). Fry were fed four times daily on weekdays and two times daily on weekends and holidays at an estimated rate of 4% body weight each day. Exposure chambers were cleaned as needed.

Test Methods

Source water for the test consisted of a mixture of onsite well water and reverse osmosis water. A conductivity controller (Oakton) maintained a constant mixture with a water hardness near 45 mg/L. The pH of the source water was maintained at a pH = 7.7 using a pH controller (Oakton). 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). An ammonia stock solution was prepared by dissolving a calculated amount of ammonium chloride in deionized water (Mallincrodt). New stock solutions were prepared as needed during the exposure period. The ammonia stock solution was delivered to the diluter via a peristaltic pump at a rate of 2.0 mls/min. The diluter delivered five concentrations of ammonia with a 50% dilution ratio and a control. Nominal total ammonia concentrations were 13, 6.6, 3.3, 1.6, 0.8 and 0 mg NH_3-N/L. The target concentrations represent approximately 4X, 2X, 1X, 0.5X, and 0.25X of the chronic criteria for ammonia, given test pH and temperature. 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 12-h/12-h light-dark photoperiod. Diluters and toxicant flow rates were monitored daily to ensure proper operation. Twenty eggs were randomly assigned and distributed to each incubation cup. Incubation cups were constructed from 53mm I.D. X 75 mm PVC pipe and 200 mesh nylon screen affixed to the PVC pipe with aquarium-grade silicone adhesive. Incubation cups were suspended in the exposure chamber and received 40 mLs/min flow of exposure water from the diluter. Eggs were monitored daily to measure hatching success. The first ten eggs to successfully hatch were removed from the incubation cup and placed in the exposure chamber. Remaining eggs were monitored for hatching and removed once hatching was completed. Consequently, hatching success is based on twenty organisms in each incubation cup, while fry survival and growth is based on ten organisms transferred to the exposure chamber. Duration of exposure was 90 days total, approximately 73 days post hatch, and approximately 52 days post swim-up. At the end of the test, surviving organisms were terminally anesthetized with MS222, blotted dry with a paper towel, and weights (0.001g) and lengths (1 mm) measured and recorded.

Test methods followed guidance provided by ASTM method E1241, *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes* (ASTM 1997) with the exception that temperature was measured weekly in each exposure level. Guidance specifies that temperature must be recorded hourly in at least one test chamber or minimum and maximum temperatures must be measured daily.

Water Quality

Water quality parameters were measured weekly in all treatment levels within a replicate. Different replicates were selected each week for sampling. 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 ammonia analysis were collected weekly. Water samples for ammonia determinations were preserved with 0.25% H_2SO_4 and refrigerated until analysis. Ammonia concentrations were measured using a Lachat Flow Injection Analyzer (QuikChem 8000) calibrated prior to each use and using EPA method 350.1. Calibration curves were verified using a commercially prepared QAQC check standard. Sample splits and spikes were collected and analyzed to assess reproducibility and recovery of analyses.

Statistics

Statistical analyses were conducted using Toxstat version 3.5 software (West Inc. 1996). Analysis of variance (ANOVA) was used to compare toxicity endpoints which included hatching success, sac fry and swim-up survival, biomass at the end of the test, and lengths, weights, and condition factor of surviving fish at test termination. Condition factor, C, was calculated using the formula $C = (\text{Weight (g)}/\text{Length (mm)}^3)/10^6$. Hatching success and survival data were arcsine square root transformed prior to ANOVA (Snedecor and Cochran 1980). Normality and homogeneity of variances were tested using Chi-square 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) at $p<0.05$. The nonparametric Steel's Many-One Rank Test was used to compare sac fry survival treatment means because data failed assumptions of normality and homogeneity of variances (Weber et al. 1989). The highest ammonia 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 ammonia 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 (biomass or standing crop).

RESULTS

Water Quality

Water quality characteristics were consistent over the duration of the experiment (Table 1). Mean pH was 7.75 and ranged between 7.46 and 7.96. Mean dissolved oxygen was near saturation levels (Ft. Collins, CO elevation 4984 ft above sea level) and did not fall below 6.73 mg/L. Temperature of exposure chambers was maintained in a narrow range near the mean temperature 11.4ºC. Conductivity of exposure solutions varied considerably due to dissolved ammonium chloride that differed among the different exposure levels.

The detection limit for ammonia analyses was 0.03 mg NH_3-N/L. Mean recovery of quality assurance samples was 99%. Mean sample spike recovery was 106%. Mean relative percent difference of sample splits was 3.0%. Measured ammonia concentrations of exposure solutions were slightly greater than target concentrations in the two higher exposure levels but otherwise near target levels and were relatively constant over the duration of the 90 days (Table 2).

Toxicity Endpoints

Mean hatching success exceeded 78% in all exposure levels (Table 2). No effect of ammonia exposure on hatching success was detected at any concentrations tested. Survival of sac fry released from the incubation cups into exposure chambers was very high and exceeded 95% in all exposure levels (Table 2). Survival of sac fry was unaffected by ammonia concentrations used in this test. Survival from hatch through swim-up stage ranged between a low of 22.5% in the highest exposure to a high of 92.5% in the control (Table 2). Exposure to 16.8 mg NH_3-N/L significantly reduced swim-up up fry survival ($p<0.05$). No effect on swim-up fry survival was detected at ammonia concentrations $\leq$ 7.44 mg NH_3-N/L. Significant reductions in length or weight at test termination were not detected at ammonia concentrations $\leq$ 7.44 mg NH_3-N/L. However, exposure to 16.8 mg NH_3-N/L greatly reduced weights and lengths of surviving organisms (Table 2). In spite of a reduction of length and weight at the highest exposure, changes in condition factor were not observed. Biomass at test termination (combined weight of surviving organisms) was also unaffected at concentrations $\leq$ 7.44 mg NH_3-N/L but significantly reduced at 16.8 mg NH_3-N/L. Condition factor was unaffected by ammonia concentrations used in this test. The LOEC for each of the endpoints affected by ammonia (swim-up fry survival, length, weight, and biomass at test termination) was 16.8 mg NH_3-N/L. The NOEC for each of the endpoints was 7.44 mg NH_3-N/L. The chronic value is 11.2 mg NH_3-N/L. The IC_{20} based on biomass was 7.72 mg NH_3-N/L.

Casual observations near the end of the sac fry stage suggested a concentration-dependent effect of ammonia on development of fry. To assess the transition from sac fry to swim-up stage, the number of free swimming fry relative to the number resting on the bottom were compared in each exposure chamber. This qualitative measure of transition to swim-up stage indicated a delay of a few days in exposures between 0.81 to 7.44 mg NH_3-N/L (Figure 1) with higher concentrations causing a greater delay. Fry exposed to 16.8 mg NH_3-N/L continued to rest on the bottom of the exposure chamber through the end of the test. Many organisms exposed to 16.8 mg NH_3-N/L failed to convert to exogenous feeding and a large amount of mortality occurred shortly after swim-up. Although surviving organisms exposed to 16.8 mg NH_3-N/L transitioned onto exogenous feeding, they exhibited edema in the abdominal area that prevented free swimming for extended periods of time.

DISCUSSION

The test organisms used in this study were Colorado River rainbow trout strain, a vigorous naturally-reproducing population. Domesticated strains have been in various hatchery programs for multiple generations and may have developed a tolerance to low levels of ammonia. The Colorado River rainbow trout should provide responses that better represent *in-situ* exposures to ammonia.

No effects on survival or growth were detected at exposure concentrations as high as 7.44 mg NH_3-N/L. The chronic value based on lethal and sublethal endpoints was 11.2 mg NH_3-N/L. The IC_{20} based on biomass at test termination was somewhat lower than the chronic value at 7.72 mg NH_3-N/L. For the pH and temperature conditions used in the test, the most recent USEPA chronic criteria in the presence of Early Life Stage fish is 3.38 mg NH_3-N/L (USEPA 1999). The results of our study indicate that current ammonia criteria are chronically protective of rainbow trout.

To facilitate comparison of toxicity test data among studies, results are often normalized to a pH = 8 (USEPA 1999). The equation used to normalize chronic values is:

$$CV_{pH}=(CV_8)[(0.0676/(1+10^{7.688-pH}))+(2.91/(1+10^{pH-7.688}))]$$

Where CV_{pH} is chronic value at test pH, CV_8 is chronic value normalized to a pH=8, and pH is test pH.

A multigeneration five-year exposure failed to detect any adverse effects on rainbow trout survival or growth at the highest concentration tested indicating that a toxic threshold would be greater than 5.4 mg NH_3-N/L, adjusted to pH = 8 (Thurston et al. 1984). In contrast to those results, a 73-day-Early-Life-Stage exposure found significant mortality at the lowest concentration tested indicating that a toxic threshold would be less than 1.45 mg NH_3-N/L, adjusted to pH = 8 (Solbe and Shurben 1989). The USEPA 1999 criteria reporting the work of Calamari et al. (1977, 1981), estimated an LC_{20} of 1.34 mg NH_3-N/L at pH=8. The chronic value and IC_{20} from our test are 5.89 mg NH_3-N/L and 4.03 mg NH_3-N/L, respectively, after adjustment to pH = 8. Our chronic value is slightly above the level reported by Thurston et al. (1984) to have no observed effect, though the IC_{20} was somewhat lower. Both the chronic value and IC_{20} developed by the current study are considerably greater than results from Solbe and Shurben (1989) and Calamari et al. (1977, 1981).

Development of fry to the swim-up stage was delayed by exposure to ammonia at all concentrations tested. The delay in development was considerable at 16.8 mg NH_3-N/L but otherwise relatively modest. Reduced lengths and weights at test termination was observed at 16.8 mg NH_3-N/L but not at ammonia concentrations ≤ 7.44 mg NH_3-N/L indicating that fry were able to compensate by test termination 52 days later. Retardation of growth and development of rainbow trout fry as well as a failure to absorb the yolk sac at higher concentrations was similarly observed by Burkhalter and Kaya (1977). Their test was terminated prior to swim-up stage so it's uncertain whether exposed fry would have recovered from early retardation of development.

No effect on hatch success was observed at ammonia concentrations as high as 16.8 mg NH_3-N/L (=12.1 mg NH_3-N/L at pH 8). In other studies, hatch success was unaffected by 5.4 mg NH_3-N/L (Thurston et al. 1984) and 58.6 mg NH_3-N/L (Burkhalter and Kaya 1977), (values adjusted to pH=8). These results are inconsistent with the high egg mortality observed by Solbe and Shurben (1989) at concentrations as low as 1.45 mg NH_3-N/L, adjusted to pH = 8. All tests cited above started exposure within 24 hours of fertilization.

No ammonia-related mortality occurred during the sac fry stage at concentrations up to 16.8 mg NH_3-N/L (=12.1 mg NH_3-N/L at pH 8). Burkhalter and Kaya (1977) did not observe sac fry mortality at 8.8 mg NH_3-N/L but reported significant mortality at 16.7 mg NH_3-N/L (values adjusted to pH=8).

Significant ammonia-related mortality was not observed until shortly after swim-up. The results suggest that this is a much more sensitive stage compared to embryo or larvae. This finding is consistent with 24 hr exposures that found that fry shortly after swim-up were about 20X more sensitive than eggs and sac fry (Rice and Stokes 1975) although contradicts 96 hr acute test results that showed tolerance increasing only slightly as trout grew from 1 day old at 0.06g to about 1.0 g (Thurston and Russo 1983).

Growth, as measured by lengths and weights at test termination, were greatly decreased at 16.8 mg NH_3-N/L (=12.1 mg NH_3-N/L at pH 8), but unaffected by ammonia levels ≤7.44 (=5.36 mg NH_3-N/L at pH 8). Despite a significant reduction in growth, condition factor was unchanged. No effects of ammonia exposure on growth were found at 5.4 mg NH_3-N/L (Thurston et al. 1984) or 14.6 mg NH_3-N/L (Solbe and Shurben 1989). Burkhalter and Kaya (1977) observed reduced growth at 16.7 mg NH_3-N/L but not 8.8 mg NH_3-N/L.

In summary, results from the current study are generally consistent with toxicity values reported by Thurston et al. (1984) and Burkhalter and Kaya (1977) but much greater than those reported by Solbe and Shurben (1989) and Calamari (1977, 1981). Unfortunately, our study does little to shed light on possible reasons for the discrepancy in toxicity values among studies. The

USEPA criteria document suggests different strains of rainbow trout used in the tests may account for some of the observed differences in ammonia sensitivity. Rainbow trout from Ennis Hatchery were used by Thurston et al. (1984) and Burkhalter and Kaya (1977), and unreported strains used by Solbe and Shurben (1989) and Calamari (1977, 1981). This study used a wild reproducing strain of rainbow trout from the Colorado River, which are expected to differ considerably from domesticated strains. Temperatures of the tests reporting lower toxicity values were 14.5ºC and 14.9ºC compared to 11.4ºC (current study), 9.5 and 12.5ºC (Burkhalter and Kaya 1977), and 7.5-10.5ºC (Thurston et al. 1984) suggesting a potential interaction of temperature and ammonia toxicity.

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Table 1. Mean, standard deviation and range of water quality characteristics of exposure water used for ammonia toxicity tests conducted with rainbow trout.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (ºC)	Conductivity (µS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	44.6	42.9	7.75	11.4	192.7	8.50
Std. Dev.	4.2	5.1	0.13	0.25	61.8	0.66
Range	34.8-54.6	32.6-51.2	7.46-7.96	10.8-11.9	109-377	6.73-9.17

Table 2. Mean ammonia concentrations (mg/L) and associated mean hatching success, sac fry survival and swim-up fry survival of rainbow trout (Colorado River strain). Standard deviations are in parentheses.

Target Concentration (mg N/L)	0	0.8	1.6	3.3	6.6	13
Measured Concentration (mg N/L)	<0.02 (0.01)	0.81 (0.20)	1.74 (0.58)	3.34 (0.53)	7.44 (0.88)	16.8 (2.07)
Measured Concentration (Normalized to pH 8) (mg N/L)	<0.02 (0.019)	0.59 (0.14)	1.25 (0.41)	2.41 (0.38)	5.36 (0.62)	12.1 (1.50)
Hatch Success (%)	83.8 (4.8)	82.5 (15.5)	86.3 (13.8)	78.8 (11.1)	88.8 (2.5)	83.8 (13.1)
Sac Fry survival (%)	100 (0)	100 (0)	100 (0)	95.0 (5.8)	100 (0)	97.5 (5.0)
Swim-up Fry Survival (%)	92.5 (9.6)	75.0 (20.6)	90.0 (14.1)	85.0 (17.3)	82.5 (5.0)	22.5* (9.6)
Weight at Termination (g)	0.431 (0.040)	0.403 (0.021)	0.409 (0.031)	0.403 (0.051)	0.396 (0.024)	0.199* (0.028)
Length at Termination (mm)	35.8 (1.3)	35.0 (0.8)	35.5 (0.8)	34.4 (2.0)	34.6 (0.9)	27.3* (2.0)
Condition Factor	0.918 (0.023)	0.917 (0.034)	0.878 (0.030)	0.935 (0.005)	0.937 (0.025)	0.900 (0.043)
Biomass at Termination (g)	3.97 (0.34)	2.95 (0.97)	3.65 (0.39)	3.48 (1.03)	3.27 (0.28)	0.44* (0.18)

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

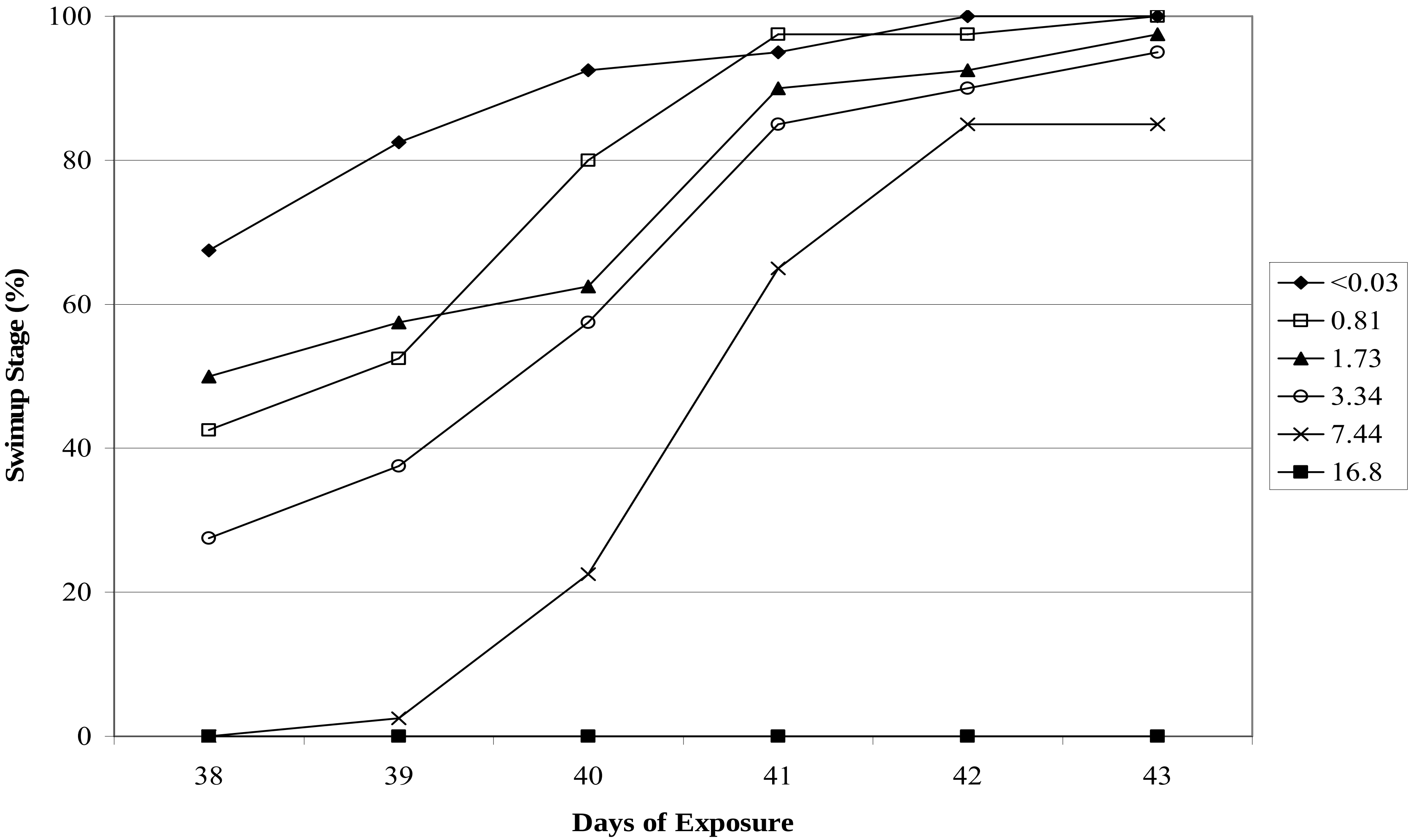

Figure 1. Percent of rainbow trout at swim-up stage between 38 and 43 days of exposure as a function of ammonia exposure concentration.

Effect of Embryo-Larval Incubation Temperature on the Toxicity of Zinc to Brown Trout (*Salmo trutta*)

INTRODUCTION

Brown trout subjected to toxicity tests in the spring of 2004 exhibited greater tolerance to zinc than previous tests. Several possible causes of increased tolerance were eliminated. The source of eggs, size of test organisms, water quality, and testing procedures were similar. One major difference for the spring 2004 tests was that the incubation temperature of the embryos and larvae was 3-5°C. The purpose of the low incubation temperature was to delay development of the eggs collected in the fall 2003 to provide appropriately sized organisms for tests conducted the following spring. Brown trout in previous toxicity tests were incubated near the 12°C test temperature. A consequence of the low incubation temperature was that organisms used in spring 2004, while similar in size to previous tests, were chronologically much older. It was hypothesized that temperature-independent development of the chronologically older brown trout resulted in increased tolerance to zinc. The objective of this study was to determine whether incubation temperature and chronologic age affects sensitivity of brown trout fry to zinc.

MATERIAL and METHODS

Organisms and Culture

Fertilized brown trout eggs from a Colorado Division of Wildlife spawning operation at the North Delaney Butte Reservoir in Northern Colorado were transported to the Colorado Division of Wildlife Research Hatchery in Bellevue, Colorado. The green eggs developed to the eyed stage prior to transport to the Colorado Division of Wildlife Aquatic Toxicology Laboratory in Fort Collins. The eyed eggs were divided into four equal lots and placed in different 100-liter glass aquaria. Each aquarium received aerated de-chlorinated Ft. Collins municipal tap water at a rate of 100 mls/min. Re-circulating chillers (VWR models 1173, 1175) and stainless steel heat exchangers chilled the four aquaria to 12, 9, 6, and 3ºC. Temperature loggers (Optic StowAway logger, Onset Computer Corporation) measured and recorded temperatures in each aquarium every four hours. Dim fluorescent lighting provided a 12 hour day/night photoperiod. Aquaria were monitored daily for mortality. At swim-up stage, fry received newly hatched (<24 hrs) brine shrimp nauplii to stimulate feeding. Appropriately sized trout food pellets (Rangen Inc., Buhl, ID) were then provided at half the recommended rate for optimal growth (Piper et al 1982). Wet weight of fry in each temperature regime was measured weekly. Once test organisms grew to a mean weight of 0.50 g, temperature of the aquarium was adjusted at a rate of 1ºC/day until the 12ºC test temperature was reached. Organisms were acclimated to the test temperature for 7 days prior to conducting the toxicity tests.

Toxicity Test

Toxicity solutions were delivered using a continuous-flow diluter (Benoit et al. 1982) constructed of teflon, polyethylene and polypropylene components. Dechlorinated municipal tap water supplied the diluters which delivered five exposures with a 50% dilution ratio, and an exposure control. A flow-splitter distributed each concentration equally among four replicate exposure chambers at a rate of 40 mls/minute each. A concentrated stock solution was prepared by dissolving a calculated amount of reagent grade zinc sulfate heptahydrate (Mallinkrodt) in deionized water and delivered to the diluter at a rate of 2.0 mls/minute using a peristaltic pump (Cole-Parmer model C/L). Fresh stock solutions were prepared as needed during the toxicity tests. Test solutions delivered to the exposure chambers (polyethylene containers with a capacity of 2.8 liters) overflowed into a temperature-controlled water bath at 12ºC. Diluters and toxicant flow rates were monitored daily to ensure proper operation. Nominal concentrations for the test conducted with fry from the 12°C incubation were 2000, 1000, 500, 250, 125, and 0 µg Zn/L. Anticipating greater tolerance, the nominal concentrations for the remaining tests were increased to 3200, 1600, 800, 400, 200, 100, and 0 µg Zn/L. Fifteen organisms were initially randomly assigned to each exposure chamber. After 24 hours of exposure, up to five organisms were sub-sampled from each experimental unit for subsequent analysis of gill metallothionein, ATPase activity, and zinc content. The number of sub-sampled organisms was adjusted based on the level of mortality after 24 hours (Table 3). Fry were not fed during the initial 96 hours of exposure but were fed twice daily thereafter. Exposure chambers were monitored hourly for mortality during the first 96 hours of the test. 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. Exposure continued for a total of 7 days, after which surviving fish from each exposure chamber were terminally anesthetized, blotted dry with a paper towel and mean weights measured and recorded. After recording the weights, these organisms were transported on ice, their gills excised and stored at-80°C for later analysis of metallothionein, ATPase activity and zinc content.

Water quality characteristics of exposure water were measured daily for the first 96 hours of the test. Hardness and alkalinity were determined according to Standard Methods (APHA 1998). A Thermo Orion 635 meter (calibrated with 4.00 and 7.00 pH buffers and two conductivity standards prior to each use) measured pH and conductivity and temperature. Dissolved oxygen was measured using an Orion 1230 dissolved oxygen meter.

Water samples were collected daily for the first 96 hours from each exposure level with surviving fry for dissolved zinc analysis. Exposure water was passed through a 0.45 Φm (micron) filter (Acrodisc), collected in disposable polystyrene tubes (Falcon), and immediately preserved with Ultrex® triple distilled nitric acid to pH <2. Concentrations 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). Sample splits were collected and spikes prepared for each sampling event to verify reproducibility and recovery.

Statistical Analyses

Toxstat version 3.5 software (West Inc. 1996) was used to perform statistical analyses. Analysis of variance (ANOVA) was used to evaluate differences of seven day survival. Survival proportions were transformed using the arcsine square root prior to ANOVA (Snedecor and Cochran 1980). Shipiro-Wilk's test and Levene's test assessed normality of data and homogeneity of variances, respectively (Weber et al. 1989). Treatment means were compared to the control using Williams' 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 exposure concentration not associated with a treatment effect was designated as the no-observed-effect concentration (NOEC). The lowest concentration of zinc 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. Median lethal concentrations (LC_{50}) after 96 hrs of exposure were estimated by the Trimmed Spearman-Karber technique (Hamilton et al. 1977, 1978).

RESULTS

Incubation

Hatching success and survival through the swim-up stage was not significantly affected by incubation temperatures and exceeded 92% (Table 4). Mean daily temperatures for 12, 9, 6, and 3ºC incubation regimes were relatively constant (Figure 2). Occasional temperature spikes resulted from chiller failures or overloaded circuit breakers but were small (usually <2°C) and of short duration. Incubation temperature had a profound effect development of embryos and larvae (Table 5). The number of days to hatch, swim-up and start of test greatly increased as temperature decreased. For example, brown trout incubated at 12° C reached the swim-up stage after 35 days whereas it required 135 days for trout at 3°C. In contrast, degree-days (defined as the sum of the daily mean temperature in °C) for each of the developmental stages was relatively constant among incubation temperatures (Table 5). Growth rate was reduced by decreased incubation temperature (Figure 3). Fry incubated at 3°C failed to grow for the first 40 days after swim-up. Concern for the health of the organisms prompted a decision to increase the holding temperature to 6°C. After increasing the temperature, the growth rate of the 3ºC fish increased to a level similar to the 6ºC fry.

Toxicity Tests

Temperature, dissolved oxygen and alkalinity were similar among the toxicity tests (Table 4). Mean water hardnesses in toxicity tests with the 12, 9, and 6ºC incubated fry were between 43.7 and 45.6 mg $CaCO_3$/L whereas the 3ºC water hardness was 54.2

mg $CaCO_3$/L. The pH ranged from a high of 7.58 for the 12ºC fry to a low of 7.28 for the 3ºC fry.

Analysis of external quality assurance samples was 99.7% (range 96.6-104.4%). Sample splits had a mean relative percent difference of 1.3% (range 0.0-5.9%). Spiked samples had a mean recovery of 100.0 % (range 96.0-104.2%). The detection limit, defined as mean measured blank concentration + three standard deviations, was 6 µg Zn/L.

Zinc exposure concentrations and associated 96-hour and seven-day mortality for the 12, 9, 6, and 3ºC incubated groups are presented in Tables 7, 8, 9, and 10, respectively. Measured concentrations were consistent over the course of the toxicity test and near nominal values. The majority of mortality occurred during the initial 96 hours of exposure. Little additional mortality was observed between 96 hours and test termination at 7 days. Mortality (96 hrs) plotted against zinc concentration indicates that brown trout incubated at 6°C were the most sensitive whereas fish incubated at 3°C were least sensitive (Figure 4). Mortality curves for toxicity tests with 12°C and 9°C incubated fish were similar to each other and intermediate. Estimated 96-hour $LC_{50's}$ for each incubation temperature regime are summarized in Table 11. Hardness-based estimates of LC_{50} calculated from previous brown trout toxicity data (Brinkman et al. 2006) are also presented in Table 11. Measured LC_{50} values were within 30% of the hardness-based estimates. The higher LC_{50} observed with the 3ºC fish is largely explained by the higher water hardness used in that test. Chronic values for fish incubated at 3, 9, and 12ºC were similar among each other and ranged between 329-392 µg/L (Table 11). The chronic value for the 6ºC incubation test could not be calculated due to high rates of mortality but must be less than the lowest exposure concentration 238 µg/L.

Mean weights of fry ranged between a low of 0.47 g for the 3ºC fish to a high of 0.62 g for the 9ºC fish. Mean weights of fry from the 12ºC and 6ºC tests were 0.53 g and 0.54 g, respectively.

DISCUSSION

Decreased incubation temperature greatly increased the time to hatch, reach swim-up and decreased growth rate. In contrast, degree-days to achieve developmental stages were relatively constant among incubation temperatures.

No relationship between 96-hour LC_{50} values and chronologic age (or incubation temperature) was observed.

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Table 3. Number of brown trout fry sub-sampled from each exposure chamber, based on 24-hour mortality.

Initial # of organisms	# dead at 24 hours	# collected for sub-sample
15	1	5
15	2	4
15	3	4
15	4	4
15	5	3
15	6	3
15	7	3
15	8	2
15	9	2
15	10	2
15	11	1
15	12	1
15	13	1
15	14	0
15	15	0

Table 4. Hatch success and survival through swim-up (%) for brown trout incubated at different temperatures.

Incubation Temperature (C)	Hatch Success (%)	Survival through Swim-up (%)
12	95.0	92.9
9	95.4	93.8
6	95.8	93.8
3	95.8	94.7

Table 5. Number of days and degree-days to initiation of hatch, swim-up and start of toxicity test for different incubation temperatures.

Incubation Temperature (°C)	Initiation of hatch		Swim-up		Start of Test	
	Days	Degree-Days	Days	Degree-Days	Days	Degree-Days
12	10	119	35	419	77	921
9	12	110	49	450	111	1043
6	20	122	80	499	134	888
3	36	103	139	430	230	1000

Table 6. Mean water quality characteristics for toxicity tests conducted using brown trout incubated at different temperatures. Standard deviations are in parentheses.

Incubation Temperature (°C)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	pH (S.U.)	Temperature (°C)	Dissolved Oxygen (mg O_2/L)
12	45.6 (2.1)	33.5 (1.7)	7.58 (0.25)	12.5 (0.4)	8.83 (0.35)
9	43.7 (1.6)	33.8 (1.0)	7.43 (0.25)	12.2 (0.3)	8.45 (0.55)
6	44.5 (1.9)	32.0 (1.0)	7.34 (0.12)	12.3 (0.3)	8.43 (0.50)
3	54.2 (1.8)	31.4 (0.9)	7.28 (0.13)	12.6 (0.7)	8.29 (0.62)
EPA	60.3 (2.0)	30.3 (1.0)	7.42 (0.10)	12.1 (0.3)	8.20 (0.82)

Table 7. Zinc concentrations and associated mortality after 96 hours and seven days for brown trout incubated at 12°C. Standard deviations are in parentheses.

Zn Concentration (µg/L)	<10 (2)	157 (4)	286 (6)	538 (4)	1044 (20)	1996 (65)
96 h mortality (%)	0 (0)	2.5 (5.0)	15.0 (12.9)	45.0 (25.2)	75.0 (12.9)	92.5 (9.6)
7 day mortality (%)	0 (0)	5.0 (5.8)	15.0 (12.9)	47.5* (29.9)	75.0* (12.9)	95.0* (5.8)

*Significantly greater than control (p>0.05)

Table 8. Zinc concentrations and associated mortality after 96-hours and seven days for brown trout incubated at 9°C. Standard deviations are in parentheses.

Zn Concentration (µg/L)	<10 (4)	266 (6)	471 (11)	858 (14)	1556 (26)	2953 (60)
96 h mortality (%)	2.3 (4.5)	11.7 (5.8)	40.4 (16.9)	59.4 (15.6)	94.7 (6.1)	100 (0)
7 day mortality (%)	2.3 (4.5)	11.7 (5.8)	40.4* (16.9)	64.7* (16.0)	97.5* (5.0)	100* (0)

*Significantly greater than control (p>0.05)

Table 9. Zinc concentrations and associated mortality after 96 hours and seven days for brown trout incubated at 6°C. Standard deviations are in parentheses.

Zn Concentration (µg/L)	<10 (1)	238 (13)	447 (20)	834 (41)	1623 (86)	3005 (64)
96 h mortality (%)	0 (0)	23.0 (5.3)	60.5 (23.5)	87.6 (5.1)	97.5 (5.0)	100 (0)
7 day mortality (%)	0 (0)	23.0* (5.3)	63.3* (24.9)	87.6* (5.1)	100* (0)	100* (0)

*Significantly greater than control (p>0.05)

Table 10. Zinc concentrations and associated mortality after 96 hours and seven days for brown trout incubated at 3°C. Standard deviations are in parentheses.

Zn Concentration (µg/L)	<10 (2)	234 (31)	462 (40)	831 (40)	1656 (51)	3305 (7)
96 h mortality (%)	2.5 (5.0)	3.3 (3.8)	26.8 (14.3)	55.8 (17.3)	89.5 (8.6)	100 (0)
7 day mortality (%)	7.5 (9.6)	8.4 (7.1)	29.4* (17.4)	55.8* (17.3)	89.5* (8.6)	100* (0)

*Significantly greater than control (p>0.05)

Table 11. Spearman-Karber and Probit median lethal zinc concentrations, and hardness-based regression estimates of zinc LC_{50}s (µg/L) for brown trout fry incubated at 12, 9, 6, and 3°C.

Incubation Temperature (°C)	Spearman-Karber LC_{50} (µg/L)	Chronic Value (µg/L)	Mean Hardness (mg $CaCO_3$/L)	Hardness-based regression estimate of LC_{50} (µg/L)
12	617	392	45.6	578
9	642	354	43.7	555
6	381	<238	44.5	565
3	757	329	54.2	683

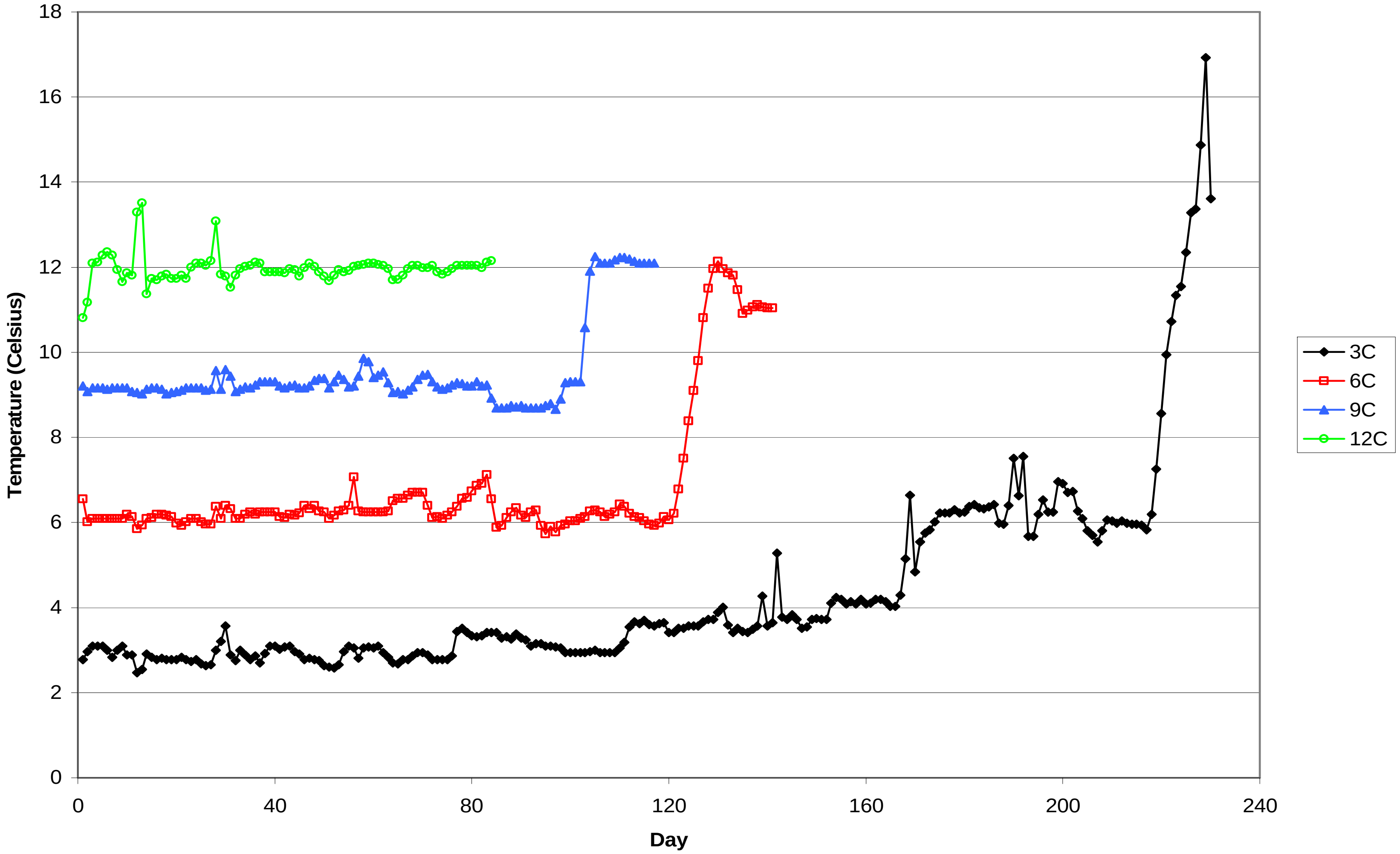

Figure 2. Mean daily temperature of 3, 6, 9, and 12°C incubation treatments.

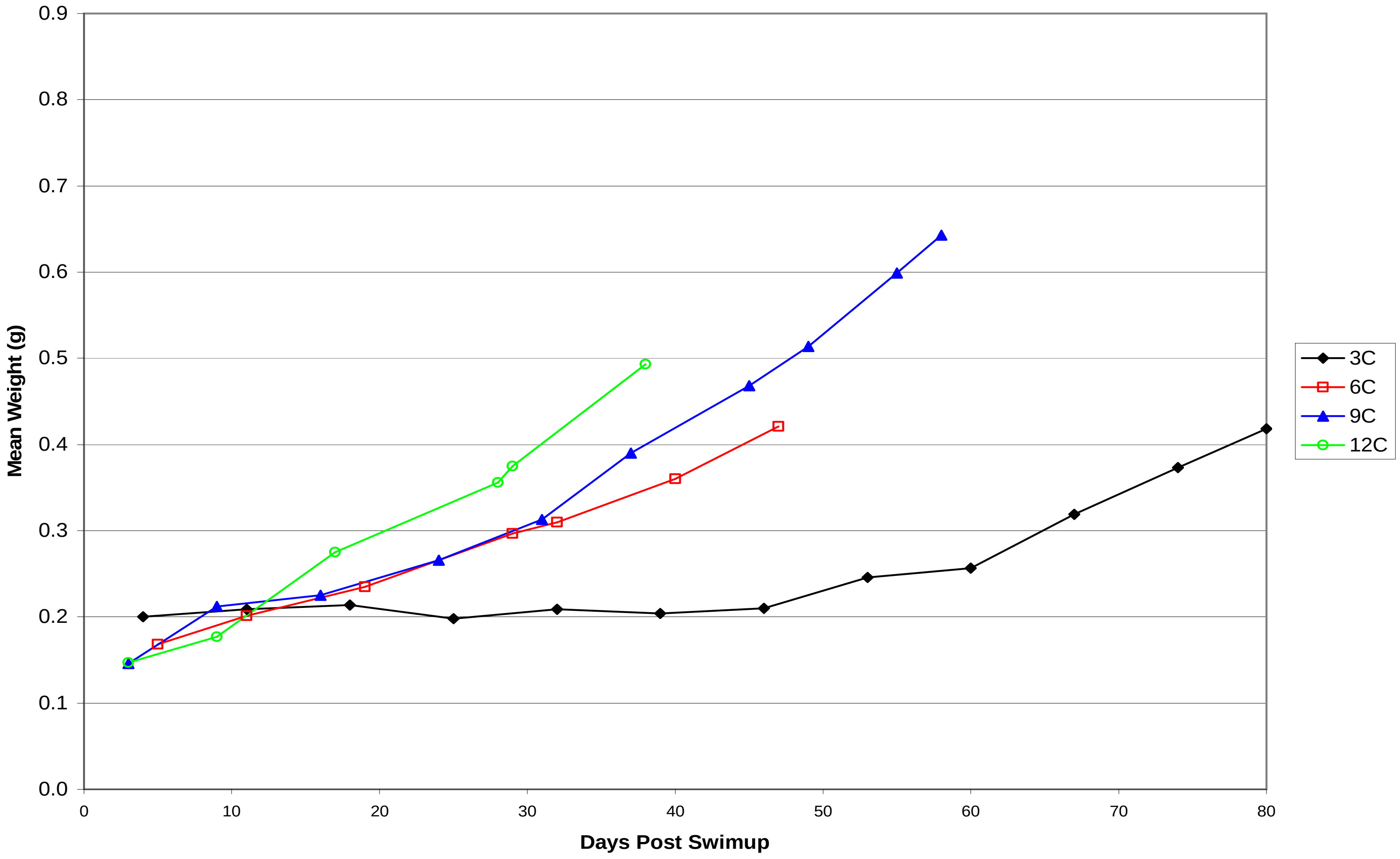

Figure 3. Mean weights of post swim-up brown trout fry incubated at 3, 6, 9, and 12°C.

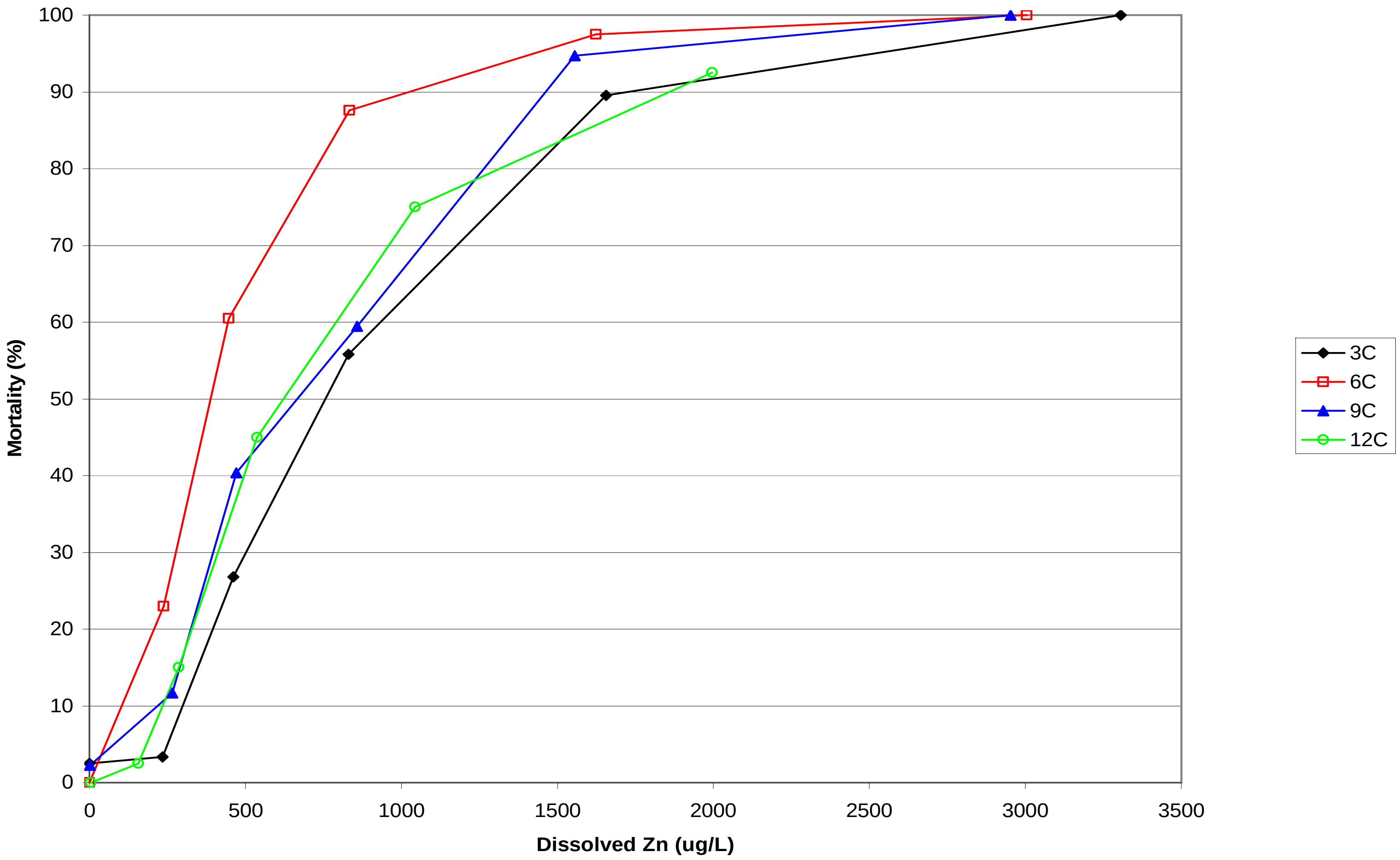

Figure 4. Zinc concentration-mortality responses of brown trout fry incubated at 3, 6, 9, and 12ºC

A Comparison of the Acute Sensitivity of Two Strains of Rainbow Trout (Oncorhynchus mykiss) to Zinc.

ABSTRACT

An acute toxicity test was conducted to compare the sensitivity of two strains of rainbow trout (Oncorhynchus mykiss) to zinc. The strains tested were a wild strain from the Colorado River and a hatchery strain from Washington. The 96-hour median lethal concentrations were 222 and 242 µg/L for the wild and hatchery strain, respectively. The 95% confidence intervals exhibited considerable overlap suggesting no difference in the sensitivity of the two strains to zinc. However, the growth rate of the hatchery strain was much greater than the wild Colorado River strain resulting in a large difference in size between the two strains at the start of the test. The size difference is a potential confounding factor in the comparison of the toxicity test results.

MATERIAL and METHODS

Organisms

The Colorado River rainbow trout (CRR) eggs were obtained from the Colorado State Hatchery in Glenwood Springs. Eggs were received as fertilized green eggs and were immediately transported to the aquatic toxicology laboratory in Ft. Collins, CO. The source for the broodstock is the Colorado River which has a wild reproducing strain of rainbow trout. The hatchery strain of rainbow trout was obtained as eyed eggs from Troutlodge Inc. in Sumner, Washington. The hatchery strain was received after the CRR eggs had hatched. Each strain was maintained in 55 gallon acrylic aquariums supplied with dechlorinated Ft. Collins municipal tap water at approximately 12ºC. Upon swim-up, fry were fed appropriately-sized trout chow at an estimate rate of 2.4% body weight per day. The mean weights of the fish at the start of the test were 0.158 g and 0.413g for the CRR and hatchery strain, respectively.

Exposure Apparatus

A continous-flow serial diluter (Benoit et al. 1982) was supplied with chilled de-chlorinated Fort Collins municipal tap water. The diluter was constructed of teflon, polyethylene and polypropylene components. A zinc stock solution was prepared by dissolving a calculated amount of reagent grade zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) (Mallinkrodt) in de-ionized water and delivered to the diluter via peristaltic pumps (Cole-Parmer model C/L) at an approximate rate of 2.0 mls/minute. The diluter 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 40 mls/minute each. Nalgene food-grade vinyl tubing delivered test solutions to 2.8 L polypropylene exposure chambers. Test solutions overflowed from the exposure chambers into water baths which were maintained at 12ºC using temperature-controlled re-circulators (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.

Test Methods

Target zinc exposure concentrations were 1600, 800, 400, 200, 100 and 0 µg Zn/L. The four replicates were divided between the two strains thus providing two replicates exposures for each strain.Mortality was monitored and recorded throughout each day. Dead fry were measured for total length to the nearest mm, blotted dry with a paper towel and weighed to the nearest 0.001 g. At the end of the 96-hour exposure, surviving fish were terminally anesthetized and measured for lengths and weights. Fry were not fed during the test.

Water quality characteristics were analyzed from a control and one randomly selected exposure level from one block daily. Blocks sampled were alternated each time. Hardness and alkalinity were determined according to Standard Methods (APHA 1985). A Thermo Orion 635 meter was used to measure pH, temperature and conductivity. The meter was calibrated using 4.00 and 7.00 pH buffers and two conductivity standards prior to each use. Dissolved oxygen was measured using an Orion model 1230 meter and was also calibrated prior to each use.

Water samples for dissolved zinc analysis were collected daily from each exposure level with surviving fry. Exposure water was passed through a 0.45 Φm filter (Acrodisc) and immediately preserved with Ultrex7 triple distilled nitric acid to pH <2. 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 were collected and samples were spiked to verify analytical reproducibility and recovery. The zinc detection limit was <10 Φg/L.

Ninety six hour median lethal concentrations (LC_{50}) were estimated by the Trimmed Spearman-Karber technique (Hamilton et al. 1977, 1978) with automatic trim.

RESULTS and DISCUSSION

Water quality characteristics were constant over the duration of the 96-hour exposure (Table 12). Hardness, alkalinity, and temperature fell within a narrow range. Mean dissolved oxygen was slightly under saturation and exceeded 7.97 at all times. Zinc concentration varied little over the duration of the test as evidenced by low standard deviations of daily measurements (Table 13). Mean recovery of quality assurance standards was 98% (range 97-100%). Relative percent difference of sample splits was 1% (range 0.4-1.8%) and percent recovery of spiked samples was 98% (range 96.5-99.5%).

The 96-hour median lethal concentration (LC_{50}) for the Colorado River strain was 222 µg/L with a 95% confidence range of 160-306 µg/L. For the hatchery strain, the LC_{50} was 242 µg/L with a 95% confidence range of 204-287 µg/L. The confidence ranges of the LC_{50}s had considerable overlap. However, the growth rate of the hatchery strain was much greater than the CRR resulting in a large weight difference between the two strains at the start of the test. The mean weights of the two strains were 0.158 g and 0.413g for the CRR and hatchery strain, respectively. The large size difference between the two strains introduces a potential confounding factor that makes comparison of the toxicity data less direct.

Survival of Colorado River strain of rainbow trout was less than hatchery strain exposed to low zinc concentrations (Table 13). However at higher zinc concentrations, survival of the Colorado strain was greater than the hatchery strain. The overall result is that hatchery strain of rainbow trout exhibit a steeper concentration-response curve compared to the Colorado River strain (Figure 5). The steeper concentration-response curve of the hatchery strain suggests less genetic variability compared to the wild CRR strain.

Table 12. Mean, standard deviation and range of water quality characteristics of exposure water used during Rainbow trout comparison Zn test.

	Hardness (ppm)	Alkalinity (ppm)	pH (S.U.)	Temperature (EC)	Conductivity (ΦS/cm)	Dissolved Oxygen (mg O_2/L)
Mean	56.8	32.3	7.33	11.8	125.1	8.73
Std. Dev.	0.9	0.9	0.12	0.17	12.0	0.36

Table 13. Mean dissolved zinc concentrations (Φg/L) and associated 96-hour survival of Colorado River (CRR) and a hatchery strain of rainbow trout. Standard deviations are in parentheses.

Dissolved Zn (Φg/L)	<10 (1.4)	140 (1.3)	259 (5.2)	447 (15)	847 (16)	1633 (39)
Colorado River RBT (%)	100 (0)	75.0 (2.1)	35.0 (0.7)	30.0 (2.8)	10.0 (0)	0 (0)
Hatchery Strain RBT (%)	100 (0)	90.0 (1.4)	45.0 (0.7)	5.0 (2.1)	0 (0)	0 (0)

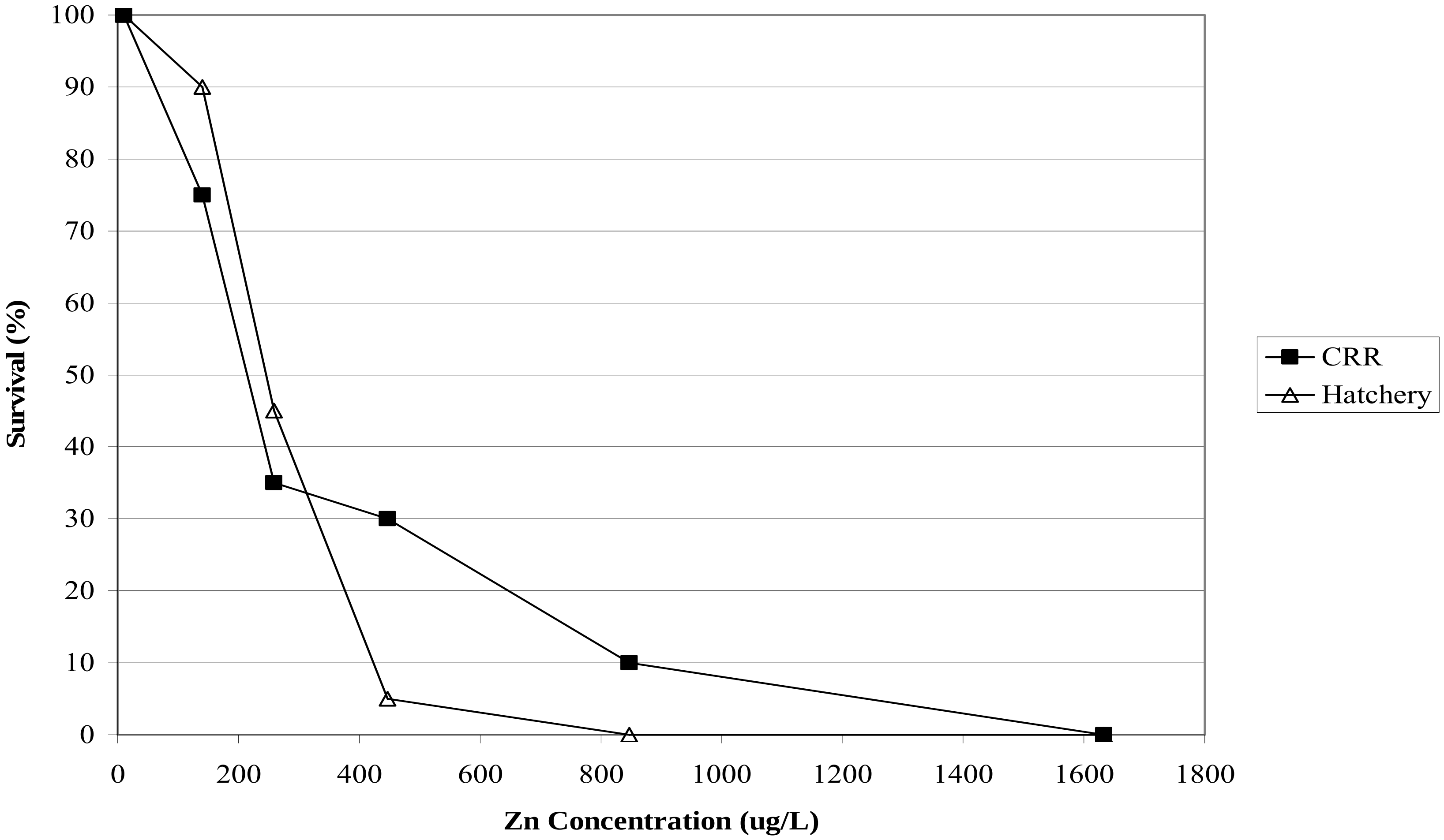

Figure 5. Survival (%) of Colorado River (CRR) and hatchery strains of rainbow trout exposed to zinc.

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Appendix One

2005 Investigative Study:

Factors Influencing Brown Trout Populations in Mine-impacted Reaches of the Eagle River following Remediation Efforts

and

Additional Appendices for Above Study

Final Report

2005 Investigative Study:

Factors Influencing Brown Trout Populations in Mine-impacted Reaches of the Eagle River following Remediation Efforts

Released October 3, 2005

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EXECUTIVE SUMMARY

The Colorado Division of Wildlife (CDOW), through a partnership with the Colorado Department of Public Health and Environment (CDPHE) and the U.S. Environment Protection Agency (EPA), has monitored fish populations, macroinvertebrate communities and water quality in the Eagle River near the Eagle Mine Superfund Site since 1990. Monitoring efforts, which were funded in part by Viacom International, sought to evaluate the progress of biological recovery in the Eagle River during and after mine remediation efforts. Another objective of monitoring activities was to provide a biological data set that would support the development of biologically-based water quality standards as required by the EPA's 1993 Record of Decision for the Eagle Mine Superfund Site. Ultimately, CDOW's data on brown trout populations were incorporated into the "Biological Approach to Defining a Healthy Biological Community", which was adopted in 2002 to develop water quality standards. Specifically, water quality standards to be developed with the Biological Approach were to protect brown trout populations and macroinvertebrates from adverse impacts of heavy metals from mining activities, including zinc, cadmium and copper contamination.

The "Biological Approach" to developing metals standards was a site-specific approach, where ambient metals concentrations at mine-sites would be considered "protective" if, for several consecutive years, measures of brown trout and their prey showed that mine-impacted sites were "in attainment". Determining attainment included comparing a brown trout metric (total density) at mine sites to metrics at reference sites. For site attainment, spring densities of brown trout at mine sites were to be equal to or greater than 95 percent of the mean of the population densities at the reference sites for three consecutive years. Biological results at each sampling location were compared against the water quality data for that location and if metric attainment had been achieved, then the water quality data for that sampling location could be used in the calculation of the new water quality standards. In 2004, attainment was not reached at multiple mine-impacted sites, thus requiring an "Investigative Study" to determine the relative roles of heavy metals vs. natural environmental influences on non-attainment, especially in the year 2004. CDOW was contracted to conduct this study in 2005, and results of this "2005 Investigative Study" are reported herein.

The objective of the 2005 Investigative Study was to determine the influence of aqueous metal concentrations on spring brown trout densities, young of year counts, average brown trout weight and site attainment in post-remediation years (1997-2005), relative to the influence of natural environmental variation in the Eagle River. Hypotheses compared the relative influences of: zinc, cadmium, and copper toxicity units (expressed as a ratio of measured concentrations to acute concentrations from hardness-based equations); high and low stream flows; amount of suitable habitat for adult and fry trout; and trout preybase. Our statistical analysis included predictive and descriptive modes of linear regression models and multivariate statistics. Agreement in results from this broad range of analyses techniques was assumed to indicate strong trends in the data, and insured that no single statistical test was favored. Below is a summary of the major findings:

1. Zinc toxicity units (usually the previous year's toxicity) was the variable most frequently included in the "best" regression models to explain annual changes in brown trout population densities, followed by copper, and then peakflow. Preybase was an important variable influencing brown trout weight. By contrast, cadmium, adult and fry habitat and other measures of streamflow were rarely important in regression models.

2. Zinc toxicity (current and/or previous year), high flows in the previous year (average flows in May and June), low flows in the previous year (baseflow), habitat and copper were all identified as important variables influencing site-attainment in multivariate analyses.

3. When zinc was a significant variable in statistical analyses, increases in zinc toxicity units were associated with reductions in brown trout metrics (e.g. lower densities, reduced weight, or lower probability of site attainment). Zinc often interacted with either copper or previous year peakflow to have a negative influence on brown trout metrics. Relationships between brown trout metrics and peakflow were not consistent; that is, they were positive or negative depending on the model. Copper was positively related to brown trout metric.

4. None of the variables that we tested explained the reduced brown trout metric (and thus non-attainment) at mine-sites in the year 2004. The sample year 2004 did not "stand out" from other years in terms of previous year flow, previous year zinc/copper/cadmium, or any other variable that we tested. That is, the metals and environmental variables that we had data for were not "extreme" in 2004 and did not contribute to the attainment problem.

In summary, our general investigation of how metals and environmental variables influence spring brown trout populations at mine-impacted sites revealed that increases in zinc are associated with decreases in the brown trout populations and site attainment. This was true in post-remediation years 1997-2005, and also was found to be the case between the years during which attainment was being assessed (2000-2005). Between the years 2000-2005, when further water quality improvements had occurred, negative impacts of peakflows were more pronounced than we found for the entire post-remediation period. However, zinc continued to have negative impacts to brown trout populations and site attainment, even after accounting for these negative effects of peakflow. These findings are not surprising, given that zinc concentrations during the spring snowmelt season are still above concentrations suggested by hardness-based LC50 equations for brown trout at some mine sites.

While zinc appears to be the variable with the most significant negative influence on spring brown trout populations across post-remediation years, zinc was not the culprit in non-attainment in the year 2004. Environmental variables that we had data for, including habitat and stream flows, also did not contribute to non-attainment in 2004. Zinc and copper concentrations in 2003 and 2004 were not notably higher than in other years where attainment occurred, and peakflows in 2004 and in the previous year (2003) were high but not extreme. Lower young of year counts in 2004, possibly due to higher flows *during* sampling, may have contributed to non-attainment at some mine sites in this year. Inter-annual fluctuation in sampling conditions (e.g. due to early or unexpected snowmelt, etc.) is an element of site-specific biological approaches that often cannot be well controlled, but which can impact development of water quality standards. Furthermore, it is difficult to statistically single out factors influencing brown trout populations for a specific year; most statistical tests deal with trends and correlations which require data points across multiple sites and multiple years. For this reason, we used a "weight of evidence" approach. Given the data that was collected in post-remediation years, most lines of evidence suggested that recent zinc concentrations during the "high metals" season may negatively influence brown trout populations in the Eagle River near the Eagle Mine Superfund Site.

BACKGROUND

Colorado Division of Wildlife Involvement in the Eagle River Project

The Colorado Division of Wildlife (CDOW) was first involved with the Eagle River remediation and monitoring project in 1989. Through a partnership with the Colorado Department of Public Health and Environment (CDPHE) and the U.S. Environment Protection Agency (EPA), CDOW has monitored fish populations, macroinvertebrate communities and water quality since 1990. The annual monitoring events were funded by Viacom International (Viacom), the Potentially Responsible Party for the Eagle Mine Superfund Site through a Cooperative Agreement with CDPHE, and also funded in-kind by CDOW. In addition to data collection over the past 15 years, CDOW has prepared reports summarizing the previous year's data to evaluate the progress of biological recovery in the Eagle River during and after mine remediation efforts. CDOW also conducted a survey of depth and velocity profiles in October 1999 to determine suitable habitat for brown trout using the model RHABSIM. CDOW contracted out the task of identifying macroinvertebrates in each year of the monitoring study. CDOW's partnership in this long-term monitoring project was initiated by researcher John Woodling, now retired from the CDOW. John Woodling continued his involvement as an outside contractor.

PURPOSE

One of the purposes of the biological monitoring activities was to provide a biological data set that would support the development of biologically-based water quality standards as required by the EPA's 1993 Record of Decision for the Eagle Mine Superfund Site. To that end, a decision had to be made about how the biological data would be used in a quantitative fashion, agreeable to all parties, to set the new water quality standards. In March 2002, EPA and CDPHE presented the "Biological Approach to Defining a Healthy Biological Community" to the public and requested public comment.

The "Biological Approach" included comparing brown trout metrics (total density, relative weight) at mine sites to metrics at reference sites. For site attainment, spring densities of brown trout at mine sites were to be equal to or greater than 95 percent of the mean of the population densities at the reference sites for three consecutive years. Attainment of this 95 percent criterion at mine sites was considered to indicate no statistically significant difference between the mine sites and the reference sites. At that time, the Biological Approach was set up to allow for lack of attainment at any individual sampling location, because simultaneous attainment of all metrics at all locations was considered unlikely to occur. The Biological Approach was designed such that the biological results at each sampling location would be compared against the water quality data for that location and if metric attainment had been achieved, then the water quality data for that sampling location could be used in the calculation of the new water quality standards.

Subsequent to the initial public review of the 2002 Biological Approach, relative weight of brown trout was removed as a metric because it does not allow for the same station-by-station comparison as the population density metric. Relative weight is best calculated as an aggregate value, where all the mine sampling locations are lumped together. When calculated for each site in each sample year, relative weight may be calculated based on only a few individual fish. In addition, when attainment of the relative weight metric occurred in the year 2001, it showed that the water quality in the previous year, 2000, would have been appropriate for use in the calculation of new water quality standards. In fact, water quality in 2000 was poor and there was failure of the population density metric. At that time it was determined that a strict comparison of relative weight to the water quality data in the fashion proposed by the Biological Approach was not appropriate and relative weight was removed from the final Biological Approach. The relative weight metric continues to be evaluated in the annual CDOW reports. The Biological Approach also included a macroinvertebrate metric, which is not discussed herein.

Brown trout population densities measured during the spring, pre-runoff season in the Eagle River have increased in reaches with heavy metals pollution since the Eagle Mine remediation began. In general, brown trout densities at mine sites were similar to densities at reference sites in the spring of 2002 and 2003, with the exception of the Bishop Gulch sample location. In the spring of 2004, however, brown trout populations were significantly reduced at both reference and mine sample locations. Concurrently, some of the mine sample locations did not attain the 95 percent criterion. Reasons for decreases in brown trout numbers in the spring of 2004 are unknown. Under the Biological Approach, such non-attainment would trigger an “Investigative Study” to attempt to determine probable causes.

Between the years 2004 -2005, CDOW agreed to produce two annual reports (after the 2004 and 2005 collecting seasons, respectively), and to complete the RHABSIM model developed for the sites. CDOW also agreed to conduct an analysis of existing data to investigate factors that may have influenced non-attainment of the brown trout metric (“2005 Investigative Study”). Following postponement of the Water Quality Control Commission’s 2005 Rulemaking Hearing for the Eagle River, CDPHE held an informational meeting in February 2005 for formal “parties” and “mailing list status” organizations to discuss details of the Investigative Study. CDOW presented their analysis approach to determine factors influencing spring brown trout population fluctuations in the Eagle River. Attendees were invited to provide criticisms on the proposed approach, to provide suggestions for alternative approaches, and/or to propose variables of interest to be included in statistical analyses. Attendees were also given the opportunity to provide additional comments to CDOW regarding selection of both response and predictor variables in statistical models via email. Discussion was limited to responses related to brown trout populations. Comments from parties regarding variable selection and statistical methodology were incorporate into a draft study plan of CDOW’s Investigative Study.

The purpose of CDOW’s Investigative Study was to use existing data to identifying factors influencing variability in spring brown trout population densities. Specifically, CDOW attempted to elucidate the relative influence of metals-related variables versus other environmental variables on non-attainment of the brown trout metric, especially in the year 2004.

Objectives and Hypotheses

The overall goal of this project was to explore relative influences of environmental variables and metals contamination on spring brown trout densities. Given the available data, specific objectives and hypotheses of CDOW’s 2005 Investigative Study were the following:

1) To compare the influence of aqueous heavy metal concentrations on brown trout population densities, young of year counts and average brown trout weight at sample sites, in each year after the majority of cleanup was completed (1997-2005), relative to the influence of natural physical, chemical, and biological aspects of the Eagle River.

   H_{1a}: Metal (especially zinc) or metal-mixture concentrations have the strongest influence on spring brown trout populations

H_{1b}: Hydrologic variables (peakflow, baseflow, and/or minimum flow) from the previous year have the strongest influence on spring brown trout populations

H_{1c}: Factors other than fluctuations in heavy metal concentrations and max/min stream flows, such as habitat suitability (estimated from RHABSIM modeling) and prey base (abundance and richness of stream invertebrates), influence spring brown trout populations

H_{1d}: Brown trout populations are influenced by a combination of metal concentrations, hydrology, habitat, and/or invertebrate prey base simultaneously.

2) To test the influence of aqueous heavy metal concentrations on attainment of mine sites for the brown trout metric (total trout density), in each year after the majority of cleanup was completed (1997-2005), relative to the influence of natural variation in physical, chemical, and biological aspects of the Eagle River.

H_{2a}: Metal (especially zinc) or metal-mixture concentrations have the strongest influence on site attainment of the brown trout density metric

H_{2b}: Hydrologic variables (peakflow, baseflow, and/or minimum flow) from the previous year have the strongest influence on site attainment of the brown trout density metric

H_{2c}: Factors other than fluctuations in heavy metal concentrations and max/min stream flows, such as habitat suitability (estimated from RHABSIM modeling) and prey base (abundance and richness of stream invertebrates), influenced site attainment of the brown trout density metric

H_{2d}: Site attainment of the brown trout metric was influenced by a combination of metals, hydrology, habitat, and/or invertebrate prey base simultaneously

Rather than relying on a single analytical method to test these hypotheses, we used a weight of evidence approach with several statistical tests. Specifically, linear regression analyses, with an information-theoretic approach to model selection, were conducted to address hypotheses 1a-1d. Both logistic regression and discriminant function analysis were employed to address hypotheses 2a-2d. Canonical discriminant analysis was employed to investigate the year 2004. Further details on these methods are provided below.

METHODS

Data Collection

A number of physical, chemical, and biological parameters have been measured since 1990 in the Eagle River and Cross Creek above and below the mine site by CDOW, Viacom, and other parties such as USGS (Appendix A). The CDOW sampled five sites from 1990-1993, and then added three more sites to the monitoring study between 1994-2005. CDOW conducted a survey of velocity and depth profiles in October 1999 to better understand habitat suitability for brown trout populations. CDOW also surveyed water surface elevations during higher flow conditions. Viacom collected macroinvertebrate and water quality data for some sites during the course of the study. Several USGS stream gage stations are available for streamflow data including gages near Redcliff, Minturn, and Avon. Flow was extrapolated from these gages to sample sites that lie between them. Flow data was obtained from the USGS for the years 1988 through 2005 in which cfs was captured every 15 minutes for the three separate stream gages. For site 1 (at Redcliff) the Redcliff flow gage data was used. For sites 1.9, 2, 2.9, 3, 4 and 5, the Minturn gage was used in combination with an adjustment coefficient to account for the station either being up or downstream of the flow gage (Table 1cfs). Sites 1.9 and 2 adjustments were determined by Viacom consultants. Sites 2.9 and 3 were not adjusted, for CDOW did not feel significant additions to the river were occurring between the stream gage location and the station locations. Sites 4 and 5 were adjusted by taking into account the addition of water from Cross Creek. An average was calculated for both low flow and high flow periods to estimate the addition of Cross Creek to the Eagle River. Six flow variables were calculated using this data (current and previous year; base flow, maximum flow and average "May and June" cfs). Base flow values were determined by calculating the average cfs during November, December and the following year January and February flows. An error was found in the provisional USGS gage data that influenced some of our flow calculations. Specifically, values of 2100 cfs and 2090 cfs were found during December 2000 and January 2001. The three records containing these values were obvious errors and were removed from the dataset. Flow values were then recalculated and our analysis was updated (presented herein).

Flow adjustment coefficients for each station based on Minturn stream gage.

Site	**Low Flow Adjustment**	**High Flow Adjustment**
1.9	Multiply by 0.9	Multiply by 0.9
2	Multiply by 0.91	Multiply by 0.91
2.9	Multiply by 1	Multiply by 1
3	Multiply by 1	Multiply by 1
4	Add 5 cfs	Add 227 cfs
5	Add 5 cfs	Add 227 cfs

CDOW also modeled habitat suitability for brown trout adults and fry at all study sites using a one dimensional RHABSIM model. We modeled stream conditions expected to produce lower habitat quality for each year (e.g. low flows for adult trout and higher, flushing flows for fry). Several different measures of "suitable habitat" were generated, including HSI values of 0, 0.25, 0.50 and 0.75 (e.g. the product of depth*velocity indices which range from 0-1). Curves for brown trout from both the USFWS and CDOW (B. Nehring, Colorado Division of Wildlife) were used. CDOW's curves for brown trout adults and fry were ultimately used to develop habitat variables for the 2005 Investigative Study analysis. Details of the RHABSIM model will be presented in a separate report (Skinner, unpublished data). Note that RHABSIM does not represent other important aspects of trout habitat such as pool depth, number of pools, etc. These habitat quality indicators were not measured.

Appendix A lists potential response and predictor variables. For this Investigative Study, we only considered data from 1997 to present, because these years represented a "post-remediation" time period where a site had a chance of "attainment". For the Biological Approach, the three consecutive years of attainment were actually not considered for mine sites until after the year 2000 (Prehearing Statement of Hazardous Materials and Waste Management Division, Viacom International Inc., and US EPA). However, using data from years 2000-2005 would have offered too few data points to capture environmental fluctuations of interest (e.g. both high flow years and drought years), and would also have had lower statistical power to detect how trends in brown trout populations matched trends in metals and other environmental variables. Using data from the years 1997-2005 allowed us to include a wider range of hydrologic conditions, habitat conditions and metals concentrations to determine impacts of these environmental variables on brown trout populations.

We selected the following response variables for analysis: 1) total brown trout density in the spring, 2) total count of young of year in the spring, 3) average brown trout weight in the spring, and 4) site attainment. We used average brown trout weight instead of the index calculation of relative weight. Sampling frequencies and spatial extent of response variables and predictor variables often differed. For example, we only sampled brown trout populations once each year, but had access to continuous streamflow data. Time series data such as streamflow needed to be condensed to a scale that was comparable to the brown trout metrics. For instance, measures of streamflow were condensed to mean daily flows in the spring and winter months of each year. Sometimes, a single value for streamflow at the gage was used (e.g. peakflow). Furthermore, hydrologic measures from the year *prior* to each brown trout sampling event were used to express flow conditions that brown trout populations experienced *between* the sampling events. Although surveys of depth and velocity profiles were only conducted in 1999, we utilized low flow conditions from stream gages for each year to estimate amount of suitable habitat for brown trout adults with the RHABSIM model. To estimate amount of suitable habitat for YOY during highflow conditions, we investigated flows in May and June of the previous year. We could not use peakflow data because they were outside the bounds of our RHABSIM model given the data (e.g. surface elevations) that the model was built on (i.e., only flows below 1400 cfs were appropriate to use in the model).

For metals and hardness data, two types were available – one-time sampling data in spring by CDOW and more frequent sampling by Viacom. We used CDOW data because these data were collected at sites where brown trout populations were sampled, and because they reflected metals concentrations in the high loading season (spring during snowmelt). Dissolved metals concentrations were translated into "toxicity units" based on CDOW's hardness-based equations for brown trout (see **Appendix A** for equations). To best reflect variation in toxicity units across sites and years, we used site- and year- specific hardness levels for our calculations. To correspond to our fish collection data, we used CDOW's point estimates of hardness measured each spring at fish collection sites, which typically ranged from 90-140 mg/L $CaCO_3$. It is important to note that we used these higher hardness values because we were only making *relative* comparisons across sites and years. It would be inappropriate to apply this hardness data to remediation goals and water quality criteria for the segments, where the values of hardness directly impact metals toxicity to fish. Lower values of hardness result in higher metals toxicity, and thus should be considered in setting water quality criteria. We used metals concentration and hardness data in the year of sampling, and in the previous year, at each site to calculate current and previous toxicity units. Although we were trying to restrict our analysis to the years 1997-2005, use of "previous year metals" required inclusion of metals concentrations in 1996.

We used qualitative measures of invertebrate richness and abundance (CDOW data) for the spring of each year to represent trout prey base. There are other environmental variables that may be important to brown trout population measures and site attainment for which we do not have data for all study years and/or sites. For instance, we were lacking data on daily temperatures and invertebrate prey biomass. Other missing variables are those associated with microhabitat that are also highly spatially structured (substrate composition, embeddedness, availability of suitable materials for redd construction, % pool and riffle, channel configuration, etc.)

Statistical Methodology

Exploring the nature of the variables

To determine whether parametric statistical methods are appropriate for testing our hypotheses, we inspected each variable's distribution shape with scatter plots and histograms. All sites and all years were combined at first, and then sites were investigated separately (across all years). When plots showed a non-normal distribution, we performed appropriate data transformations to reduce skew and to better approximate a normal curve. While parametric tests require that variables fit a normal curve, some deviation is typically acceptable. Statistical outliers, or data point that are "unusual" compared to the general trend of all other data points, and collinearity (high correlation) of predictor variables are more problematic in parametric tests. These problems were investigated with diagnostic tests available in the statistical software packages used (SAS/STAT and SPSS). Since "time" is an inherent factor in any monitoring dataset, we explored the relationship of each variable versus time (year) to understand whether variables were autocorrelated. Autocorrelation results in serially correlated error terms (residuals) in parametric models, violating the assumption that "residuals are identically and independently distributed".

To avoid data dredging (at least initially), response variables (e.g. brown trout density) and predictor variables (e.g. metals, flow, etc.) for statistical analyses were selected from the list of available variables *before* relationships between these variables were explored (see "Information-Theoretic Approach to Model Selection and Multi-model Inference" section below). Variable selection was driven by discussions at the February 2005 Informational Meeting, by personal communications with Parties in regards to the 2005 Investigative Study Proposal Draft, by attainment concerns and issues, and by our expert knowledge of stream ecology. Once model selection analyses were complete, additional "data mining" techniques were employed to determine whether our *a priori* selected variables and modeling efforts were worthwhile.

Information-Theoretic Approach to Model Selection and Multi-model Inference

To determine the relative influence of predictor variables on a response, model selection techniques such as forward, backward, and stepwise regression are typically used. These techniques are useful when the number of predictors relative to the sample size is low, and for data mining in larger datasets. However, such methods of model selection 1) do not include *a priori* scientific knowledge, 2) have a strong chance of including meaningless predictors in a model due to spurious correlations, and 3) run the risk of over-fitting, where precision of parameter estimates are over-estimated. An alternative approach to model selection is the Information-Theoretic Approach. This approach employs criteria such as Akaike's Information Criterion (AIC) that operate on the principle of parsimony, where both bias and variance are simultaneously minimized (Burnham and Anderson

2002). In other words, this technique seeks to balance the problem of over-fitting models (more predictors than necessary are included in a model, and some may be spurious) and under-fitting models (too few predictors are included in the model). Furthermore, this technique allows several models, rather than a single model, to be identified as important, and parameter estimates can be derived by averaging across these models. Model averaging results in final parameter estimates that are more reflective of the data, leading to stronger inference to the population from which the samples were collected.

Anderson et al. (2001) give an excellent example of how the information-theoretic approach to model selection can be used in conflict resolution. Here is a summary of how the approach works, and how it will be applied to the Eagle River:

1) Proponents work together to select a set of candidate *a priori* models that reflect their knowledge and concerns regarding relationships between response and predictor variables. In this case, CDOW constructed a suite of suggested models, and added additional models after the February 2005 Informational Meeting and after personal correspondence with Parties.

2) An AIC calculation is selected based on: a) whether the sample size is small (i.e., if the ratio of sample size to the # of estimated parameters is < 40), and b) whether the data are over-dispersed (estimated with Chi-squared goodness of fit statistic divided by the degrees of freedom for the global largest model).

In the case of the Eagle River, we utilized data from post remediation years 1997-2005, and used the AICc equation for smaller sample sizes:

$$\text{AICc} = -2\log(\text{MLE}) + 2K + (2K(K+1)/n-K-1)$$

MLE= maximum likelihood estimator
K = # of parameters to estimate, n = sample size

3) Regression models are run with a statistical program and when diagnostic tests reveal that assumptions are met, the residual sums of squares (SS) from the regression output is used to approximate the MLE in a “least squares” case of AICc:

$$\text{AICc} = n*\log(\text{RSS}/n) + 2K + (2K(K+1)/n-K-1)$$

RSS = residual sums of squares from regression model output
K = # of regression parameters +1 for the intercept and +1 for the residual variance
n = sample size

For the Eagle River analysis, we employed SAS/STAT software (PROC LOGISTIC and PROC REG) to generate output needed to calculate AIC values in an excel spreadsheet. PROC LOGISTIC provided direct calculations of AIC and MLE’s.

4) Models in the candidate set are compared by:

- Calculating Raw AICc values (see equation above)

- Calculating Δ AICc for each model "*i*" relative to the model with lowest AICc value to rank models:

$$\Delta \text{ AICc}_i = \text{AICc}_i - \text{AICc}_{\text{minimum}}$$

- Calculating Akaike weights to determine the relative likelihood of a model given the data and given the set of candidate models:

$$W_i = (\exp(-0.5\ \Delta \text{ AICc}_i))/(\Sigma \exp(-0.5\ \Delta \text{ AICc}) \text{ over all models})$$

In addition to the AIC model selection steps outlined above, adjusted R-squared values of the regression models were examined and compared to determine how much total variance in the response was explained by each model. This measure helps identify whether we tested a suite of meaningful hypotheses (e.g. our *a priori* models), because models that included variables strongly related to the response should have high explanatory power (high adjusted R-squared values). We considered an adjusted R-squared value of >0.30 to be noteworthy, >0.40 to be "good", and >0.60 to be "very good" for an environmental monitoring dataset. Assumptions of models and statistical outliers were checked with diagnostic tests (PROC UNIVARIATE PLOT, PROC PLOT with student residuals, VIF and COOK's D). After we ran our *a priori* models, we conducted a data mining exercise with Pearson's correlation statistics (PROC CORR) and traditional model selection techniques (PROC REG/STEPWISE and PROC LOGISTIC/STEPWISE, both with rules for variable entry/exit into and out of models of $p = 0.10$). Data mining procedures evaluated whether our suite of *a priori* models did, in fact, include variables that were most strongly correlated with the response variables. To explore the potential influence of the variable "previous year metals" in early post-remediation years (e.g. 1996, 1997 data), we also conducted a *post-hoc* analysis (with mine sites) using data only from years where attainment was possible (2000-2005). These analyses provided another check as to whether data for the years 1997-1999 were driving relationships discovered in the full set analyses.

Discriminant Function Analysis

Discriminant function analysis (DFA) is a dimension-reducing discrimination technique, where linear combinations of predictor variables (e.g. flow, metal levels, habitat suitability, etc.) are summarized to maximize separation between groups (e.g. GROUP1: mine sites in attainment vs. GROUP2: mine sites not in attainment). DFA can be used to estimate parameters and to build a linear model, constructed with significant predictor variables, to forecast the response variable. That is, DFA creates a model describing the probability of biological attainment for each sample location within each sample year beginning in 1997 to present, and predicts the probability of attainment of sites given future scenarios in flow, metals concentrations, etc. Coefficients of variables in discriminant functions were used to assess the relative contributions of the predictor variables to the final discrimination model. DFA is a multivariate normal statistical procedure and must meet assumptions typical of parametric tests.

We used data from 1997-2005 for reasons described previously. We further filtered the data set by removing the three reference stations, the below Minturn station and all years prior to 1997. Reference sites were used to calculate "attainment" for mine sites, and thus it did not make sense to include them in the DFA models. The remaining dataset consisted of data from 1997 to 2005 for stations 2, 2.9, 3 and 4. These stations were determined to either be "passing" or "failing" the brown trout biological criteria (95% of the average reference site brown trout population estimate for that particular year).

Principle Components Analysis (PCA) was then performed to reduce the number of potential variables as well as to group similar/correlated variables. Removal of highly correlated variables reduces the problems of collinearity and variance inflation in parametric statistical analyses, thereby increasing our confidence in the final DFA model, and in the relative importance of predictor variables. Major components (eigenvectors) were chosen from the PCA and individual variables from each PCA component (e.g. variables that were strongly weighted along each vector) were chosen to be a part of the DFA. Utilizing DFA, an equation was developed using the variables indicated from the PCA. These variables were then tested for Skewness, Kurtosis, equality of group means, equality of variance-covariance and equality of error variance. Secondly, the variables were then tested for collinearity using Pearson's Correlation. The data reduction techniques were used to remove highly correlated covariates from the final analysis.

Canonical Discriminant Analysis

One question that the aforementioned statistical analyses do not directly address is "what happened in 2004 that resulted in non-attainment of the brown trout metric at so many sites"? To better explore this question, we conducted canonical discriminant analysis. CDA is a descriptive multivariate technique, similar to discriminant function analysis, which can be used to maximize separation of data points based on linear combinations of predictor variables. In this case, we asked CDA to best separate mine sites based on sample year, using predictor variables identified as important in the aforementioned analyses. That is, we conducted CDA to best distinguish data collected at mine sites sampled in 2004 from data collected at these sites in 1997-2003 and 2005. Like the aforementioned analyses, CDA does not establish cause and effect. However, this analysis can indicate which variables differed most in 2004 compared to other years. For instance, if CDA separated sites in the year 2004 from sites in other years along an axis of "reduced fry habitat", then it could be conjectured that this variable, especially if it was significant in the best regression and DFA models, contributed to non-attainment in 2004.

RESULTS

For a lay-person "friendly" interpretation of results, please refer to the "roadmap" provided in Appendix B. Otherwise, details of statistical analyses are provided below.

Information-Theoretic (AIC) Model Selection

A priori models compared with the AIC model selection method are listed in **Tables 1a-1d**. Suites of models were created for our four response variables: total brown trout density (**Table 1a**), YOY count (**Table 1b**), average brown trout weight (**Table 1c**), and site attainment of the brown trout metric (**Table 1d**). Suites of models for the responses were very similar, with the exception that 1) prey base and young of year counts were only included in models for brown trout weight. The number of young of year trout, like measures of average trout length, accounts for the negative influence of smaller fish on the average weight of trout at a site. Young of year count, rather than average length, was used because these small fish may also have been a prey item (thus, it was a more informative variable). Models for the first three response variables were conducted with two datasets. First, we used data for all of the sites between the years 1997-2005 (mine and reference sites). In this analysis, reference sites provided additional variation in brown trout responses and metal toxicity units (e.g. higher and lower values compared to mine sites, respectively). Second, we ran the same models using only mine sites (1997-2005) to better isolate factors that may be influencing the brown trout metric at these sites. For the response variable "site attainment", only data from mine sites between the years 1997-2005 were used.

Table 2a presents the P-value, the adjusted R-squared value, the AIC_c value, the delta AIC_c value, and the Akaike weights (w_i) for each model (using all sites between 1997-2005). Models with a delta AIC of 2 or less are considered to be the most parsimonious, and thus the "best" models (Burnham and Anderson 2002). For brown trout density, models ***BT15a*** was the "best" model based on AIC model selection and was highly statistically significant (overall model: $p < 0.0001$). Model ***BT15a*** included toxicity units of Zn, Cd, Cu from the previous year and the interaction of the latter two metals with Zn. This model had notable explanatory power in terms of explaining variation in the response ($R^2_{adj} = 36.7\%$). When data from only the mine sites was used, the same model was selected as "best" (**Table 2b**) and this model now explained substantially more of the variation ($R^2_{adj} = 69.9\%$) in spring brown trout densities. In the ***BT15a*** model for the full dataset (**Table 3a**) and the "mine-site only" dataset (**Table 3b**), brown trout density was negatively related to Zn toxicity in the previous year (see parameter estimates of the ***BT15a*** model and their significance levels in **Table 3a** and **Table 3b**). For mine sites, previous year concentrations of Zn, Cd and the interaction between Zn and Cu were negatively related to brown trout population estimates. The interaction between Zn and Cd was positively associated with brown trout populations, for unexplained reasons. For the full dataset, model ***BT5a*** was also a runner-up model, with a similar R^2_{adj}. Previous year copper toxicity (positive relationship), and the interaction between previous year copper and previous year zinc (negative relationship), were the only significant variables predicting brown trout density in this model (Table 3a). A negative effect of the interaction between previous year zinc and previous year peakflow was close to significant ($p = 0.0805$).

For counts of YOY, the "best" model was ***YOY5*** when the entire dataset was used (**Table 2a**). When only the mine sites were considered, models ***YOY3***, ***YOY5*** and ***YOY7*** were selected as the "best" models (**Table 2b**). All of these models were statistically significant (overall model: $P<0.001$), but other than ***YOY5***, did not explain enough variation in the data to be considered "worthwhile" and informative ($R^2_{adj} < 30\%$). Low adjusted R-squared values suggest that even our best models did not capture the key variables that influence counts of young of year trout from year to year. These best models *did* indicate that, of the variables that we *did* measure and included in our *a priori* suite, current year metals toxicity (zinc and copper) and peak flows were the most influential (see parameter estimates and their significance levels in **Table 3a** and **Table 3b**). In the models with all sites, zinc toxicity units in the current year negatively influenced young of year counts when it interacted with peakflow (**Table 3a**). Otherwise, the relationship with YOY was positive for both zinc and peakflow, and negative for copper. In ***BT5a*** for only the mine sites, the negative relationship between YOY and the interaction between zinc toxicity and peakflow in the previous year was close to significant (p=0.0764).

For brown trout weight, a single "best" model (***WT16***) was identified using all of the data (**Table 2a**), and ***WT16*** , ***WT17*** and ***WT19*** were identified as the best models when using the dataset with only the mine sites (**Table 2b**). These models were highly significant in both scenarios ($p<0.0001$). ***WT16*** explained a good proportion of the variation in brown trout weight across sites and years (R^2_{adj} = 54.6% for the entire dataset and = 51.0% for mine-only sites). ***WT17*** explained 54.6% of the variation and ***WT19*** explained 50.1% of the variation when only mine-sites were used in regression analyses. The number of young of year negatively influenced trout weight, and prey base positively influenced trout weight, in all models with the whole dataset and with mine-only sites (see parameter estimates and their significance levels in **Table 3a** and **Table 3b**). Previous year peakflow, Zn toxicity in the previous year, and their interaction appeared repeatedly in "best" models, but were typically not significantly associated with trout weight (except to ***WT16*** –see Table 3a). YOY counts accounted for the fact that a high YOY count would result in lower averages of weight measurements. Model ***WT17*** was similar to ***WT16*** but with Cu toxicity added (**Table 3a**), and WT19 included habitat suitability for adults at low flow conditions (**Table 3b**).

For the response "site attainment of the brown trout metric", ***SA5a*** was the "best model" (**Table 2b**). Model ***SA5a*** was significant ($P < 0.0001$) and explained a notable proportion of the variation in the response (R^2_{adj} = 34.3 %). Previous year Zn and Cu toxicity units, and their interaction, were again included in the model. Previous year peakflow and the interaction with previous year Zn toxicity were also included. However, based on Chi-squared goodness of fit statistics, only Cu toxicity units from the previous year had a significant association with site attainment (see parameter estimates and their significance levels in **Table 3b**), even though the overall model was significant. As we found for other brown trout responses, there was a trend where the interaction between previous year Zn and Cu, and the interaction between zinc and peakflow, both negatively impacted site attainment (increases in these parameters contributed to site failure). However, these relationships were not significant with chi-squared statistics.

Data Mining with Stepwise Model Selection

We conducted stepwise regression model selection (both logistic and linear) to investigate relative importance of the following variables: Zn toxicity units in the current and previous years, Cu toxicity units in the current and previous years, previous year peakflow, previous year minimum flow, previous year habitat suitability (at higher flows in May and June for fry, and at low fall-winter flows for adults), invertebrate abundance and invertebrate richness. Results are found in **Table 4**. We only used data for the mine sites, since the purpose of this data dredging exercise was to better identify factors contributing to non-attainment of the brown trout metric at these sites. We also included variables that were not considered in the *a priori* regression models (such as minimum flow in the previous year, taxa richness) to see if we "missed" any important variables. We did not include interaction terms to reduce the possibility that collinearity would result in spurious results. That is, we allowed models to choose the most influential "raw" variables without highly correlated interactions "masking" these relationships.

For the brown trout density response variable, previous year Zn and Cu toxicity units, current year zinc, as well as peakflow, minimum flow and invertebrate abundance, were selected for inclusion in the best model ($R^2 = 75.1\%$). The best model from AIC model selection had similar explanatory power ($R^2 = 69.9\%$). Relationships between predictor variables and brown trout densities were all significant at the $p = 0.010$ level except minimum flow, and were all in a negative direction with the exception of Cu toxicity units. The zinc variable had a partial R-squared value of 41%, and thus contributed a majority of the explanatory power to the stepwise model. A negative relationship with previous year Zn toxicity units and a positive relation with previous year Cu were also found in the "best" AIC model. Peakflow was negatively associated with brown trout density in our stepwise model, but was not included in our best AIC model for mine-only sites. Our AIC model may have been improved if we had included prey base as a variable. However, both the AIC "best" model and the stepwise selection model identified zinc as the primary factor negatively influencing brown trout density.

Stepwise regression identified previous year Zn toxicity units as the primary variable that negatively influenced young of year counts (partial R^2 for zinc = 35.2%, compared to the overall model R^2 of 63%). Current year and previous year copper toxicity, previous year peakflow, invertebrate abundance, and habitat suitability for fry during high flows were also included, but contributed little to the overall explanatory power of the model (partial R^2 's all were < 6%). Zinc, copper and peakflow were also included in our top AIC models, but were not significantly associated with young of year counts. Our stepwise model had stronger explanatory power than our best AIC models (all adjusted R^2 values for AIC models were less than 34%). For brown trout weight, toxicity units for Zn and Cu from the previous year, young of year count, and invertebrate abundance and richness were included in the best stepwise model. This model explained 59.7% of the variability in the response. Our best AIC models were similar, but also included previous year peakflow (or habitat) and explained somewhat less variability (R^2_{range}=50-55%). In the stepwise model and in the AIC models, young of year counts were negatively related to weight and invertebrate prey base was associated with increases in weight. Previous year zinc toxicity was negatively associated with weight in the stepwise model, but was not significant in the AIC models.

Our stepwise logistic model for site attainment was more informative than our best AIC models, in that more variables included in the final stepwise model were statistically significant, and the stepwise model had higher explanatory power (R^2=50.5% vs. 34% for our best AIC model). Both the AIC models and the stepwise model included a positive relationship with Cu (both measured in the previous year). However, our AIC model included peakflow in the previous year, while our stepwise model included a measure of habitat suitability and young of year counts. Increases in both of these variables led to an increase in site attainment.

We further evaluated the relative importance of metals toxicity units versus environmental variables by conducting analyses with data in the years 2000-2005. Since sample sites in the years 1997-1999 were removed, sample sizes for analysis were reduced. Therefore, results were interpreted with caution, because the low sample size may have given us inadequate statistical power to detect relationships. For this reason, results of this "diagnostic" analysis are discussed but are not presented in table form. For spring brown trout densities at mine sites between 2000-2005, model **15a** (our "best model from AIC analysis for years 1997-2005) still showed that previous year zinc had a negative impact (p = 0.0703). This relationship was weaker than what was found when mine-site data between 1997-2005 was used (p = 0.0004). Also, the explanatory power of the overall model for 2000-2005 was halved (R^2 = 37.8% using years 2000-2005 vs. 69.9% using years 1997-2005). Stepwise regression with the 2000-2005 dataset identified peakflow and invertebrate richness as important variables, both with significant negative relationships with density (p=0.0134 and p=0.0439, respectively). For brown trout weight and young of year counts, metals were not significantly related to these responses in any model from our suite of *a priori* models for AIC. Peakflow was negatively associated with brown trout weight in one AIC model (p=0.0283), and negatively interacted with habitat suitability in another AIC model (p=0.0365). Stepwise regression identified invertebrate abundance and copper toxicity as a positive influence on average trout weight, and significant negative variables were previous year zinc toxicity and young of year counts . For the response variable "young of year" count, peakflow and copper were identified in stepwise regression as the only variables of interest, and the partial R-squared value for these variables were very low ($R^2 < 13\%$). For site attainment in the years 2000-2005, the AIC models were again poor in their explanatory power, and no statistically significant relationship was found with previous year zinc or any other environmental variable. However, logistic stepwise regression identified previous year zinc and minimum flow as significant predictors of site attainment (e.g., increases in these variables led to site failure between 2000-2005). Young of year count and previous year copper toxicity showed the opposite relationship.

Discriminant Function Analysis

Principal Component Analysis (PCA)

PCA was performed on the filtered dataset. A total of 8 components were found to have influence, but only variables from the first 4 components were utilized within the DFA model. The first 4 components explained 75.5% of the variance (**Table 5**). Variables were chosen from each component for DFA analysis. For example, component 1 contained 16 variables with physical habitat (e.g. low and high flow values for both adult and fry brown trout) that were found to have significant influence in explaining overall variance. From these 16, four physical habitat variables were chosen: the natural log of adult and fry brown trout habitat, at low and high flows respectively, in which the product of depth and velocity HSI was equal or greater to 0.75. The list of variables utilized in the DFA can be found in **Table 6**.

Discriminant Function Analysis (DFA)

DFA was utilized to determine if the independent variables could be weighted such that they significantly discriminated whether or not a sample site met the biological attainment level for brown trout. The initial DFA equation created using the variables obtained from PCA is as follows (see **Appendix 1sa** for model output and descriptions of output):

> $\boldsymbol{DFA_{PCA}}$ *= (LnZnTU_previous year*-3.725) + (ave may-june cfs_previous year*-0.005) + (lnBaseflow_previous year*0.925) + (lnZnTU_current year*1.976) + (ave may-june cfs current year*0.004) + (lnphab75_fry high flow*1.840) + (lnphab75_fry low flow*-3.442) + (lnCdTU_previous year*-2.260) + (EPT_richness*-0.094) + (lnCuTU_previous year*5.506) + (lnBaseflow_current year*2.208) + (lnCdTU_current year*-4.496) + (lnphab75_adult low flow*7.192) + (lnphab75_adult high flow*-4.012) + (lnCuTU_current year*1.558) – 18.976.*

This equation correctly classified 90.6% of the original cases and 59.4% of the cross-validated cases. The contribution of each variable to the function can be viewed in **Table 6**. A negative relationship indicates that the variable contributed to site failure, while a positive variable indicates that it contributed to site attainment (with DFA, a variable can be said to be positive, yet have a negative effect. The vice verse is also a potential outcome. This is a result of which axis in multivariate space the variable is placed). This equation must be viewed with caution because multicollinearity occurred and normal distribution assumptions were violated. Upon removing the "failing" variables (e.g. those not meeting assumptions), the DFA equation classification power was reduced to 87.5% of original cases and 65.6% of cross-validated cases (equation **DFA_{PCA2}**)(**Appendix 2sa**).

For the **DFA_{PCA}** model (the first model developed) the concentration of Zn from the previous year had the most negative influence in determining site attainment. Previous and current Cd concentrations, high flow adult physical habitat, previous and current year base flow and previous and current average "May through June" flow also had negative influences. Previous Cu concentrations, high and low flow fry physical habitat, low flow adult physical habitat and EPT taxa richness positively influenced site attainment.

A *post-hoc* approach was then initiated to see if the DFA model could be refined. Using the same variable list as the **DFA_{PCA2}** equation and adding the variable 'countyoy' (count of young of year brown trout), a new DFA model was developed.

> **DFA_{YOY}** = *(*Ln*ZnTU_current year*-0.423) + (*Ln*ZnTU_previous year*-1.058) + (*Ln*CuTU_current year*1.223) + (*Ln*CuTU_previous year*1.804) + (*Ln*phab75adult-lowflow*4.618) + (EPT_richness*-0.009) + (ave may_june cfs*-0.001) + (ave may_june cfs_previous year*-0.004) + (*Ln*baseflow*0.215) + (*Lnbase*flow_p*-0.029) + (count_YOY*0.057) – 9.670*

The contribution of each variable to the function can be viewed in **Table 7.** Equation **DFA_{YOY}** correctly classified 96.9% of the original cases and 78.1% of the cross-validated cases, an improvement upon the original **DFA_{PCA}** model (**Appendix 3sa**).

For the **DFA_{YOY}** model the count of YOY had the greatest positive influence on site attainment. Other positive variables were previous Cu concentration, adult low flow physical habitat, and EPT taxa richness. Variables that negatively influenced site attainment were previous and current Zn, current Cu, previous and current year base flow, and previous and current year average "May through June" flow. Previous year Zn had the greatest negative influence on the model.

In an attempt to simplify the **DFA_{YOY}** model, an iterative process utilizing different combinations of variables was used to determine the influence on model results. **DFA_{YOY2}** was generated using this iterative process. **DFA_{YOY2}** correctly classified 93.8% of the original cases and 93.8% of the cross-validated cases (**Appendix 4sa**). The simplified model is:

> **DFA_{YOY2}** = *(*Ln*baseflow*0.719) + (*Ln*ZnTU_previous year*-2.232) + (*Ln*CuTU_previous year*2.068) + (*Ln*phab75adult_lowflow*1.316) + (count of YOY*0.044) - 6.364*

Previous year Zn and base flow negatively influenced the model. Count of YOY, previous year Cu and physical habitat positively influenced the model.

Data Mining with Canonical Discriminant Analysis

An exploratory canonical discriminant analysis was conducted, where environmental variables (toxicity units in previous and current years for zinc, copper and cadmium, minimum and maximum flows in the previous year, trout habitat for adults and fry at low and high flows, and prey base measures) separated mine site samples in the years 1997-2005. Our results indicate that the year 2004 was not singled out in this analysis as being “unusual” in any of our measured variables (**Figure 1**). Variables that best separated mine site samples in different years were associated with the first canonical axis (**Table 8**). This axis explained 60.7% of the variation among years and separated year with high minimum flows in the previous year (e.g. 2005) from years with lower minimum flows and/or higher peakflows in the previous year (e.g. 2000). The second canonical axis explained 23.2% of the variation and separated years with higher zinc toxicity in the previous year (e.g. 1997, 1998) in the positive direction from years with lower zinc concentrations and higher young of year counts in the negative direction (**Figure 1 and Table 8**). In general, the year 2004 was characterized by having a lower young of year count and low previous year zinc toxicity, but very high minimum flow during that 2004 sampling event (indicated by the fact that year 2005 was strongly associated with “high previous year minimum flow”). Supporting graphs to better understand how the year 2004 compared to other years are presented in **Figures 2a-2e**.

CONCLUSION

Zinc toxicity unit, which expresses the ratio of ambient zinc concentration to LC50 toxicity predicted from hardness-based equations for brown trout, was the primary environmental variable that negatively influenced brown trout densities and contributed to site failure in the spring seasons of 1997 through 2005. Zinc toxicity in the current or previous year was included in the "best" models in AIC analysis and/or in stepwise selection models for brown trout density, trout weight, young of year counts and site attainment. Both current year and previous year zinc toxicity units were also selected as important variables that influence site attainment in models constructed with discriminant function analyses. When the relationship between response variables and zinc was statistically significant it was usually negative; that is, zinc negatively influenced measures of brown trout and contributed to non-attainment of the brown trout metric at mine sites. Zinc also interacted with copper and previous year peakflow to have a negative impact on brown trout responses.

Previous year peakflow was negatively associated with brown trout population responses or mine site attainment during the years 1997-2005 in post-hoc stepwise models for brown trout density and young of year counts. We had anticipated that higher peakflows would negatively impact trout eggs and fry, leading to lower densities of young of year fish (and thus overall trout densities) in the following year (Nehring and Anderson 1993). Such a relationship was found when all data years were used (1990-2005) and analysis was conducted on a site-by-site basis (Woodling and Rollings 2004, 2005). Also, average flow in spring (May and June), was negatively associated with site attainment in the DFA models. Some counterintuitive relationships with brown trout responses were the positive association between responses and copper toxicity, and negative associations with increases in minimum flow. The relationship with low flow might be explained by the fact that in years with lower flows leading up to spring sampling, fish are more concentrated into available habitat and may be more available to sampling gear/methods. This would lead to a situation where brown trout densities would increase with decreased minimum flows. However, this "sampling availability" phenomenon does not explain why increased baseflows were associated with reduced probability of site attainment in multivariate analysis. Positive relationships with copper were probably not meaningful, since copper levels were well below acute levels for brown trout (toxicity units ratio < 0.25).

Our post-hoc modeling suggests that we selected appropriate and informative *a priori* models. That is, the R^2 values and variables in our best *a priori* AIC models were usually similar to those in our best *post hoc* models from stepwise selection, and the direction of significant relationships (e.g. negative impacts of increased zinc, positive impacts of increased prey base) were fairly consistent. Results from linear and logistic regression matched results using a multivariate approach, lending further evidence that our results are meaningful. *Post hoc* data mining suggested that there were additional variables that may have contributed to fluctuations in brown trout responses. For instance, some of our *post hoc* models showed that brown trout responses were negatively impacted by increased average spring (May and June) flows. It may be that higher spring flows, in addition to one-time peakflow events, negatively impact eggs and juvenile fish to a greater degree, but this can only be conjectured. There are other variables that are known to influence trout survival and growth, but for which we did not have adequate data for statistical testing. For instance, variation in stream temperature was not considered in either *a priori* models or in data mining models. Snow-pack and timing of snowmelt were also likely important, not only to brown trout populations, but also to our ability to sample young of year fish. For instance, the sampling event in 2004 is one of the few years where CDOW sampled at streamflows near 125 cfs, because of an unexpected early runoff. Streamflows during sampling in other years were typically 50-75 cfs. The fact that brown trout densities at reference sites also tended to be lower in 2004 supports the idea that small brown trout individuals were not as available for sampling in this year.

Why were so many sites not in attainment in 2004? Our approach to this question was two-fold. First we identified general trends in how site-attainment and brow trout populations tracked fluctuations in metals, flow, and habitat. Second, we honed in on the year 2004 to attempt to identify what made this year "unique". Our analyses suggest that there is no one "smoking gun" in regards to an environmental variable that explains reduced brown trout metrics in this particular year. Zinc and copper concentrations in 2003 and 2004 were not notably higher than in other years where attainment occurred, and peakflows in 2004 and in the previous year (2003) were high but not extreme. We showed in several of our analyses that young of year counts were highly associated with brown trout densities, and thus site attainment. Young of year counts were lower in 2004, which likely contributed to non-attainment at some mine sites, yet the reason for reductions in young fish cannot ultimately be determined from our regression models. Our "young of year" models showed that zinc, peakflow and flow-related habitat were negatively related to YOY counts, but these models had very low explanatory power (low R-squared values). Our canonical discriminant analysis identified young of year count as a major variable separating years, but did not separate 2004 from the other years. Thus, flushing of eggs and juvenile fish by a peakflow event is not likely to be the primary factor in site failure in 2004. In general, canonical discriminant analysis did not reveal any one natural environmental factor that made 2004 an "unusual" year.

Although our analysis did not reveal the ultimate reason behind non-attainment of the brown trout metric in 2004, we *did* identify the continued negative impact of zinc on brown trout populations. Even with fluctuations in flow and habitat variables between 1997-2005, zinc still surfaced as the single most important variable explaining reductions in brown trout metrics. Zinc also interacted with peakflow and copper toxicity to negatively influence brown trout populations. When we investigated mine sites in years where attainment was possible (2000-2005), we still found that total brown trout weights and the probability of site attainment were negatively associated with zinc toxicity units (albeit at a lower statistical significance than was previous found in the 1997-2005 dataset). These findings are not surprising, given that zinc concentrations during the spring snowmelt season are still above concentrations suggested by hardness-based LC50 equations for brown trout at some mine sites. Higher zinc concentrations in the spring season may result in fish mortality or fish movement out of mine-sites. At this point in the post-remediation phase, it appears that zinc and other metals concentrations are fairly stable in that they fluctuate annually based on environmental conditions (e.g. snowmelt, flows). That is, there does not appear to be a continued trend of decreasing metal concentrations from year to year. Additional removal of zinc sources, if possible, would likely improve the probability of site attainment for the brown trout metric in the spring season. Remediation efforts thus far have been very effective, and have resulted in major improvements to brown trout populations in these segments of the Eagle River.

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Table 1a. *A priori* linear regression models (BT1-BT15a) tested with AIC model selection for the response variable "brown trout density estimate" (POP EST). Sample sizes reflect models where all data from all sites between the years 1997-2005 (reference and mine sites) were used. The same models were run with data from mine sites only (reference sites excluded; n = 45 for all models). Metals toxicity units in the current sample year are expressed as ZNTU, CDTU and CUTU while toxicity units in the previous year are denoted as PNZTU, PCUTU, and PCDTU. TU_ALL is the total toxicity units in the current year. PYPEAK= previous year peakflow, while HAB-SUIT indicates habitat suitability with an HSI of 0.75 calculated with RHABSIM for adult brown trout at low flow conditions (using DOW curves).

Model	Response	N	K		TENTATIVE LIST OF SUGGESTED MODELS FOR AIC MODEL SELECTION							
					Predictors and Interesting Interactions							
					Metals			*Peakflow and Habitat*		*Prey, Other Flow and Chemical Measures, Interactions*		
BT1	POP EST	71	3		ZNTU							
BT2	POP EST	71	3					PYPEAK				
BT3	POP EST	71	5		ZNTU			PYPEAK		PYPEAK*ZNTU		
BT4	POP EST	71	5		ZNTU	CUTU					ZNTU*CUTU	
BT5	POP EST	71	7		ZNTU	CUTU		PYPEAK		PYPEAK*ZNTU	ZNTU*CUTU	
BT6	POP EST	71	3	TU_ALL								
BT7	POP EST	71	5	TU_ALL				PYPEAK		PYPEAK*TU_ALL		
BT8	POP EST	63	3						HAB_SUIT			
BT9	POP EST	63	5		ZNTU				HAB_SUIT			HAB_SUIT*ZNTU
BT10	POP EST	63	7		ZNTU	CUTU			HAB_SUIT		ZNTU*CUTU	HAB_SUIT*ZNTU
BT11	POP EST	63	5	TU_ALL					HAB_SUIT			HAB_SUIT*TU_ALL
BT12	POP EST	63	8		ZNTU			PYPEAK	HAB_SUIT	PYPEAK*ZNTU	ZNTU*HAB_SUIT	HAB_SUIT*PYPEAK
BT13	POP EST	63	8	TU_ALL				PYPEAK	HAB_SUIT	PYPEAK*TU_ALL	TU_ALL*HAB_SUIT	HAB_SUIT*PYPEAK
BT14	POP EST	63	5					PYPEAK	HAB_SUIT		HAB_SUIT*PYPEAK	
BT15	POP EST	71	7		ZNTU	CUTU	CDTU				ZNTU*CUTU	ZNTU*CDTU
BT1a	POP EST	71	3		PZNTU							
BT3a	POP EST	71	5		PZNTU			PYPEAK		PYPEAK*PZNTU		
BT5a	POP EST	71	7		PZNTU	PCUTU		PYPEAK		PYPEAK*PZNTU	ZNTU*PCUTU	
BT9a	POP EST	71	5		PZNTU				HAB_SUIT			HAB_SUIT*PZNTU
BT12a	POP EST	63	8		PZNTU			PYPEAK	HAB_SUIT	PYPEAK*PZNTU	PZNTU*HAB_SUIT	HAB_SUIT*PYPEAK
BT15a	POP EST	71	7		PZNTU	PCUTU	PCDTU				PZNTU*PCUTU	PZNTU*PCDTU

Table 1b. *A priori* linear regression models (YOY1-YOY15) tested with AIC model selection for the response variable “count of brown trout young of year” (YOY CNT). Sample sizes reflect models where data from all sites between the years 1997-2005 (reference and mine-impacted sites) were used. The same models were run with data from mine sites only (reference sites excluded; n = 45 for all models). Metals toxicity units in the current sample year are expressed as ZNTU, CDTU and CUTU. TU_ALL is the total toxicity units in the current year. PYPEAK= previous year peakflow, while HAB-SUIT indicates habitat suitability with an HSI of 0.75 calculated with RHABSIM for fry brown trout at high flow (using DOW curves).

				TENTATIVE LIST OF SUGGESTED MODELS FOR AIC MODEL SELECTION								
Model	**Response**	**N**	**K**	**Predictors and Interesting Interactions**								
				Metals				***Peakflow and Habitat***		***Prey, Other Flow and Chemical Measures, Interactions***		
YOY1	YOY CNT	71	3		ZNTU							
YOY2	YOY CNT	71	3					PYPEAK				
YOY3	YOY CNT	71	5		ZNTU			PYPEAK		PYPEAK*ZNTU		
YOY4	YOY CNT	71	5		ZNTU	CUTU					ZNTU*CUTU	
YOY5	YOY CNT	71	7		ZNTU	CUTU		PYPEAK		PYPEAK*ZNTU	ZNTU*CUTU	
YOY6	YOY CNT	71	3	TU_ALL								
YOY7	YOY CNT	71	5	TU_ALL				PYPEAK		PYPEAK*TU_ALL		
YOY8	YOY CNT	63	3						HAB_SUIT			
YOY9	YOY CNT	63	5		ZNTU				HAB_SUIT			HAB_SUIT*ZNTU
YOY10	YOY CNT	63	7		ZNTU	CUTU			HAB_SUIT		ZNTU*CUTU	HAB_SUIT*ZNTU
YOY11	YOY CNT	63	5	TU_ALL					HAB_SUIT			HAB_SUIT*TU_ALL
YOY12	YOY CNT	63	8		ZNTU			PYPEAK	HAB_SUIT	PYPEAK*ZNTU	ZNTU*HAB_SUIT	HAB_SUIT*PYPEAK
YOY13	YOY CNT	63	8	TU_ALL				PYPEAK	HAB_SUIT	PYPEAK*TU_ALL	TU_ALL*HAB_SUIT	HAB_SUIT*PYPEAK
YOY14	YOY CNT	63	5					PYPEAK	HAB_SUIT		HAB_SUIT*PYPEAK	
YOY15	YOY CNT	71	7		ZNTU	CUTU	CDTU				ZNTU*CUTU	ZNTU*CDTU

Table 1c. *A priori* linear regression models (WT1-WT22) tested with AIC model selection for the response variable "brown trout average weight" (BT WEIGHT). Sample sizes reflect models where data from all sites between the years 1997-2005 (reference and mine-impacted sites) were used. The same models were run with data from mine sites only (reference sites excluded; n = 44 for all models). Metals toxicity units in the current sample year are expressed as ZNTU, CDTU and CUTU while toxicity units in the previous year are denoted as PNZTU, PCUTU, and PCDTU. TU_ALL is the total toxicity units in the current year. PYPEAK= previous year peakflow, while HAB-SUIT indicates habitat suitability with an HSI of 0.75 calculated with RHABSIM for adult brown trout at low flow (using DOW curves). PREY indicates insect abundance, while #YOY indicates the number of YOY contributing the measure of average weight. More YOY would result in a lower average.

Model	Response	N	K		**TENTATIVE LIST OF SUGGESTED MODELS FOR AIC MODEL SELECTION** **Predictors and Interesting Interactions**								
					Metals			***Peakflow and Habitat***		***Prey, Other Flow and Chemical Measures, Interactions***			
WT1	BT WEIGHT	69	4		PZNTU								#YOY
WT2	BT WEIGHT	69	4					PYPEAK					#YOY
WT3	BT WEIGHT	69	6		PZNTU			PYPEAK		PYPEAK*PZNTU			#YOY
WT4	BT WEIGHT	69	6		PZNTU	PCUTU					PZNTU*PCUTU		#YOY
WT5	BT WEIGHT	69	8		PZNTU	PCUTU		PYPEAK		PYPEAK*PZNTU	PZNTU*PCUTU		#YOY
WT6	BT WEIGHT	69	4	TU_ALL									#YOY
WT7	BT WEIGHT	69	6	TU_ALL				PYPEAK		PYPEAK*TU_ALL			#YOY
WT8	BT WEIGHT	61	4						HAB_SUIT				#YOY
WT9	BT WEIGHT	61	6		PZNTU				HAB_SUIT			HAB_SUIT*PZNTU	#YOY
WT10	BT WEIGHT	61	8		PZNTU	PCUTU			HAB_SUIT		PZNTU*PCUTU	HAB_SUIT*PZNTU	#YOY
WT11	BT WEIGHT	61	6	TU_ALL					HAB_SUIT			HAB_SUIT*TU_ALL	#YOY
WT12	BT WEIGHT	61	9		PZNTU			PYPEAK	HAB_SUIT	PYPEAK*PZNTU	PZNTU*HAB_SUIT	HAB_SUIT*PYPEAK	#YOY
WT13	BT WEIGHT	61	9	TU_ALL				PYPEAK	HAB_SUIT	PYPEAK*TU_ALL	TU_ALL*HAB_SUIT	HAB_SUIT*PYPEAK	#YOY
WT14	BT WEIGHT	61	6					PYPEAK	HAB_SUIT		HAB_SUIT*PYPEAK		#YOY
WT15	BT WEIGHT	69	8		PZNTU	PCUTU	PCDTU				PZNTU*PCUTU	PZNTU*PCDTU	#YOY
WT16	BT WEIGHT	69	7		PZNTU			PYPEAK		PYPEAK*PZNTU		PREY	#YOY
WT17	BT WEIGHT	69	9		PZNTU	PCUTU		PYPEAK		PYPEAK*PZNTU	PZNTU*PCUTU	PREY	#YOY
WT18	BT WEIGHT	69	7	TU_ALL				PYPEAK		PYPEAK*TU_ALL		PREY	#YOY
WT19	BT WEIGHT	61	7		PZNTU				HAB_SUIT		HAB_SUIT*PZNTU	PREY	#YOY
WT20	BT WEIGHT	61	9		PZNTU	PCUTU			HAB_SUIT	PZNTU*PCUTU	HAB_SUIT*PZNTU	PREY	#YOY
WT21	BT WEIGHT	61	7	TU_ALL					HAB_SUIT		HAB_SUIT*TU_ALL	PREY	#YOY
WT22	BT WEIGHT	61	7					PYPEAK	HAB_SUIT		HAB_SUIT*PYPEAK	PREY	#YOY

Table 1d. *A priori* linear regression models (BT1-BT15a) tested with AIC model selection for the response variable "site attainment of the brown trout metric" (ATTAIN?). Sample sizes reflect data from all mine sites between the years 1997-2005.
Metals toxicity units in the current sample year are expressed as ZNTU, CDTU and CUTU while toxicity units in the previous year are denoted as PNZTU, PCUTU, and PCDTU. TU_ALL is the total toxicity units in the current year. PYPEAK= previous year peakflow, while HAB-SUIT indicates habitat suitability with an HSI of 0.75 calculated with RHABSIM for adult brown trout at low flow (using DOW curves).

					TENTATIVE LIST OF SUGGESTED MODELS FOR AIC MODEL SELECTION							
Model	Response	N	K			Predictors and Interesting Interactions						
					Metals			*Peakflow and Habitat*		*Prey, Other Flow and Chemical Measures, Interactions*		
SA1	ATTAIN?	45	3		ZNTU							
SA2	ATTAIN?	45	3					PYPEAK				
SA3	ATTAIN?	45	5		ZNTU			PYPEAK		PYPEAK*ZNTU		
SA4	ATTAIN?	45	5		ZNTU	CUTU					ZNTU*CUTU	
SA5	ATTAIN?	45	7		ZNTU	CUTU		PYPEAK		PYPEAK*ZNTU	ZNTU*CUTU	
SA6	ATTAIN?	45	3	TU_ALL								
SA7	ATTAIN?	45	5	TU_ALL				PYPEAK		PYPEAK*TU_ALL		
SA8	ATTAIN?	45	3						HAB_SUIT			
SA9	ATTAIN?	45	5		ZNTU				HAB_SUIT			HAB_SUIT*ZNTU
SA10	ATTAIN?	45	7		ZNTU	CUTU			HAB_SUIT		ZNTU*CUTU	HAB_SUIT*ZNTU
SA11	ATTAIN?	45	5	TU_ALL					HAB_SUIT			HAB_SUIT*TU_ALL
SA12	ATTAIN?	45	8		ZNTU			PYPEAK	HAB_SUIT	PYPEAK*ZNTU	ZNTU*HAB_SUIT	HAB_SUIT*PYPEAK
SA13	ATTAIN?	45	8	TU_ALL				PYPEAK	HAB_SUIT	PYPEAK*TU_ALL	TU_ALL*HAB_SUIT	HAB_SUIT*PYPEAK
SA14	ATTAIN?	45	5					PYPEAK	HAB_SUIT		HAB_SUIT*PYPEAK	
SA15	ATTAIN?	45	7		ZNTU	CUTU	CDTU				ZNTU*CUTU	ZNTU*CDTU
SA1a	ATTAIN?	45	3		PZNTU							
SA3a	ATTAIN?	45	5		PZNTU			PYPEAK		PYPEAK*PZNTU		
SA5a	ATTAIN?	45	7		PZNTU	PCUTU		PYPEAK		PYPEAK*PZNTU	ZNTU*PCUTU	
SA9a	ATTAIN?	45	5		PZNTU				HAB_SUIT			HAB_SUIT*PZNTU
SA12a	ATTAIN?	45	8		PZNTU			PYPEAK	HAB_SUIT	PYPEAK*PZNTU	PZNTU*HAB_SUIT	HAB_SUIT*PYPEAK
SA15a	ATTAIN?	45	7		PZNTU	PCUTU	PCDTU				PZNTU*PCUTU	PZNTU*PCDTU

Table 2a. Results of AIC model selection for the response "brown trout population estimate" (BT1-BT15a), "count of young of year" (YOY1-YOY15), and "brown trout average weight" (WT1-WT22). Data from reference and mine sites for the years 1997-2005 were used for this analysis. Models with a delta AICc = 2 or less are considered "the best" based on the principle of parsimony. **These "best" models are in boldface type.** The Akaike weight (wi, ranging from 0-1) is another indication of "strength" of the model. P-values and Adjusted R-squared values are given for each model (Pmodel and Adj-R2). Refer to Table 1a-1c to determine what parameters are in each model.

Model	Pmodel	Adj-R2	AICc	Δ AICc	wi
BT1	0.0257	0.057	-221.0	23.2	0.00
BT2	0.3064	0.000	-216.9	27.3	0.00
BT3	0.0001	0.274	-237.1	7.1	0.02
BT4	0.0069	0.127	-224.0	20.1	0.00
BT5	0.0001	0.312	-238.2	6.0	0.03
BT6	0.1600	0.014	-217.9	26.3	0.00
BT7	0.0001	0.271	-236.8	7.4	0.01
BT8	0.9157	0.000	-188.0	56.2	0.00
BT9	0.0095	0.133	-195.4	48.8	0.00
BT10	0.0178	0.139	-193.1	51.1	0.00
BT11	0.1027	0.053	-189.9	54.3	0.00
BT12	0.0001	0.327	-207.1	37.1	0.00
BT13	0.0002	0.303	-204.8	39.4	0.00
BT14	0.0227	0.105	-193.4	50.8	0.00
BT15	0.0001	0.280	-235.0	9.2	0.01
BT1a	0.0001	0.187	-231.5	12.7	0.00
BT3a	0.0001	0.244	-234.3	9.9	0.00
BT5a	**0.0001**	**0.363**	**-243.7**	**0.5**	**0.41**
BT9a	0.0001	0.331	-211.8	32.4	0.00
BT12a	0.0001	0.360	-210.2	34.0	0.00
BT15a	**0.0001**	**0.367**	**-244.2**	**0.0**	**0.53**

Model	Pmodel	Adj-R2	AICc	Δ AICc	wi
YOY1	0.5879	0.000	116.5	27.8	0.00
YOY2	0.9988	0.000	116.8	28.1	0.00
YOY3	0.0033	0.147	106.9	18.2	0.00
YOY4	0.0787	0.055	114.2	25.5	0.00
YOY5	**0.0001**	**0.365**	**88.7**	**0.0**	**1.00**
YOY6	0.8971	0.000	116.7	28.1	0.00
YOY7	0.0104	0.116	109.5	20.8	0.00
YOY8	0.1400	0.020	105.7	17.0	0.00
YOY9	0.1599	0.037	107.1	18.4	0.00
YOY10	0.0387	0.110	104.9	16.2	0.00
YOY11	0.3061	0.011	108.7	20.1	0.00
YOY12	0.0172	0.152	103.4	14.7	0.00
YOY13	0.0666	0.097	107.4	18.7	0.00
YOY14	0.1870	0.031	107.5	18.8	0.00
YOY15	0.0088	0.146	109.7	21.1	0.00

Table 2a. CONTINUED

Model	Pmodel	Adj-R2	AICc	Δ AICc	wi
WT1	0.0004	0.1846	-172.5	36.3	0.00
WT2	0.0001	0.2303	-178.8	30.0	0.00
WT3	0.0001	0.4588	-198.1	10.7	0.00
WT4	0.0003	0.2326	-172.7	36.1	0.00
WT5	0.0001	0.5145	-202.8	6.0	0.03
WT6	0.0035	0.1320	-168.2	40.6	0.00
WT7	0.0001	0.4555	-197.7	11.1	0.00
WT8	0.0001	0.3462	-162.5	46.3	0.00
WT9	0.0001	0.5185	-178.4	30.4	0.00
WT10	0.0001	0.5971	-186.3	22.5	0.00
WT11	0.0001	0.4942	-175.4	33.4	0.00
WT12	0.0001	0.6338	-190.5	18.3	0.00
WT13	0.0001	0.6351	-190.8	18.0	0.00
WT14	0.0001	0.5263	-179.4	29.3	0.00
WT15	0.0004	0.2553	-173.3	35.5	0.00
WT16	**0.0001**	**0.5455**	**-208.8**	**0.0**	**0.61**
WT17	0.0001	0.5466	-205.9	2.8	0.15
WT18	0.0001	0.5306	-206.6	2.2	0.20
WT19	0.0001	0.5902	-186.8	22.0	0.00
WT20	0.0001	0.6287	-189.7	19.1	0.00
WT21	0.0001	0.5758	-184.7	24.1	0.00
WT22	0.0001	0.6082	-189.5	19.2	0.00

Table 2b. Results of AIC model selection for the response “brown trout population estimate” (BT1-BT15a), “count of young of year” (YOY1-YOY15), “brown trout average weight” (WT1-WT22) and “site attainment of the brown trout metric” (SA1-SA15a). Data from only the mine-impacted sites for the years 1997-2005 were used for this analysis. Models with a delta AICc = 2 or less are considered “the best” based on the principle of parsimony. **These “best” models are in boldface type.** The Akaike weight (wi, ranges from 0-1) is another indication of “strength” of the model. P-values and Adjusted R-squared values are given for each model (Pmodel and Adj-R2). Refer to Table 1a-1d to see parameters in each model.

Model	Pmodel	Adj-R2	AICc	Δ AICc	wi
BT1	0.0063	0.1416	-133.4	41.1	0.00
BT2	0.0046	0.1529	-134.0	40.5	0.00
BT3	0.3426	0.3426	-142.5	31.9	0.00
BT4	0.0043	0.2189	-134.8	39.6	0.00
BT5	0.0003	0.3617	-140.7	33.8	0.00
BT6	0.1006	0.0396	-128.3	46.1	0.00
BT7	0.0004	0.3108	-118.0	56.5	0.00
BT8	0.5874	0.0000	-125.8	48.7	0.00
BT9	0.0603	0.1020	-128.6	45.9	0.00
BT10	0.0185	0.1936	-130.2	44.3	0.00
BT11	0.4161	0.0000	-123.6	50.8	0.00
BT12	0.0019	0.3151	-135.7	38.7	0.00
BT13	0.0031	0.2942	-134.3	40.1	0.00
BT14	0.0279	0.1384	-118.0	56.5	0.00
BT15	0.0001	0.4469	-147.1	27.3	0.00
BT1a	0.0001	0.3961	-149.2	25.2	0.00
BT3a	0.0001	0.4314	-149.0	25.4	0.00
BT5a	0.0001	0.6283	-165.0	9.4	0.01
BT9a	0.0001	0.3723	-144.7	29.8	0.00
BT12a	0.0003	0.3894	-140.9	33.5	0.00
BT15a	**0.0001**	**0.6985**	**-174.4**	**0.0**	**0.99**

Model	Pmodel	Adj-R2	AICc	Δ AICc	wi
YOY1	0.0087	0.1297	63.9	6.6	0.02
YOY2	0.0057	0.1454	63.1	5.8	0.02
YOY3	**0.0011**	**0.2714**	**58.7**	**1.4**	**0.21**
YOY4	0.0614	0.1012	68.1	10.9	0.00
YOY5	**0.0006**	**0.3434**	**57.2**	**0.0**	**0.43**
YOY6	0.0203	0.0985	65.5	8.2	0.01
YOY7	**0.0009**	**0.2798**	**58.2**	**0.9**	**0.27**
YOY8	0.7791	0.0000	71.1	13.8	0.00
YOY9	0.0345	0.1285	66.7	9.5	0.00
YOY10	0.095	0.1052	71.2	13.9	0.00
YOY11	0.0637	0.0994	68.2	11.0	0.00
YOY12	0.0069	0.2576	64.6	7.3	0.01
YOY13	0.0046	0.2762	63.4	6.2	0.02
YOY14	0.0536	0.1078	67.8	10.6	0.00
YOY15	0.0112	0.2178	65.1	7.9	0.01

Table 2b. CONTINUED

Model	Pmodel	Adj-R2	AICc	Δ AICc	wi
WT1	0.0026	0.2159	-137.1	16.0	0.00
WT2	0.0007	0.2624	-139.8	13.3	0.00
WT3	0.0013	0.2956	-137.9	15.2	0.00
WT4	0.0001	0.4325	-148.2	4.8	0.03
WT5	0.0001	0.4744	-148.1	5.0	0.03
WT6	0.0117	0.1558	-133.8	19.2	0.00
WT7	0.0028	0.2651	-136.9	16.2	0.00
WT8	0.0026	0.2158	-137.1	16.0	0.00
WT9	0.001	0.3064	-139.4	13.6	0.00
WT10	0.0001	0.4382	-145.2	7.9	0.01
WT11	0.016	0.1873	-132.4	20.6	0.00
WT12	0.0017	0.3441	-136.4	16.7	0.00
WT13	0.0033	0.3157	-134.5	18.5	0.00
WT14	0.0008	0.3152	-140.0	13.1	0.00
WT15	0.0001	0.4352	-144.9	8.1	0.01
WT16	**0.0001**	**0.5104**	**-153.1**	**0.0**	**0.31**
WT17	**0.0001**	**0.5459**	**-152.6**	**0.5**	**0.25**
WT18	0.0001	0.4515	-148.0	5.0	0.03
WT19	**0.0001**	**0.5012**	**-152.3**	**0.8**	**0.21**
WT20	0.0001	0.5242	-150.5	2.5	0.09
WT21	0.0001	0.4091	-144.8	8.3	0.00
WT22	0.0001	0.4672	-149.3	3.7	0.05

Model	Pmodel	Adj-R2	AICc	Δ AICc	wi
SA1	0.8794	0.0000	66.7	15.9	0.00
SA2	0.0068	0.1337	59.4	8.6	0.01
SA3	0.0046	0.1980	58.7	7.8	0.01
SA4	0.1231	0.0562	65.9	15.1	0.00
SA5	0.0111	0.2022	62.3	11.5	0.00
SA6	0.4963	0.0000	66.2	15.4	0.00
SA7	0.0043	0.2092	58.5	7.7	0.02
SA8	0.3838	0.0000	66.0	15.1	0.00
SA9	0.7678	0.0000	70.6	19.7	0.00
SA10	0.1377	00689	68.9	18.0	0.00
SA11	0.6705	0.0000	70.2	19.3	0.00
SA12	0.0151	0.1954	64.4	13.5	0.00
SA13	0.0095	0.2265	63.2	12.4	0.00
SA14	0.0169	0.1447	61.5	10.6	0.00
SA15	0.0237	0.1552	64.2	13.4	0.00
SA1a	0.0531	0.0536	63.0	12.1	0.00
SA3a	0.0104	0.1531	60.5	9.6	0.01
SA5a	**0.0001**	**0.3426**	**50.9**	**0.0**	**0.71**
SA9a	0.1936	0.0314	66.0	15.1	0.00
SA12a	0.0187	0.1536	65.0	14.1	0.00
SA15a	0.0002	0.3077	53.1	2.2	0.23

Table 3a. Means (ESTIMATE), standard errors (SE) and statistical significance (P) of parameters in the "best models" from AIC model selection. Direction of association between responses and parameters is also shown in the sign of the estimate, where a negative sign indicates that the variable contributes to reductions in brown trout density, young of year counts, or weights. These models reflect data collected from both reference sites and min-impacted sites in the years 1997-2005. **Significant P-values are in boldface type.**

BEST MODELS	*Parameters*	Parameter Code	Estimate	SE	P
Brown trout density					
BT5a	*Intercept*	Bo	2.509	0.053	**0.0001**
	Zn toxicity units previous year	PZNTU	0.050	0.258	0.8458
	Cu toxicity units previous year	PCUTU	1.656	0.441	**0.0004**
	Peakflow previous year	PYPEAK	0.001	0.001	**0.4745**
	Interaction 1	PYPEAK*PZNTU	-0.001	0.001	0.0805
	Interaction 2	ZNTU*CUTU	-4.031	1.812	**0.0296**
BT15a	*Intercept*	Bo	2.535	0.042	**0.0001**
	Zn toxicity units previous year	PZNTU	-0.516	0.219	**0.0214**
	Cu toxicity units previous year	PCUTU	0.927	0.719	0.2023
	Cd toxicity units previous year	PCDTU	1.330	0.646	**0.0432**
	Interaction 1	PZNTU*PCDTU	-1.199	1.406	0.3970
	Interaction 2	PZNTU*PCUTU	-0.800	2.205	0.7178
Young of Year Count					
YOY5	*Intercept*	Bo	4.579	0.577	**0.0001**
	Zn toxicity units	ZNTU	23.104	3.911	**0.0001**
	Cu toxicity units	CUTU	-14.582	5.429	**0.0092**
	Peakflow previous year	PYPEAK	0.001	0.001	**0.0009**
	Interaction 1	PYPEAK*PZNTU	-0.013	0.002	**0.0001**
	Interaction 2	ZNTU*CUTU	-28.269	18.105	0.1233
Brown Trout Weight					
WT16	*Intercept*	Bo	4.184	0.229	**0.0001**
	Young of year count	Sqrt (#YOY)	-0.052	0.012	**0.0001**
	Zn toxicity units previous year	PZNTU	0.592	0.279	**0.0385**
	Peakflow previous year	PYPEAK	0.001	0.001	**0.0001**
	# Invertebrates	Ln(PREY)	0.282	0.078	**0.0006**
-	*Interaction 1*	PYPEAK*PZNTU	-0.001	0.001	**0.0012**

Table 3b. Best models (See Table 3a for details) using mine sites only.

BEST MODELS	*Parameters*	Parameter Code	Estimate	SE	P
Brown trout density					
BT15a	*Intercept*	Bo	2.655	0.081	**0.0001**
	Zn toxicity units previous year	PZNTU	-0.985	0.254	**0.0004**
	Cu toxicity units previous year	PCUTU	8.211	1.663	**0.0001**
	Cd toxicity units previous year	PCDTU	-3.709	0.169	**0.0030**
	Interaction 1	PZNTU*PCDTU	8.522	2.305	**0.0007**
	Interaction 2	PZNTU*PCUTU	-14.130	3.424	**0.0002**
Young of Year Count					
YOY3	*Intercept*	Bo	7.730	1.736	**0.0001**
	Zn toxicity units	ZNTU	0.993	5.752	0.8638
	Peakflow previous year	PYPEAK	0.001	0.002	0.9539
	Interaction 1	PYPEAK*PZNTU	-0.005	0.005	0.2435
YOY5	*Intercept*	Bo	7.952	2.341	**0.0016**
	Zn toxicity units	ZNTU	7.396	8.752	0.4032
	Cu toxicity units	CUTU	-18.360	12.744	0.1577
	Peakflow previous year	PYPEAK	0.001	0.001	0.7476
	Interaction 1	PYPEAK*PZNTU	-0.010	0.005	0.0764
	Interaction 2	ZNTU*CUTU	11.034	36.193	0.7621
YOY7	*Intercept*	Bo	7.922	1.587	**0.0001**
	Total toxicity units	TU_ALL	0.499	2.835	0.8610
	Peakflow previous year	PYPEAK	-0.001	0.001	0.9493
	Interaction 1	PYPEAK*TU_ALL	-0.003	0.003	0.2087
Brown Trout Weight					
WT16	*Intercept*	Bo	4.376	0.279	**0.0001**
	Young of year count	Sqrt (#YOY)	-0.069	0.015	**0.0001**
	Zn toxicity units previous year	PZNTU	0.283	0.409	0.4937
	Peakflow previous year	PYPEAK	0.001	0.001	0.4606
	# Invertebrates	Ln(PREY)	0.300	0.071	**0.0001**
	Interaction 1	PYPEAK*PZNTU	-0.001	0.001	0.1517
WT17	*Intercept*	Bo	4.768	0.322	**0.0001**
	Young of year count	Sqrt (#YOY)	-0.079	0.015	**0.0001**
	Zn toxicity units previous year	PZNTU	-0.347	0.493	0.4863
	Cu Toxicity units previous year	PCUTU	0.119	0.762	0.8768
	Peakflow previous year	PYPEAK	0.001	0.001	0.6660
	# Invertebrates	Ln(PREY)	0.209	0.080	**0.0130**
	Interaction 1	PZNTU*PCUTU	2.127	1.519	0.1700
	Interaction 2	PYPEAK*PZNTU	-0.001	0.001	0.2437
WT19	*Intercept*		4.231	0.311	**0.0001**
	Young of year count	Sqrt (#YOY)	-0.065	0.014	**0.0001**
	Zn toxicity units previous year	PZNTU	0.502	0.507	0.3279
	Habitat suitability low flow	HAB_SUIT (adult)	0.012	0.010	0.2106
	# Invertebrates	Ln(PREY)	0.300	0.075	**0.0003**
	Interaction 1	PZNTU*HAB_SUIT	-0.040	0.024	0.1067

Site Attainment					
SA5a	*Intercept*	Bo	-4.212	3.065	0.1693
(a negative sign on a parameter means that it contributes to site failure if it increases)	*Zn toxicity units previous year*	PZNTU	5.811	9.042	0.5204
	Cu toxicity units previous year	PCUTU	61.433	29.806	**0.0393**
	Peakflow previous year	PYPEAK	0.002	0.002	0.4135
	Interaction 1	PYPEAK*PZNTU	-0.008	0.007	0.2213
	Interaction 2	ZNTU*PCUTU	-71.497	70.673	0.3117

Table 4. Regression models from stepwise selection using data from mine sites only between the years 1997-2005. Means (ESTIMATE), standard errors (SE) and statistical significance (P) of parameters in the "best models" are given. Direction of association between responses and parameters is also shown in the sign of the estimate, where a negative sign indicates that the variable contributes to reductions in brown trout density, young of year counts, or weights. A negative sign also indicates variables that contribute to site "failure" (versus attainment). Partial R-squared values show the relative contributions of each variable (na = not available for logistic stepwise modeling for site attainment model). **Significant P-values are in boldface type.**

BEST MODELS	*Parameters*	Parameter Code	Estimate	SE	P	Partial R-sqr
Brown trout density	*Overall model significance*				**0.0001**	
	Intercept	Bo	3.485	0.182	**0.0001**	
(R-squared = 0.7510)	*Zn toxicity units current year*	ZNTU	-0.434	0.183	**0.0235**	0.024
	Peakflow previous year	PYPEAK	-0.001	0.001	**0.0067**	0.039
	Minimum flow previous year	PYMINFL	-0.004	0.003	0.0914	0.020
	Invertebrate abundance	Ln(PREY)	-0.091	0.027	**0.0023**	0.055
	Cu toxicity units previous year	PCUTU	2.348	0.441	**0.0001**	0.203
	Zn toxicity units previous year	PZNTU	-0.789	0.131	**0.0001**	0.410
Young of Year Count	*Overall model significance*				**0.0001**	
	Intercept	Bo	15.147	1.948	**0.0001**	
(R-squared = 0.6304)	*Zn toxicity units previous year*	PZNTU	-5.776	1.231	**0.0001**	0.352
	Cu toxicity units current year	CUTU	-17.406	4.733	**0.0007**	0.042
	Cu toxicity units previous year	PCUTU	18.110	5.077	**0.0010**	0.096
	Peakflow previous year	PYPEAK	-0.001	0.001	**0.0015**	0.056
	Habitat suitability at high flow	HAB_SUIT (fry)	-0.852	0.432	0.0559	0.038
	# invertebrates	Ln(PREY)	-0.603	0.297	**0.0495**	0.047
Brown Trout Weight	*Overall model significance*				**0.0001**	
	Intercept	Bo	4.480	0.229	**0.0001**	
	Young of year count	Sqrt(#YOY)	-0.064	0.015	**0.0001**	0.169
(R-squared = 0.5968)	*Zn toxicity units current year*	ZNTU	-0.492	0.162	**0.0044**	0.061
	Cu toxicity units previous year	PCUTU	0.995	0.513	0.0600	0.039
	# Invertebrates	Ln(PREY)	0.399	0.114	**0.0012**	0.296
	# Invertebrate Taxa	PREY_RICH	-0.011	0.007	0.0898	0.032

Site Attainment	*Overall model significance*				**0.0001**	
	Intercept	Bo	-23.330	7.510	**0.0019**	na
(R-squared = 0.5049)	*Copper toxicity units current year*	CUTU	50.610	17.627	**0.0041**	na
	Young of year count	Sqrt (#YOY)	1.814	0.556	**0.0011**	na
	Habitat suitability at low flow (adult)	HAB_SUIT (adult)	0.285	0.285	**0.0199**	na

Table 5. First four PCA components, variables describing each component, and percent of explained variance.

Component	% of Variance	Cumulative %
1. Physical Habitat and Average May and June cfs (current and previous year)	29.285	29.285
2. Macroinvertebrates	17.366	46.651
3. Current year Cu, Cd and Zn Toxicity Units and current year base flow	14.629	61.279
4. Previous year Cu, Cd and Zn Toxicity Units and previous year base flow	14.231	75.511

Table 6. List of variables and their contributions (associations) with DFA axis for model DFA_{PCA}.

Variable	Contribution to Function
Ln(zinc toxicity units previous year)	-0.339
Ave. May-June cfs previous year	-0.281
Ln (baseflow previous year)	-0.242
Ln(zinc toxicity current year)	-0.180
Ave. May-June cfs	-0.177
Ln(cadmium toxicity units previous year)	-0.141
Ln (baseflow)	-0.067
Ln(cadmium toxicity units current year)	-0.060
Ln (habitat suitability =0.75 for adults at higher flow)	-0.018
Ln(copper toxicity units current year)	-0.014
Ln (habitat suitability =0.75 for adults at low flow)	0.023
Ln(copper toxicity units previous year)	0.075
EPT richness	0.111
Ln (habitat suitability =0.75 for fry at low flow)	0.153
Ln (habitat suitability =0.75 for fry at higher flow)	0.175

Table 7. List of variables and their contributions (associations) with DFA axis model DFA_{YOY}

Variable	**Contribution to Function**
Ln(zinc toxicity units previous year)	-0.305
Ave. May-June cfs previous year	-0.253
Ln (baseflow previous year)	-0.218
Ln(zinc toxicity units current year)	-0.162
Ave. May-June cfs current year	-0.159
Ln (baseflow)	-0.061
Ln(copper toxicity current year)	-0.013
Ln(habitat suitability = 0.75 adults low flow)	0.021
Ln (copper toxicity units previous year)	0.068
EPT taxa richness	0.100
Count of young of year brown trout	0.527

Table 8. Canonical discriminant function analysis results showing how linear combinations of environmental variables separate mine sites in each year (1997-2005). The first canonical variable explained 98.4% of the total variation in the dataset and separated years based on previous year peakflow in the positive direction, and previous year minimum flow in the negative direction. Note that 2004, the year where attainment was reduced at multiple sites, does not “stand out” in that the class mean values is not on either the positive or negative extreme of canonical axis 1 or 2. Figure 1 shows this.

OVERALL CDA MODEL SIGNIFICANCE

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.001043	7.29	64	173.76	<.0001

PRIMARY CDA CANONINCAL AXES (TOP 2) and their SIGNIFICANCE

	Eigenvalue	Cumulative variation explained	F Value	P-Value
1	26.1866	0.6074	7.29	<.0001
2	7.8254	0.8391	4.77	<.0001

Total Canonical Structure

(Correlations of each variable with the two Canonical Axes)

Variable	AXIS 1 (explains 61% variation)	AXIS 2 (explains 23%)
Young of year count	0.2006	-0.6713
Prev. Zinc TU’s	-0.0779	0.7765
Prev. Copper TU’s	-0.0185	0.2085
Prev. Cadmium TU’s	-0.0829	0.4470
Prev. Peakflow	-0.1657	0.6951
Prev. Minimum flow	0.9179	0.1937
Adult habitat	-0.1094	0.0951
Invert Abundance	0.1160	-0.1031
Invert richness	0.0665	-0.2214
Fry habitat	-0.1123	0.0132

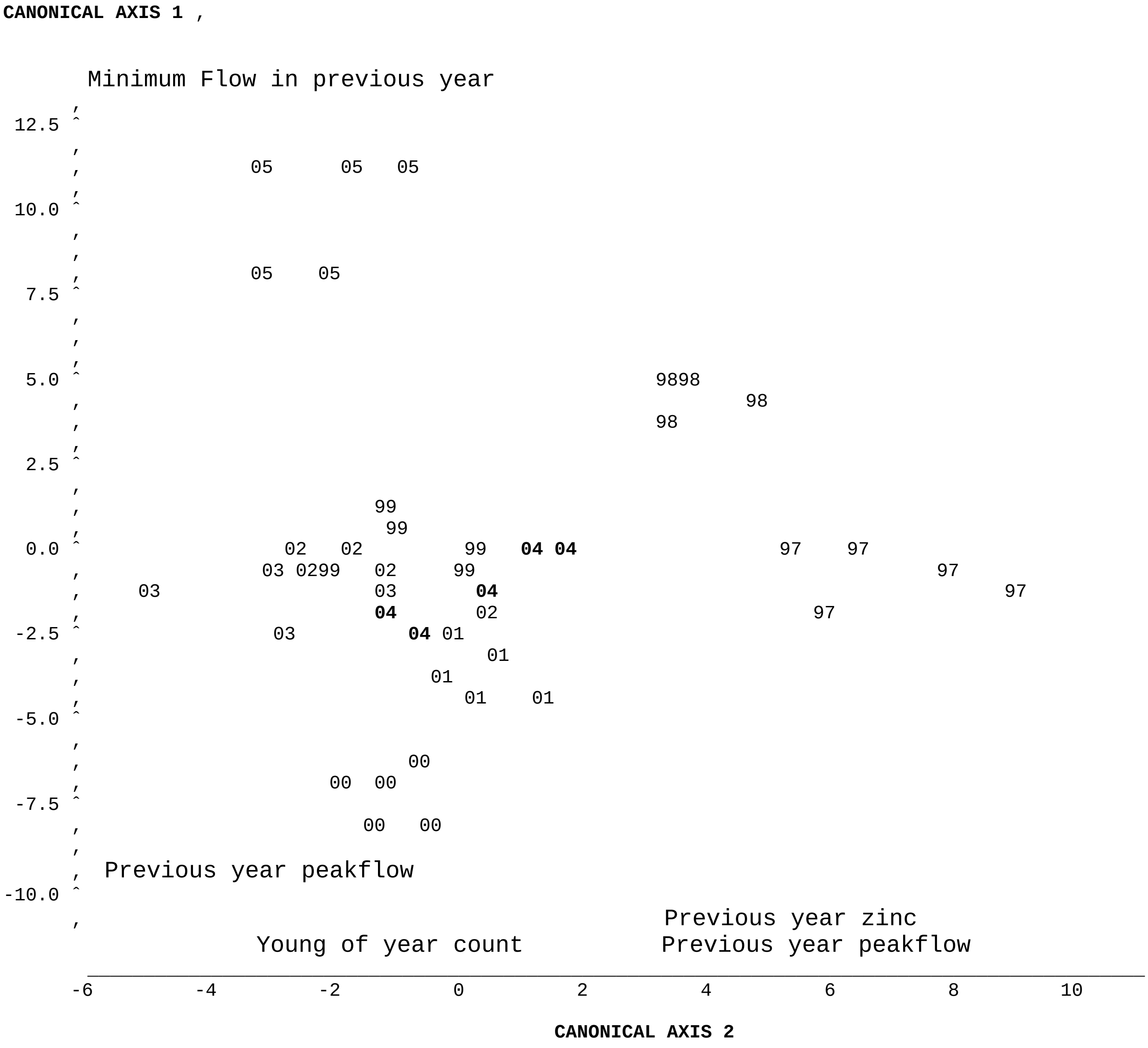
Class Means on Canonical Variables

(average values across sites for each year-these are plotted in Figure 1)

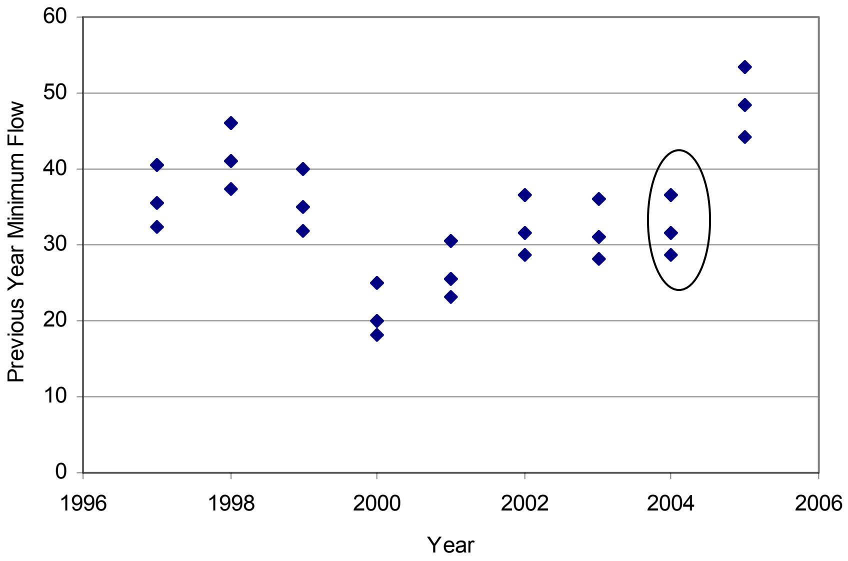
YEAR	Can1	Can2
1997	-0.836	6.090
1998	4.376	3.620
1999	0.078	-1.144
2000	-7.263	-1.281
2001	-3.467	0.159
2002	-0.412	-1.787
2003	-1.352	-3.097
2004	**-1.153**	**-0.121**
2005	10.028	-2.441

FIGURE 1. CDA results for mine sites years 1997-2005. Primary variables describing (strongly correlated with) canonical axes 1 and 2 are presented in parentheses and are also found in Table 8.

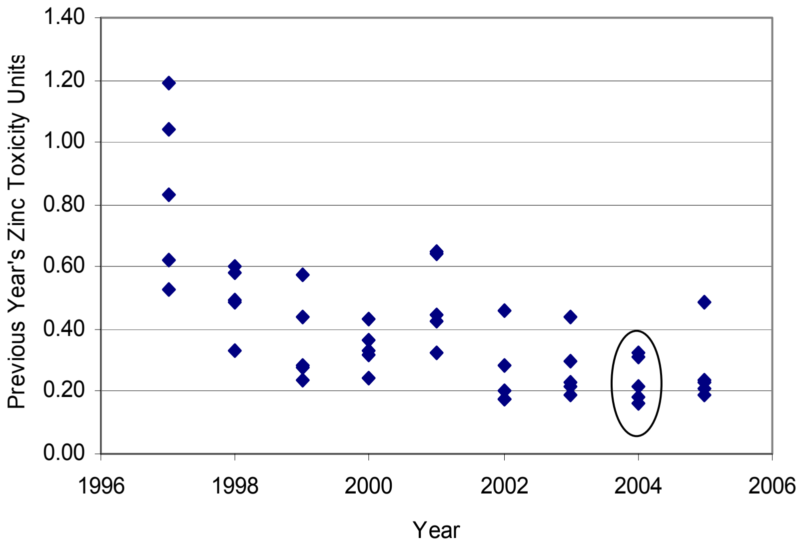
Plot of Can1*Can2 for years 1997-2005. Numbers indicate sample sites in each year

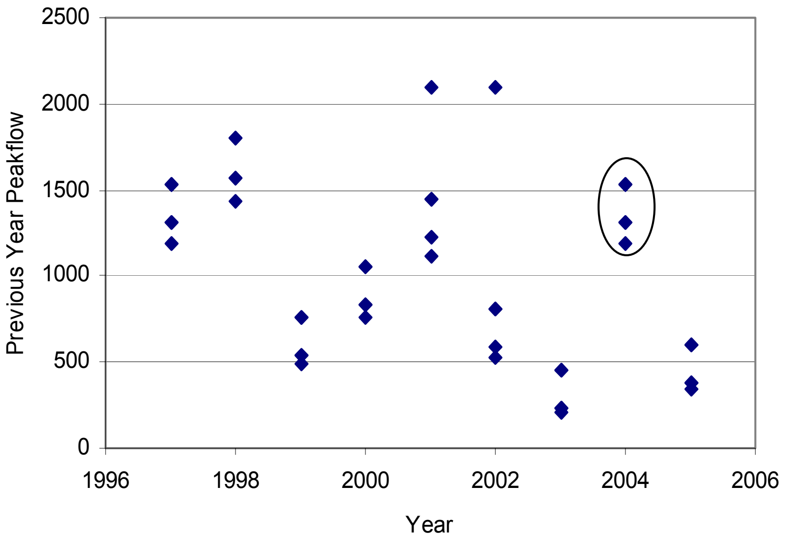


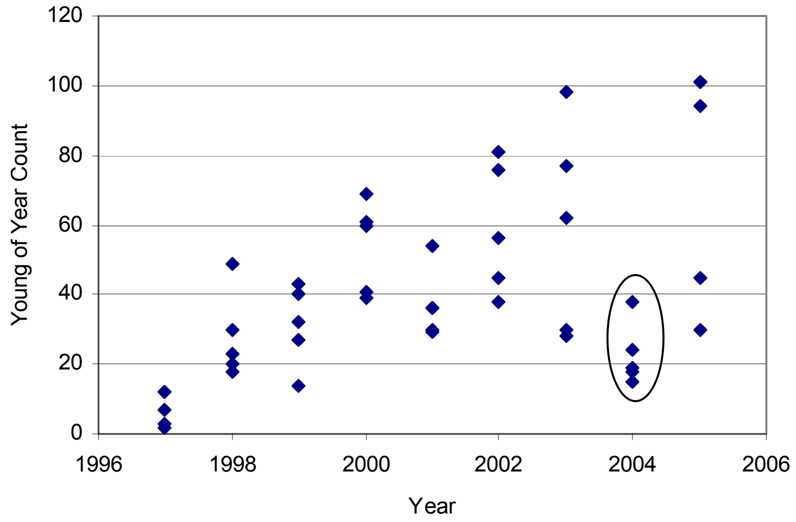
Figures 2a-2e. Graphs showing how variables selected as being “important” in teasing years 1997-2005 apart in canonical discriminant analysis differ across years. These graphs demonstrate how the year 2004 differed from other years to determine what might have influenced non-attainment in this particular year. Points represent all mine-sites combined for each year.

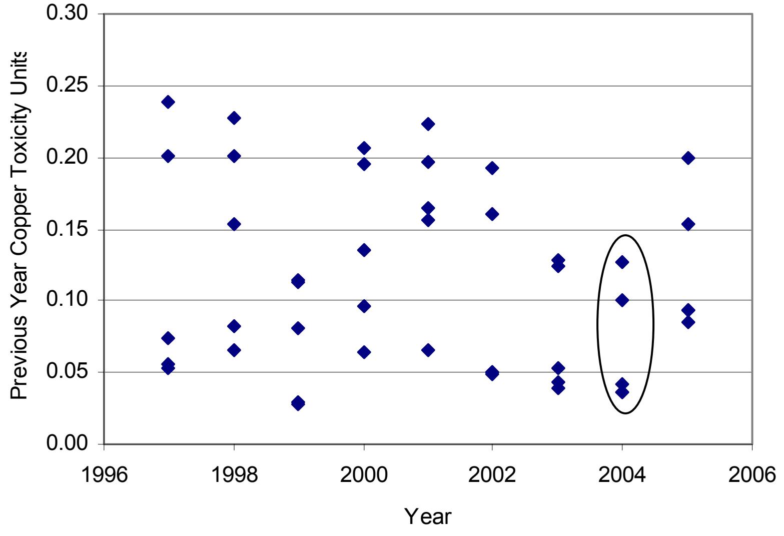


(More on next page)


Previous Year's Zinc Toxicity Units
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1996
1998
2000
2002
2004
2006
Year


Previous Year Peakflow
2500
2000
1500
1000
500
0
1996
1998
2000
2002
2004
2006
Year


Young of Year Count
120
100
80
60
40
20
0
1996
1998
2000
2002
2004
2006
Year


Previous Year Copper Toxicity Units
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1996
1998
2000
2002
2004
2006
Year

Appendix A. Data Dictionary for Investigative Study Metrics: only data from 1997-2005 were used in analyses. Highlighted data were those chosen to be included in the "filtered" dataset.

Equations for LC50's for brown trout are from:

Brinkman, S.F., and D. L. Hansen. 2004. Federal Aid in Fish and Wildlife Restoration. Job Progress Report F-243R-11. Colorado Division of Wildlife, Fort Collins, CO, USA.

Davies, P. H., S. F. Brinkman and D. L. Hansen. 2003. Federal Aid in Fish and Wildlife Restoration. Job Progress Report F-243R-10. Colorado Division of Wildlife, Fort Collins, CO, USA.

Data Dictionary for Investigative Study Metrics

Brown Trout statistics- All DOW data

1). Column Name: **popesta** Column Description: *LOC pop est w/o YOY*
Definition: Brown trout population estimates that do not include young of the year size class <130 using 2 pass empirical formula developed by Seber-LeCren, 1967.

2). Column Name: **popestb** Column Description: *LOC pop est w/ YOY*
Definition: Brown trout population estimates that include young of the year using 2 pass empirical formula developed by Seber-LeCren, 1967.

3). Column Name: **popestc** Column Description: *LOC pop est YOY only*
Definition: Brown trout population estimates that only include young of the year size class <130 using 2 pass empirical formula developed by Seber-LeCren, 1967.

4). Column Name: **count1** Column Description: *Count of 1yr*
Definition: Count of 1 year brown trout based on length (specified by Woodling, varies year to year)

5). Column Name: **count2** Column Description: *Count of 2yr*
Definition: count of 2 year brown trout based on length (specified by Woodling, varies year to year)

6). Column Name: **countad1** Column Description: *Count of Adults*
Definition: Count of adult brown trout based on length (specified by Woodling, varies year to year)

7). Column Name: **countAll** Column Description: *Count All*
Definition: Count of all brown trout

8) Column Name: **countYOY** Column Description: *Count YOY*
Definition: Count of young of the year browns (<140)

9). Column Name: **countad2** Column Description: *Count >139*
Definition: Count of adult brown trout (>139mm)

10). Column Name: **popest1yr** Column Description: *Popest 1yr*
Definition: Population estimate of 1 yr olds by length (specified by Woodling, varies year to year). Using 2 pass empirical formula developed by Seber-LeCren, 1967. The gaps are where there was a negative population estimate due to more small fish caught on 2nd pass rather than 1st pass (does not allow for a regression).

11). Column Name: **popest2yr** Column Description: *Popest 2yr*
Definition: Population estimate of 2 yr olds by length (specified by Woodling, varies year to year). Using 2 pass empirical formula developed by Seber-LeCren, 1967. The gaps are where there was a negative population estimate due to more small fish caught on 2nd pass rather than 1st pass (does not allow for a regression).

12). Column Name: **popestadult** Column Description: *Popest Adult*
Definition: Population estimate of adult trout by length (specified by Woodling, varies year to year). Using 2 pass empirical formula developed by Seber-LeCren, 1967.

13). Column Name: **AveLen** Column Description: *Ave Length*
Definition: Average brown trout length in millimeters

14). Column Name: **AveW** Column Description: *Ave Weight*
Definition: Average brown trout weight in grams

15). Column Name: **AveWr** Column Description: *Ave Wr*
Definition: Average brown trout relative weight (ratio O/E). This measurement provides an assessment of the body condition of brown trout in metal contaminated reaches versus non contaminated reaches.
$\log_{10}\underline{Wcl}(g)=-4.8052+2.9186*\log_{10}TL(mm)$

Water Quality, Flow and Physical Habitat statistics
1). Column Name: **Totalhar_D** Column Description: *TOTAL_HARD*
Definition: Total measured hardness (DOW data)

2). Column Name: **Totalhar_D2** Column Description: *TOTAL_HARD previous*
Definition: Total measured hardness for previous year (DOW data)

3). Column Name: **AveHar_V** Column Description: *Ave Hard*
Definition: Average hardness for the entire year. Used all available data for calcium and magnesium for the year to calculate hardness and then took an average hardness for the year. (Viacom data)
Hardness equation: hardness= 2.5 *(dissolved calcium µg/L) + 4.1 *(dissolved magnesium µg/L)

4). Column Name: **AveHar_V2** Column Description: *Ave Hard previous*
Definition: Average hardness for the entire year. Used all available data for calcium and magnesium for the year to calculate hardness and then took an average hardness for the year. (Viacom data)
Hardness equation: hardness= 2.5 *(dissolved calcium µg/L) + 4.1 *(dissolved magnesium µg/L)

5). Column Name: **Har85_V** Column Description: *85th Hard*
Definition: Used all available data for calcium and magnesium for the year to calculate hardness using the above mentioned equation and then took the 85th percentile of those values for the year. (Viacom data)

6). Column Name: **Har85_V2** Column Description: *85th Hard previous*

Definition: Used all available data for calcium and magnesium for the previous year to calculate hardness using the above mentioned equation and then took the 85th percentile of those values for the previous year. (Viacom data)

7). Column Name: **Har15_V** Column Description: *15th Hard*
Definition: Used all available data for calcium and magnesium for the year to calculate hardness using the above mentioned equation and then took the 15th percentile of those values for the year. (Viacom data)

8). Column Name: **Har15_V2** Column Description: *15th Hard previous*
Definition: Used all available data for calcium and magnesium for the previous year to calculate hardness using the above mentioned equation and then took the 15th percentile of those values for the previous year. (Viacom data)

9). Column Name: **Cu_D** Column Description: *CU_D*
Definition: Measured dissolved copper ug/L. (DOW data)

10). Column Name: **Cu_D2** Column Description: *CU_D previous*
Definition: Measured dissolved copper ug/L for previous year (DOW data)

11). Column Name: **Cutu_D** Column Description: *Cu toxicity*
Definition: Copper toxicity units. Calculated by using the following equation:
Toxicity Unit = (Dissolved Cu μg/L) / ($e^{0.9422*\ln \text{(hardness)}-0.2022}$) (DOW data)

12). Column Name: **Cutu_D2** Column Description: *Cu toxicity previous*
Definition: Copper toxicity units for the previous year. Calculated by using the following equation:
Copper Toxicity Unit = (Dissolved Cu μg/L) / ($e^{0.9422*\ln \text{(hardness)}-0.2022}$)(DOW data)

13). Column Name: **AveCu_V** Column Description: *Ave Cu ug/L*
Definition: Average taken of all measured dissolved copper μg/L taken throughout the year. (Viacom data)

14). Column Name: **AveCu_V2** Column Description: *Ave Cu ug/L previous*
Definition: Average taken of all measured dissolved copper μg/L taken throughout the previous year. (Viacom data)

15). Column Name: **Cu85_V** Column Description: *Cu85th*
Definition: 85th percentile of all measured dissolved copper μg/L taken throughout the year. (Viacom data)

16). Column Name: **Cu85_V2** Column Description: *Cu85th previous*
Definition: 85th percentile of all measured dissolved copper μg/L for the previous year (Viacom data)

17). Column Name: **Zn_D** Column Description: *ZN_D*
Definition: Measured dissolved zinc μg/L (DOW data)

18). Column Name: **Zn_D2** Column Description: *ZN_D previous*
Definition: Measured dissolved zinc μg/L for previous year (DOW data)

19). Column Name: **Zntu_D** Column Description: *Zn toxicity*
Definition: Zinc toxicity units. Calculated by using the following equation:
Zinc Toxicity Unit = $(e^{1.062*\ln\text{ (hardness)}+2.151})$ (DOW data)

20). Column Name: **Zntu_D2** Column Description: *Zn toxicity previous*
Definition: Zinc toxicity units for previous year. Calculated by using the following equation:
Zinc Toxicity Unit = $(e^{1.062*\ln\text{ (hardness)}+2.151})$ (DOW data)

21). Column Name: **AveZn_V** Column Description: *Ave Zn ug/L*
Definition: Average taken of all measured dissolved zinc µg/L taken throughout the year. (Viacom data)

22). Column Name: **AveZn_V2** Column Description: *Ave Zn ug/L previous*
Definition: Average taken of all measured dissolved zinc µg/L taken throughout the previous year. (Viacom data)

24). Column Name: **Zn85_V** Column Description: *Zn85th*
Definition: 85th percentile of all measured dissolved zinc µg/L taken throughout the year. (Viacom data)

25). Column Name: **Zn85_V2** Column Description: *Zn85th previous*
Definition: 85th percentile of all measured dissolved zinc µg/L taken throughout the previous year. (Viacom data)

26). Column Name: **Cd_D** Column Description: *CD_D*
Definition: Measured dissolved cadmium µg/L. (using DOW data)

27). Column Name: **Cd_D2** Column Description: *CD_D previous*
Definition: Measured dissolved cadmium µg/L for previous year. (using DOW data)

28). Column Name: **Cdtu_D** Column Description: *Cd toxicity*
Definition: Cadmium toxicity units. Calculated by using the following equation:
Cadmium Toxicity Unit = $(e^{1.258*\ln\text{ (hardness)}-3.999})$ (DOW data)

29). Column Name: **Cdtu_D2** Column Description: *Cd toxicity previous*
Definition: Cadmium toxicity units for previous year. Calculated by using the following equation:
Cadmium Toxicity Unit = $(e^{1.258*\ln\text{ (hardness)}-3.999})$ (DOW data)

30). Column Name: **AveCd_V** Column Description: *Ave Cd ug/L*
Definition: Average taken of all measured dissolved cadmium µg/L taken throughout the year. (Viacom data)

31). Column Name: **AveCd_V2** Column Description: *Ave Cd ug/L previous*
Definition: Average taken of all measured dissolved cadmium µg/L taken throughout the previous year (Viacom data)

32). Column Name: **Cd85_V** Column Description: *Cd85th*
Definition: 85th percentile of all measured dissolved cadmium µg/L taken throughout the year (Viacom data)

34). Column Name: **Cd85_V2** Column Description: *Cd85th previous*
Definition: 85th percentile of all measured dissolved cadmium µg/L taken throughout the previous year. (Viacom data)

35). Column Name: **Tutotal_D** Column Description: *Tutotal*
Definition: Total toxicity unit = copper toxicity unit + zinc toxicity unit + cadmium toxicity unit. All toxicity units calculated using above stated equations. (DOW data)

36). Column Name: **Tutotal_D2** Column Description: *Tutotal previous*
Definition: Total toxicity unit = copper toxicity unit + zinc toxicity unit + cadmium toxicity unit; for previous year. All toxicity units calculated using above stated equations. (DOW data)

37). Column Name: **cuzntu_D** Column Description: *cuzntu*
Definition: Summation of copper toxicity unit and zinc toxicity unit (DOW data)

38). Column Name: **cuzntu_D2** Column Description: *cuzntu previous*
Definition: Summation of copper toxicity unit and zinc toxicity unit for previous year (DOW data)

39). Column Name: **cucdtu_D** Column Description: *cucdtu*
Definition: Summation of copper toxicity unit and cadmium toxicity unit. (DOW data)

40). Column Name: **cucdtu_D2** Column Description: *cucdtu previous*
Definition: Summation of copper toxicity unit and cadmium toxicity unit for previous year. (DOW data)

41). Column Name: **zncdtu_D** Column Description: *zncdtu*
Definition: Summation of zinc toxicity unit and cadmium toxicity unit. (DOW data)

42). Column Name: **zncdtu_D2** Column Description: *zncdtu previous*
Definition: Summation of zinc toxicity unit and cadmium toxicity unit for previous year. (DOW data)

43). Column Name: **AveSpZn_V** Column Description: *Ave Spring Zn ug/L*
Definition: Average of March and April dissolved zinc concentrations µg/L. (Viacom data)

44). Column Name: **AveSpZn_V2** Column Description: *Ave Spring Zn ug/L previous*
Definition: Average of March and April dissolved zinc concentrations µg/L for previous year (Viacom data)

45). Column Name: **AveSpCu_V** Column Description: *Ave Spring Cu ug/L*
Definition: Average of March and April dissolved copper concentrations µg/L (Viacom data)

46). Column Name: **AveSpCu_V2** Column Description: *Ave Spring Cu ug/L previous*
Definition: Average of March and April dissolved copper concentrations µg/L for previous year (Viacom data)

47). Column Name: **AveSpCd_V** Column Description: *Ave Spring Cd ug/L*
Definition: Average of March and April dissolved cadmium concentrations µg/L (Viacom data)

48). Column Name: **AveSpCd_V2** Column Description: *Ave Spring Cd ug/L previous*
Definition: Average of March and April dissolved cadmium concentrations µg/L for previous year (Viacom data)

49). Column Name: **AveCFS1** Column Description: *AvgOfCFS (11-2)*
Definition: Average cubic feet per second (cfs) for November thru February low flow period.

50). Column Name: **AveCFS1p** Column Description: *AvgOfCFS (11-2) previous*
Definition: Average cubic feet per second (cfs) for November thru February low flow period of the previous year.

51). Column Name: **AveCFS2** Column Description: *AvgOfCFS (10-2)*
Definition: Average cubic feet per second (cfs) for October thru February low flow period.

52). Column Name: **AveCFS2p** Column Description: *AvgOfCFS (10-2) previous*
Definition: Average cubic feet per second (cfs) for October thru February low flow period of the previous year.

53). Column Name: **AveCFS3** Column Description: *AvgOfCFS (Spring)*
Definition: Average cubic feet per second (cfs) of spring flows (March-June).

54). Column Name: **AveCFS3p** Column Description: *AvgOfCFS (Spring) previous*
Definition: Average cubic feet per second (cfs) of spring flows for the previous year (March-June).

55). Column Name: **bflow** Column Description: *baseflow*
Definition: Base flow in cubic feet per second (cfs) is calculated using the previous year average monthly flow for November and December and the current year average monthly flow for January and February; then all the monthly averages are averaged into an average base flow.

56). Column Name: **bflow_p** Column Description: *prev_baseflow*
Definition: Base flow in cubic feet per second (cfs) for previous year. Base flow is calculated using the previous year average monthly flow for November and December and the current year average monthly flow for January and February; then all the monthly averages are averaged into an average base flow.

57). Column Name: **maxcfs** Column Description: *maxcfs*
Definition: Maximum cubic feet per second (cfs) for the current year

58). Column Name: **maxcfs_p** Column Description: *prev_maxcfs*
Definition: Maximum cubic feet per second (cfs) for the previous year

59). Column Name: **mincfs** Column Description: *mincfs*
Definition: Minimum cubic feet per second (cfs) for the current year

60). Column Name: **mincfs_p** Column Description: *prev_mincfs*
Definition: Minimum cubic feet per second (cfs) for the previous year

61). Column Name: **phab0** Column Description: *phab0*

Definition: Weighted Useable Area (WUA) for cfs greater than 0 for low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using USFWS HSI adult curves.

62). Column Name: **phab25** Column Description: *phab25*
Definition: Weighted Useable Area (WUA) for cfs greater than 25 for low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using USFWS HSI adult curves.

63). Column Name: **phab50** Column Description: *phab50*
Definition: Weighted Useable Area (WUA) for cfs greater than 50 for low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using USFWS HSI adult curves.

64). Column Name: **phab75** Column Description: *phab75*
Definition: Weighted Useable Area (WUA) for cfs greater than 75 for low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using USFWS HSI adult curves.

65). Column Name: **hphab0** Column Description: *hphab0*
Definition: Weighted Useable Area (WUA) for cfs greater than 0 for high flow period (average May and June), using the RHABSIM model and using USFWS HSI adult curves.

66). Column Name: **hphab25** Column Description: *hphab25*
Definition: Weighted Useable Area (WUA) for cfs greater than 25 for high flow period (average May and June), using the RHABSIM model and using USFWS HSI adult curves.

67). Column Name: **hphab50** Column Description: *hphab50*
Definition: Weighted Useable Area (WUA) for cfs greater than 50 for high flow period (average May and June), using the RHABSIM model and using USFWS HSI adult curves.

68). Column Name: **hphab75** Column Description: *hphab75*
Definition: Weighted Useable Area (WUA) for cfs greater than 75 for high flow period (average May and June), using the RHABSIM model and using USFWS HSI adult curves.

69). Column Name: **phab0a** Column Description: *phab0a*
Definition: Weighted Useable Area (WUA) for cfs greater than 0 for the low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using Nehring HSI adult curves.

70). Column Name: **phab25a** Column Description: *phab25a*
Definition: Weighted Useable Area (WUA) for cfs greater than 25 for the low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using Nehring HSI adult curves.

71). Column Name: **phab50a** Column Description: *phab50a*
Definition: Weighted Useable Area (WUA) for cfs greater than 50 for the low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using Nehring HSI adult curves.

72). Column Name: **phab75a** Column Description: *phab75a*
Definition: Weighted Useable Area (WUA) for cfs greater than 75 for the low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using Nehring HSI adult curves.

73). Column Name: **hphab0a** Column Description: *hphab0a*
Definition: Weighted Useable Area (WUA) for cfs greater than 0 for the high flow period (average of May and June), using the RHABSIM model and using Nehring HSI adult curves.

74). Column Name: **hphab25a** Column Description: *hphab25a*
Definition: Weighted Useable Area (WUA) for cfs greater than 25 for the high flow period (average of May and June), using the RHABSIM model and using Nehring HSI adult curves.

75). Column Name: **hphab50a** Column Description: *hphab50a*
Definition: Weighted Useable Area (WUA) for cfs greater than 50 for the high flow period (average of May and June), using the RHABSIM model and using Nehring HSI adult curves.

76). Column Name: **hphab75a** Column Description: *hphab75a*
Definition: Weighted Useable Area (WUA) for cfs greater than 75 for the high flow period (average of May and June), using the RHABSIM model and using Nehring HSI adult curves.

77). Column Name: **phab0f** Column Description: *phab0f*
Definition: Weighted Useable Area (WUA) for cfs greater than 0 for the low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using Nehring HSI fry curves.

78). Column Name: **phab25f** Column Description: *phab25f*
Definition: Weighted Useable Area (WUA) for cfs greater than 25 for the low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using Nehring HSI fry curves.

79). Column Name: **phab50f** Column Description: *phab50f*
Definition: Weighted Useable Area (WUA) for cfs greater than 50 for the low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using Nehring HSI fry curves.

80). Column Name: **phab75f** Column Description: *phab75f*
Definition: Weighted Useable Area (WUA) for cfs greater than 75 for the low flow period (average of November and December of the previous year and January and February of the current year), using the RHABSIM model and using Nehring HSI fry curves.

81). Column Name: **hphab0f** Column Description: *hphab0f*
Definition: Weighted Useable Area (WUA) for cfs greater than 0 for the high flow period (average of May and June), using the RHABSIM model and using Nehring HSI fry curves.

82). Column Name: **hphab25f** Column Description: *hphab25f*
Definition: Weighted Useable Area (WUA) for cfs greater than 25 for the high flow period (average of May and June), using the RHABSIM model and using Nehring HSI fry curves.

83). Column Name: **hphab50f** Column Description: *hphab50f*
Definition: Weighted Useable Area (WUA) for cfs greater than 50 for the high flow period (average of May and June), using the RHABSIM model and using Nehring HSI fry curves.

84). Column Name: **hphab75f** Column Description: *hphab75f*
Definition: Weighted Useable Area (WUA) for cfs greater than 75 for the high flow period (average of May and June), using the RHABSIM model and using Nehring HSI fry curves.

85). Column Name: **minesite** Column Description: *minesite*
Definition: This column is used to filter the mine sites from the reference stations. 1 = mine 0 = reference

86). Column Name: **crit_fsh** Column Description: *crit_fsh*
Definition: This column used to filter the years in which the sites passed the biological criteria for brown trout. 0= failed, 1=passed, 2= reference or year not used in assessment.

87). Column Name: **Zntu_V** Column Description: *Viacom Zntu*
Definition: Zinc toxicity units calculated using Viacom spring (March, April) average for dissolved zinc and hardness.
Zinc Toxicity Unit = $(e^{1.062*\ln(\text{hardness})+2.151)})$

88). Column Name: **Zntu_V2** Column Description: *Viacom Zntu previous*
Definition: Zinc toxicity units calculated using Viacom spring (March, April) average for dissolved zinc and hardness for previous year. Zinc Toxicity Unit = $(e^{1.062*\ln(\text{hardness})+2.151})$

89). Column Name: **Cutu_V** Column Description: *Viacom Cutu*
Definition: Copper toxicity units using Viacom spring (March, April) average for dissolved copper and hardness
Copper Toxicity Unit = (Dissolved Cu μg/L) / $(e^{0.9422*\ln(\text{hardness})-0.2022})$

90). Column Name: **Cutu_V2** Column Description: *Viacom Cutu previous*
Definition: Copper toxicity units calculated using Viacom spring (March, April) average for dissolved copper and hardness for previous year. Copper Toxicity Unit = (Dissolved Cu μg/L) / $(e^{0.9422*\ln(\text{hardness})-0.2022})$

91). Column Name: **Cdtu_V** Column Description: *Viacom Cdtu*
Definition: Cadmium toxicity units calculated using Viacom spring (March, April) average for dissolved cadmium and hardness. Cadmium Toxicity Unit = $(e^{1.258*\ln(\text{hardness})-3.999})$

92). Column Name: **Cdtu_V2** Column Description: *Viacom Cdtu previous*
Definition: Cadmium toxicity units using Viacom spring (March, April) average for dissolved cadmium and hardness for previous year. Cadmium Toxicity Unit = $(e^{1.258*\ln(\text{hardness})-3.999})$

93). Column Name: **SpAveCu_DV** Column Description: *Ave Spring Comb Cu*
Definition: Average of Viacom's spring average data for dissolved copper µg/L (March and April) and the measured concentrations of CDOW's spring data for dissolved copper µg/L.

94). Column Name: **SpAveCu_DV2** Column Description: *Ave Spring Comb Cu previous*
Definition: Average of Viacom’s spring average data for dissolved copper µg/L (March and April) of the previous year and the measured concentrations of CDOW’s spring data for dissolved copper µg/L for the previous year.

95). Column Name: **SpAveZn_DV** Column Description: *Ave Spring Comb Zn*
Definition: Average of Viacom’s spring average data for dissolved zinc µg/L (March and April) and the measured concentrations of CDOW’s spring data for dissolved zinc µg/L.

96). Column Name: **SpAveZn_DV2** Column Description: *Ave Spring Comb Zn previous*
Definition: Average of Viacom’s spring average data for dissolved zinc µg/L (March and April) of the previous year and the measured concentrations of CDOW’s spring data for dissolved zinc µg/L for the previous year.

97). Column Name: **SpAveCd_DV** Column Description: *Ave Spring Comb Cd*
Definition: Average of Viacom’s spring average data for dissolved cadmium µg/L (March and April) and the measured concentrations of CDOW’s spring data for dissolved cadmium µg/L.

98). Column Name: **SpAveCd_DV2** Column Description: *Ave Spring Comb Cd previous*
Definition: : Average of Viacom’s spring average data for dissolved cadmium µg/L (March and April) of the previous year and the measured concentrations of CDOW’s spring data for dissolved cadmium µg/L for the previous year.

99). Column Name: **Sp85Cu_DV** Column Description: *Spring 85th Comb Cu*
Definition: 85th percentile of Viacom’s spring average and CDOW measured concentrations for spring dissolved copper µg/L.

100). Column Name: **Sp85Cu_DV2** Column Description: *Spring 85th Comb Cu previous*
Definition: 85th percentile of Viacom’s spring average and CDOW measured concentrations for spring dissolved copper µg/L for previous year.

101). Column Name: **Sp85Zn_DV** Column Description: *Spring 85th Comb Zn*
Definition: 85th percentile of Viacom’s spring average and CDOW measured concentrations for spring dissolved zinc µg/L.

102). Column Name: **SP85Zn_DV2** Column Description: *Spring 85th Comb Zn previous*
Definition: 85th percentile of Viacom and CDOW values for spring dissolved zinc for previous year.

103). Column Name: **SP85Cd_DV** Column Description: *Spring 85th Comb Cd*
Definition: 85th percentile of Viacom and CDOW values for spring dissolved cadmium.

104). Column Name: **SP85Cd_DV2** Column Description: *Spring 85th Comb Cd previous*

Definition: 85th percentile of Viacom and CDOW values for spring dissolved cadmium for previous year.

105). Column Name: **Zntu85_DV** Column Description: *85th Zntu comb*
Definition: 85th percentile of the zinc toxicity units for Viacom and CDOW's data combined

106). Column Name: **Cutu85_DV** Column Description: *85th Cutu comb*
Definition: 85th percentile of the copper toxicity units for Viacom and CDOW's data combined

107). Column Name: **Cdtu85_DV** Column Description: *85th Cdtu comb*
Definition: 85th percentile of the zinc toxicity units for Viacom and CDOW's data combined

108). Column Name: **Zntu85_DV2** Column Description: *85th Zntu comb previous*
Definition: 85th percentile of the zinc toxicity units for Viacom and CDOW's data combined for the previous year.

109). Column Name: **Cutu85_DV2** Column Description: *85th Cutu comb previous*
Definition: 85th percentile of the copper toxicity units for Viacom and CDOW's data combined for the previous year.

110). Column Name: **Cdtu85_DV2** Column Description: *85th Cdtu comb previous*
Definition: 85th percentile of the zinc toxicity units for Viacom and CDOW's data combined for the previous year.

Macroinvertebrate Statistics
1). Column Name: **Ttaxa**
Definition: Total number of taxa (excluding chironomidae) (DOW)

2). Column Name: **Ephem_D**
Definition: total number of mayfly taxa (DOW data)

3). Column Name: **Plecoptera_D**
Definition: Total number of stonefly taxa (DOW data)

4). Column Name: **Trichoptera_D**
Definition: Total number of caddisfly taxa (DOW data)

5). Column Name: **MI_D**
Definition: Total number of metal intolerant taxa. The taxa used in the metric for metal intolerance "were mayflies in the genra *Cinygmula, Drunella, Epeorus, Paraleptophlebia*, and *Rhithrogena*; stoneflies in the genera *Skwala, Suwallia,* and *Sweltsa*; and caddisflies in the genus *Rhyacophila*; and true flies in the genus *Pericoma*" (Fore, 2001). (DOW data)

6). Column Name: **Clingers_D**
Definition: Total number of clinger taxa. The taxa defined as clingers were determined using "An Introduction to Aquatic Insects of North America, Thrid Edition" Edited by R.W. Merritt and K.W. Cummins. (DOW data)

7). Column Name: **Hept_D**
Definition: Percent of heptageniid mayflies (DOW data)

8). Column Name: **mIBI_D**
Definition: Macroinvertebrate index of biological integrity based on Leska Fore, 2001 report criteria, using the above metrics.

9). Column Name: **mTotal**
Definition: total count of organisms collected

10). Column Name: **ntaxa**
Definition: Total number of taxa

11). Column Name: **perEphem**
Definition: Percent of Ephemeroptera

12). Column Name: **nephtaxa**
Definition: Total number of Ephemeroptera taxa

13). Column Name: **EPT-tr**
Definition: EPT taxa richness calculated by the sum of Ephemeroptera taxa, Plecoptera taxa and Trichoptera taxa

Appendix B. How to Interpret Results

1. **AIC Regression models:**

    5. **What variables are in the "best models" (AIC value < 2) and how often does each variable appear in these "best" models (identified in Tables 2a and 2b)?**

        i. Variables that appear in multiple "best" models are more influential than variables that only appear occasionally in best models, or appear only in "lesser" models.

        ii. Variables in "best models" may not be statistically significant (p-values for each variable given in **Tables 3a and 3b**). However, their inclusion in a "best model" makes the overall model "strong" (e.g., low AIC value, high R-squared value, significant p-value)

    6. **Why do some of the lesser models have a stronger significance (lower p-value) for the overall model, and/or a better R-squared value) than the "best models" that AIC selected?**

        i. AIC works on the principle of parsimony – it finds models that strongly explain the brown trout response but with the least number of variables in the model

        ii. Models with more variables than the "best models" sometimes have higher R-squared values, but these models may be "over-fitted" due to spurious correlations between additional variables and trout responses

    7. **Do the variables have a positive or negative impact on brown trout responses (Tables 3a and 3b)?**

        i. First look at whether the p-values for each variable included in the "best models" are significant (P < 0.05, or 0.10 depending on your personal preference).

        ii. Then, look to see whether the "estimates" of the slopes of significant variables are positive or negative – this indicates a positive or negative influence on the brown trout response

        iii. The direction of non-significant variables needs to be interpreted with caution. Statistical analyses "sees" a variable with a high p-value as having "ZERO" influence (slope). If the p-value is not significant but pretty close (e.g. between 0.06 and 0.15), then the analyses may have “almost” detected a positive or negative influence on trout. Often, one says that a “trend” may be present.

2. **Data mining with stepwise selection regression models (Table 4):**

    a. **What variables were included in the “best” model and what is their significance levels and direction (positive or negative estimate)?**

        1. This is similar to interpretation of the AIC models EXCEPT – there in only one best model, so you don’t get that extra level of information discussed above. Also, the principle of parsimony is not used so variables that are not truly influencing brown trout may accidentally be included (e.g. redundant variables)

        2. Note that we did not include interactions between variables in this process. One could do that – we did not, because we wanted to compare the influence of the raw variables against each other. There are other more complicated problems not presented here.

3. Discriminant Function Analysis (DFA):

a. **What does the Principle Component Analysis (PCA) tell us?**

1. PCA reduced the number of variables that would go into DFA, because many variables are redundant or are too highly correlated for the statistical analysis to handle (same problem discussed in light of the stepwise selection process).

2. In **Table 5**, four major components were identified and you can see which variables “characterized” each component. Visualize a “component” as an axis, just like the “x and y” axes in a plot. Variables correlated with each component contribute strongly to the creation of that axis.

b. **In the DFA analyses, which variables are identified as “important” in determining whether a site meets attainment criteria for the brown trout metric?**

1. DFA creates a “function”, or equation (these are presented in the text) that “predicts” whether a site would meet attainment given newly measured environmental variables. These functions are difficult to interpret in terms of “positive or negative” influence of a variable on attainment, so look instead at **Tables 6-7**.

2. The value and direction of variables in **Tables 6 and 7** tells us whether a variable is positively or negatively associated with site attainment. The

value can be used to compare relative strength of association (bigger number is stronger).

4. Canonical Discriminant Analysis (CDA):

a. What does Table 8 show?

1. **Table 8** shows that the overall CDA model was useful (significant) in separating mine sites in different years. It also shows that only two axes (like the PCA and DFA components) were needed to separate years.

2. **Table 8** also shows which environment variables “characterize” (are correlated with) the two axes. Like in CDA, correlations that are larger are “stronger” in characterizing that axis, and the sign indicates whether the variables contribute to the negative or positive direction of that axis (shown in **Figure 1**).

b. How do we interpret Figure 1?

1. **Figure 1** summarizes the information presented in **Table 8** regarding how variables are correlated with each axis. Also, this figure plots mine-sites for each year along these two axes, based on what values of each environmental variable a mine-site had.

2. This figure does NOT display attainment vs. non-attainment. It only shows how the sites differed in each year based on the environmental variables measured in each year (NOT measures of brown trout metrics or site attainment)

c. What do Figures 2a-2e show?

These are straight forward. They just show how, with the exception of young of year counts, the year 2004 (all mine sites combined) was not “unusual” compared to other post-remediation years.

Final Report ADDITIONAL APPENDICES

2005 Investigative Study:

Factors Influencing Brown Trout Populations in Mine-impacted Reaches of the Eagle River following Remediation Efforts

Released October 3, 2005

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Appendix 1sa

DFA$_{PCA}$ Equation Results

Correlations

		lnzntu_D	lnzntu_D2	lncdtu_D	lncdtu_D2	lncutu_D	lncutu_D2	lnbflow	lnbflow_p	LNPH75A	LNHPH75A	LNPH75F	LNHPH75F	EPT_tr	avgm_jcfs	avgm_jcfs_p
lnzntu_D	Pearson Correlation	1	.572(**)	.857(**)	.346(*)	.616(**)	.364(*)	.573(**)	.428**	-.283	-.315	-.271	-.305	.163	.289	.232
	Sig. (2-tailed)	.	.000	.000	.039	.000	.029	.001	.009	.095	.061	.110	.070	.341	.088	.174
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
lnzntu_D2	Pearson Correlation	.572(**)	1	.435(**)	.823(**)	.317	.566(**)	.328	.737**	-.182	-.329	-.147	-.280	.240	.355(*)	.407(*)
	Sig. (2-tailed)	.000	.	.008	.000	.059	.000	.067	.000	.287	.050	.394	.098	.158	.034	.014
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
lncdtu_D	Pearson Correlation	.857(**)	.435(**)	1	.359(*)	.814(**)	.495(**)	.539(**)	.243	-.413(*)	-.476**	-.247	-.283	.246	.162	-.023
	Sig. (2-tailed)	.000	.008	.	.031	.000	.002	.001	.153	.012	.003	.147	.094	.149	.344	.893
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
lncdtu_D2	Pearson Correlation	.346(*)	.823(**)	.359(*)	1	.400(*)	.836(**)	.098	.541**	-.335(*)	-.51***	-.149	-.264	.392(*)	-.084	.244
	Sig. (2-tailed)	.039	.000	.031	.	.016	.000	.592	.001	.046	.001	.384	.120	.018	.628	.152
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
lncutu_D	Pearson Correlation	.616(**)	.317	.814(**)	.400(*)	1	.541(**)	.358(*)	.063	-.506(**)	-.550**	-.181	-.173	.388(*)	-.025	-.243
	Sig. (2-tailed)	.000	.059	.000	.016	.	.001	.045	.717	.002	.001	.290	.313	.019	.883	.153
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
lncutu_D2	Pearson Correlation	.364(*)	.566(**)	.495(**)	.836(**)	.541(**)	1	.070	.258	-.441(**)	-.622**	-.119	-.203	.629**	-.234	-.016
	Sig. (2-tailed)	.029	.000	.002	.000	.001	.	.703	.128	.007	.000	.489	.234	.000	.169	.927
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
lnbflow	Pearson Correlation	.573(**)	.328	.539(**)	.098	.358(*)	.070	1	.507**	.246	.162	.089	.043	.213	.651(**)	.390(*)
	Sig. (2-tailed)	.001	.067	.001	.592	.045	.703	.	.003	.174	.376	.626	.817	.243	.000	.027
	N	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
lnbflow_p	Pearson Correlation	.428(**)	.737(**)	.243	.541(**)	.063	.258	.507(**)	1	.263	.055	.161	.021	.067	.567(**)	.678**
	Sig. (2-tailed)	.009	.000	.153	.001	.717	.128	.003	.	.121	.748	.347	.901	.698	.000	.000
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
LNPH75A	Pearson Correlation	-.283	-.182	-.413(*)	-.335(*)	-.506(**)	-.441(**)	.246	.263	1	.838**	.812**	.807**	-.034	.557(**)	.58(**
	Sig. (2-tailed)	.095	.287	.012	.046	.002	.007	.174	.121	.	.000	.000	.000	.844	.000	.000
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36

LNHPH75A	Pearson Correlation	-.315	-.329	-.476(**)	-.515(**)	-.550(**)	-.622(**)	.162	.055	.838(**)	1	.482**	.612**	-.135	.503(**)	.338(*)
	Sig. (2-tailed)	.061	.050	.003	.001	.001	.000	.376	.748	.000	.	.003	.000	.431	.002	.043
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
LNPH75F	Pearson Correlation	-.271	-.147	-.247	-.149	-.181	-.119	.089	.161	.812(**)	.482**	1	.933**	.235	.378(*)	.400(*)
	Sig. (2-tailed)	.110	.394	.147	.384	.290	.489	.626	.347	.000	.003	.	.000	.169	.023	.016
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
LNHPH75F	Pearson Correlation	-.305	-.280	-.283	-.264	-.173	-.203	.043	.021	.807(**)	.612**	.933**	1	.274	.336(*)	.318
	Sig. (2-tailed)	.070	.098	.094	.120	.313	.234	.817	.901	.000	.000	.000	.	.106	.045	.059
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
EPT_tr	Pearson Correlation	.163	.240	.246	.392(*)	.388(*)	.629(**)	.213	.067	-.034	-.135	.235	.274	1	.057	.033
	Sig. (2-tailed)	.341	.158	.149	.018	.019	.000	.243	.698	.844	.431	.169	.106	.	.743	.849
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
avgm_jcfs	Pearson Correlation	.289	.355(*)	.162	-.084	-.025	-.234	.651(**)	.567**	.557(**)	.503**	.378(*)	.336(*)	.057	1	.396(*)
	Sig. (2-tailed)	.088	.034	.344	.628	.883	.169	.000	.000	.000	.002	.023	.045	.743	.	.017
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36
avgm_jcfs_p	Pearson Correlation	.232	.407(*)	-.023	.244	-.243	-.016	.390(*)	.678**	.582(**)	.338(*)	.400(*)	.318	.033	.396(*)	1
	Sig. (2-tailed)	.174	.014	.893	.152	.153	.927	.027	.000	.000	.043	.016	.059	.849	.017	.
	N	36	36	36	36	36	36	32	36	36	36	36	36	36	36	36

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Pearson Correlation values should not exceed 0.8. If the value is > 0.8, the multicollinearity assumption is violated.

	Skewness	Std Error	Result	Kurtosis	Std Error	Result
avgm_jcfs	0.61	0.393	1.552163	0.241	0.768	0.313802
avgm_jcfs_p	0.344	0.393	0.875318	-0.34	0.768	-0.442708
EPT_tr	-0.507	0.393	-1.290076	0.204	0.768	0.265625
lnzntu_D	0.092	0.393	0.234097	-1.141	0.768	-1.485677
lnzntu_D2	0.33	0.393	0.839695	-0.431	0.768	-0.561198
lncdtu_D	-0.168	0.393	-0.427481	-0.748	0.768	-0.973958
lncdtu_D2	-0.208	0.393	-0.529262	-0.781	0.768	-1.016927
lncutu_D	-0.731	0.393	-1.860051	-0.45	0.768	-0.585938
lncutu_D2	-0.616	0.393	-1.56743	-0.718	0.768	-0.934896
LNPH75A	0.859	0.393	2.185751	-0.688	0.768	-0.895833
LNHPH75A	1.013	0.393	2.577608	-0.588	0.768	-0.765625
LNPH75F	0.033	0.393	0.083969	-1.825	0.768	-2.376302
LNHPH75F	0.042	0.393	0.10687	-1.674	0.768	-2.179688
lnbflow	0.457	0.414	1.103865	-0.547	0.809	-0.676143
lnbflow_p	0.625	0.393	1.590331	-0.535	0.768	-0.696615

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
lnzntu_D2	.792	7.856	1	30	.009
avgm_jcfs_p	.847	5.418	1	30	.027
lnbflow_p	.882	4.017	1	30	.054
lnzntu_D	.931	2.223	1	30	.146
avgm_jcfs	.933	2.141	1	30	.154
LNHPH75F	.935	2.090	1	30	.159
LNPH75F	.949	1.606	1	30	.215
lncdtu_D2	.957	1.364	1	30	.252
EPT_tr	.973	.841	1	30	.366
lncutu_D2	.987	.386	1	30	.539
lnbflow	.990	.311	1	30	.581
lncdtu_D	.992	.249	1	30	.621
LNPH75A	.999	.036	1	30	.851
LNHPH75A	.999	.022	1	30	.884
lncutu_D	1.000	.014	1	30	.908

These variables do not meet the equality of group means assumption

Analysis 1

Box's Test of Equality of Covariance Matrices

Log Determinants

crit_fsh	Rank	Log Determinant
0	.(a)	.(b)
1	15	-33.007
Pooled within-groups	15	-22.950

The ranks and natural logarithms of determinants printed are those of the group covariance matrices.
a Rank < 15
b Too few cases to be non-singular

If Box's M returns a value of 0.05 or less, then the assumption of equal variance-covariance is violated. In this example the assumption is assumed to be violated.

Test Results(a)

Tests null hypothesis of equal population covariance matrices.
a No test can be performed with fewer than two nonsingular group covariance matrices.

Summary of Canonical Discriminant Functions

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	2.285(a)	100.0	100.0	.834

a First 1 canonical discriminant functions were used in the analysis.

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	.304	26.760	15	.031

These results demonstrate the DFA equation has strong correlation that is significant. In this example, both values indicate a strong model. However, we must remember that some violations have been violated.

Structure Matrix

	Function
	1
lnzntu_D2	-.339
avgm_jcfs_p	-.281
lnbflow_p	-.242
lnzntu_D	-.180
avgm_jcfs	-.177
LNHPH75F	.175
LNPH75F	.153
lncdtu_D2	-.141
EPT_tr	.111
lncutu_D2	.075
lnbflow	-.067
lncdtu_D	-.060
LNPH75A	.023
LNHPH75A	-.018
lncutu_D	-.014

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions Variables ordered by absolute size of correlation within function.

These values show the contribution of each variable to the model. The greater the value, the more contribution the variable has on the prediction of the model. In this example, lnzntu_D2 has the greatest contribution to the model.

Canonical Discriminant Function Coefficients

	Function
	1
lnzntu_D2	-3.725
avgm_jcfs_p	-.005
lnbflow_p	.925
lnzntu_D	1.976
avgm_jcfs	.004
LNHPH75F	1.840
LNPH75F	-3.442
lncdtu_D2	-2.260
EPT_tr	-.094
lncutu_D2	5.506
lnbflow	2.208
lncdtu_D	-4.496
LNPH75A	7.192
LNHPH75A	-4.012
lncutu_D	1.558
(Constant)	-18.976

Unstandardized coefficients

DFA$_{PCA}$ = (LnZnTU_previous year*-3.725) + (ave may-june cfs_previous year*-0.005) + (lnBaseflow_previous year*0.925) + (lnZnTU_current year*1.976) + (ave may-june cfs current year*0.004) + (lnphab75_fry high flow*1.840) + (lnphab75_fry low flow*-3.442) + (lnCdTU_previous year*-2.260) + (EPT_richness*-0.094) + (lnCuTU_previous year*5.506) + (lnBaseflow_current year*2.208) + (lnCdTU_current year*-4.496) + (lnphab75_adult low flow*7.192) + (lnphab75_adult high flow*-4.012) + (lnCuTU_current year*1.558) – 18.976

This is the DFA model.

Functions at Group Centroids

crit_fsh	Function
	1
0	-1.558
1	1.375

Unstandardized canonical discriminant functions evaluated at group means

This demonstrates the group centroids are distant from each other..

Classification Statistics

Prior Probabilities for Groups

crit_fsh	Prior	Cases Used in Analysis	
		Unweighted	Weighted
0	.500	15	15.000
1	.500	17	17.000
Total	1.000	32	32.000

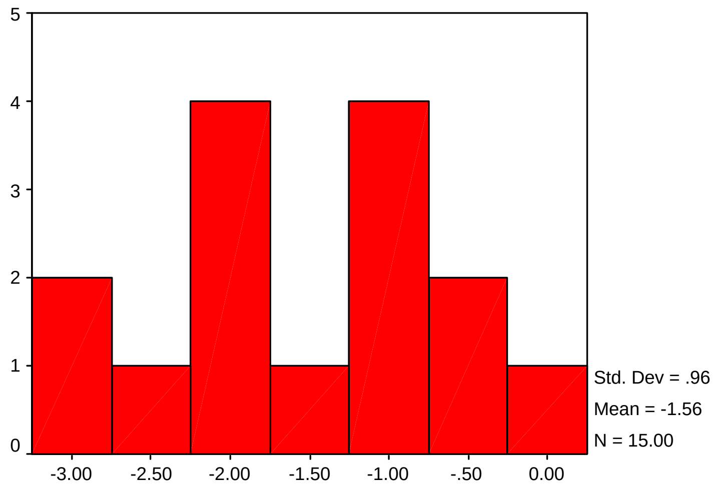
Classification Function Coefficients

	crit_fsh	
	0	1
lnzntu_D2	567.104	556.178
avgm_jcfs_p	-.646	-.661
lnbflow_p	457.914	460.628
lnzntu_D	-228.984	-223.190
avgm_jcfs	-1.477	-1.467
LNHPH75F	394.761	400.157
LNPH75F	-5.198	-15.293
lncdtu_D2	-357.524	-364.153
EPT_tr	6.315	6.040
lncutu_D2	-99.810	-83.662
lnbflow	572.656	579.131
lncdtu_D	237.717	224.531
LNPH75A	161.771	182.866
LNHPH75A	107.595	95.828
lncutu_D	-128.384	-123.816
(Constant)	-2622.791	-2678.179

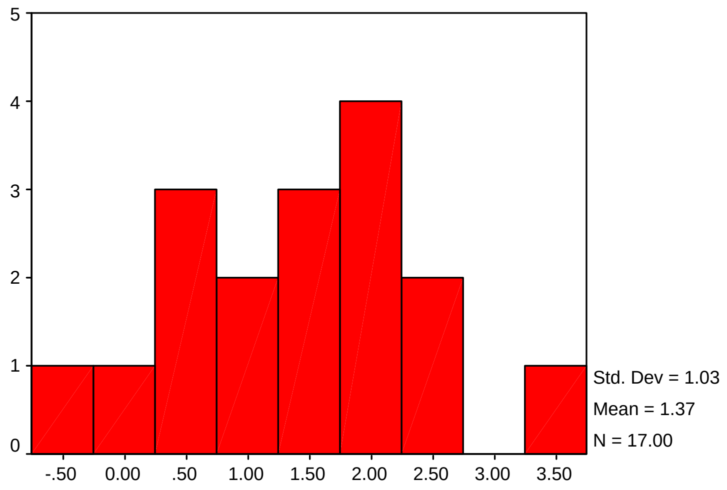
Fisher's linear discriminant functions

The greater the value, the more influence the variable has on the model. In this example, lnzntu_D2 and lnbflow have the most influence.

Separate-Groups Graphs


Canonical Discriminant Function 1

crit_fsh = 0


Canonical Discriminant Function 1

crit_fsh = 1

Classification Results(b,c)

			Predicted Group Membership		
		crit_fsh	0	1	Total
Original	Count	0	14	1	15
		1	2	15	17
	%	0	93.3	6.7	100.0
		1	11.8	88.2	100.0
Cross-validated(a)	Count	0	8	7	15
		1	6	11	17
	%	0	53.3	46.7	100.0
		1	35.3	64.7	100.0

a Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
b 90.6% of original grouped cases correctly classified.
c 59.4% of cross-validated grouped cases correctly classified.

The graphs show visually that the two groups can be separated by the independent variables.

The DFA function correctly places 90.6% of the cases. The model is both sensitive and specific.

Appendix 2sa

DFA$_{PCA2}$ Equation Results

Correlations

		lnzntu_D	lnzntu_D2	lncutu_D	lncutu_D2	lnbflow	lnbflow_p	LNPH75A	EPT_tr	avgm_jcfs	avgm_jcfs_p
lnzntu_D	Pearson Correlation	1	.572(**)	.616(**)	.364(*)	.573(**)	.428(**)	-.283	.163	.289	.232
	Sig. (2-tailed)	.	.000	.000	.029	.001	.009	.095	.341	.088	.174
	N	36	36	36	36	32	36	36	36	36	36
lnzntu_D2	Pearson Correlation	.572(**)	1	.317	.566(**)	.328	.737(**)	-.182	.240	.355(*)	.407(*)
	Sig. (2-tailed)	.000	.	.059	.000	.067	.000	.287	.158	.034	.014
	N	36	36	36	36	32	36	36	36	36	36
lncutu_D	Pearson Correlation	.616(**)	.317	1	.541(**)	.358(*)	.063	-.506(**)	.388(*)	-.025	-.243
	Sig. (2-tailed)	.000	.059	.	.001	.045	.717	.002	.019	.883	.153
	N	36	36	36	36	32	36	36	36	36	36
lncutu_D2	Pearson Correlation	.364(*)	.566(**)	.541(**)	1	.070	.258	-.441(**)	.629(**)	-.234	-.016
	Sig. (2-tailed)	.029	.000	.001	.	.703	.128	.007	.000	.169	.927
	N	36	36	36	36	32	36	36	36	36	36
lnbflow	Pearson Correlation	.573(**)	.328	.358(*)	.070	1	.507(**)	.246	.213	.651(**)	.390(*)
	Sig. (2-tailed)	.001	.067	.045	.703	.	.003	.174	.243	.000	.027
	N	32	32	32	32	32	32	32	32	32	32
lnbflow_p	Pearson Correlation	.428(**)	.737(**)	.063	.258	.507(**)	1	.263	.067	.567(**)	.678(**)
	Sig. (2-tailed)	.009	.000	.717	.128	.003	.	.121	.698	.000	.000
	N	36	36	36	36	32	36	36	36	36	36
LNPH75A	Pearson Correlation	-.283	-.182	-.506(**)	-.441(**)	.246	.263	1	-.034	.557(**)	.582(**)
	Sig. (2-tailed)	.095	.287	.002	.007	.174	.121	.	.844	.000	.000
	N	36	36	36	36	32	36	36	36	36	36
EPT_tr	Pearson Correlation	.163	.240	.388(*)	.629(**)	.213	.067	-.034	1	.057	.033
	Sig. (2-tailed)	.341	.158	.019	.000	.243	.698	.844	.	.743	.849
	N	36	36	36	36	32	36	36	36	36	36
avgm_jcfs	Pearson Correlation	.289	.355(*)	-.025	-.234	.651(**)	.567(**)	.557(**)	.057	1	.396(*)

	Sig. (2-tailed)	.088	.034	.883	.169	.000	.000	.000	.743	.	.017
	N	36	36	36	36	32	36	36	36	36	36
avgm_jcfs_p	Pearson Correlation	.232	.407(*)	-.243	-.016	.390(*)	.678(**)	.582(**)	.033	.396(*)	1
	Sig. (2-tailed)	.174	.014	.153	.927	.027	.000	.000	.849	.017	.
	N	36	36	36	36	32	36	36	36	36	36

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
lnzntu_D2	.792	7.856	1	30	.009
avgm_jcfs_p	.847	5.418	1	30	.027
lnbflow_p	.882	4.017	1	30	.054
lnzntu_D	.931	2.223	1	30	.146
avgm_jcfs	.933	2.141	1	30	.154
EPT_tr	.973	.841	1	30	.366
lncutu_D2	.987	.386	1	30	.539
lnbflow	.990	.311	1	30	.581
LNPH75A	.999	.036	1	30	.851
lncutu_D	1.000	.014	1	30	.908

Box's Test of Equality of Covariance Matrices

Log Determinants

crit_fsh	Rank	Log Determinant
0	10	-1.147
1	10	-.767
Pooled within-groups	10	2.129

The ranks and natural logarithms of determinants printed are those of the group covariance matrices.

Test Results

Box's M		92.210
F	Approx.	1.058
	df1	55
	df2	2804.592
	Sig.	.360

Tests null hypothesis of equal population covariance matrices.

Summary of Canonical Discriminant Functions

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	1.535(a)	100.0	100.0	.778

a First 1 canonical discriminant functions were used in the analysis.

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	.395	23.253	10	.010

Structure Matrix

	Function
	1
lnzntu_D2	-.413
avgm_jcfs_p	-.343
lnbflow_p	-.295
lnzntu_D	-.220
avgm_jcfs	-.216
EPT_tr	.135
lncutu_D2	.092
lnbflow	-.082
LNPH75A	.028
lncutu_D	-.017

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions Variables ordered by absolute size of correlation within function.

Canonical Discriminant Function Coefficients

	Function
	1
lnzntu_D2	-2.878
avgm_jcfs_p	-.006
lnbflow_p	1.477
lnzntu_D	.099
avgm_jcfs	-.001
EPT_tr	.006
lncutu_D2	2.389
lnbflow	1.995
LNPH75A	3.698
lncutu_D	-.522
(Constant)	-21.994

Unstandardized coefficients

Functions at Group Centroids

crit_fsh	Function
	1
0	-1.277
1	1.127

Unstandardized canonical discriminant functions evaluated at group means

Classification Statistics

Prior Probabilities for Groups

crit_fsh	Prior	Cases Used in Analysis	
		Unweighted	Weighted
0	.500	15	15.000
1	.500	17	17.000
Total	1.000	32	32.000

Classification Function Coefficients

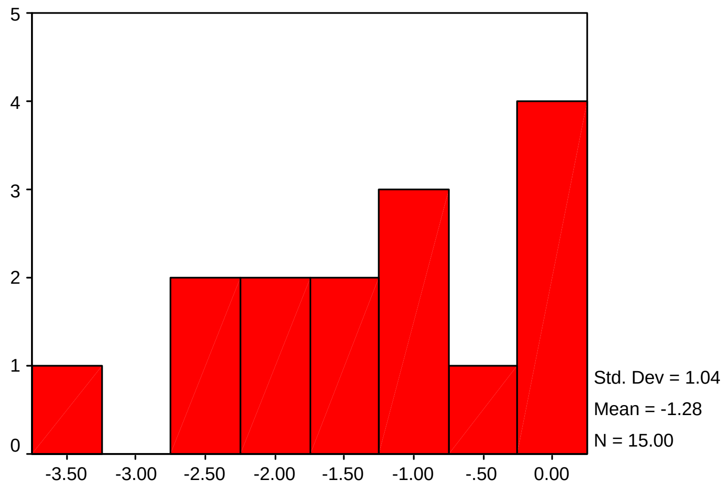
	crit_fsh	
	0	1
lnzntu_D2	66.255	59.338
avgm_jcfs_p	-.501	-.516
lnbflow_p	273.940	277.491
lnzntu_D	33.517	33.756
avgm_jcfs	-.616	-.618
EPT_tr	7.080	7.094
lncutu_D2	-86.201	-80.458
lnbflow	312.243	317.037
LNPH75A	321.237	330.125
lncutu_D	-31.299	-32.554
(Constant)	-1669.321	-1722.008

Fisher's linear discriminant functions

Separate-Groups Graphs

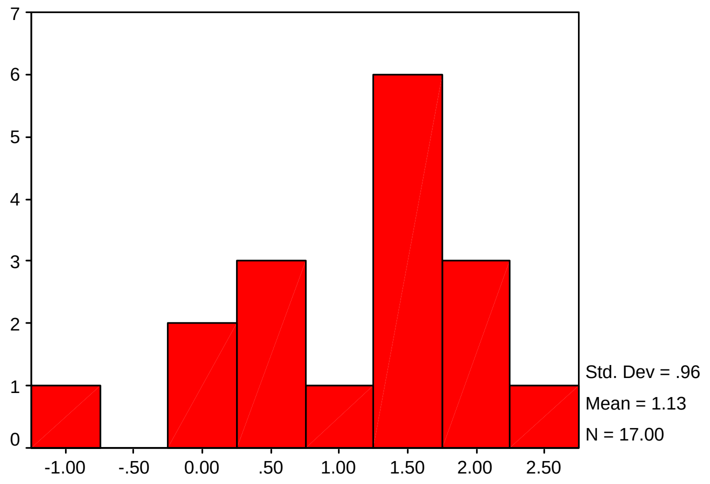
Canonical Discriminant Function 1

crit_fsh = 0



Canonical Discriminant Function 1

crit_fsh = 1



Classification Results(b,c)

		crit_fsh	Predicted Group Membership		Total
			0	1	
Original	Count	0	13	2	15
		1	2	15	17
	%	0	86.7	13.3	100.0
		1	11.8	88.2	100.0
Cross-validated(a)	Count	0	10	5	15
		1	6	11	17
	%	0	66.7	33.3	100.0
		1	35.3	64.7	100.0

a Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
b 87.5% of original grouped cases correctly classified.
c 65.6% of cross-validated grouped cases correctly classified.

Appendix 3sa

DFA$_{YOY}$ Equation Results

Correlations

		lnzntu_D	lnzntu_D2	lncutu_D	lncutu_D2	lnbflow	lnbflow_p	LNPH75A	EPT_tr	avgm_jcfs	avgm_jcfs_p	countyoy
lnzntu_D	Pearson Correlation	1	.572(**)	.616(**)	.364(*)	.573(**)	.428(**)	-.283	.163	.289	.232	-.356(*)
	Sig. (2-tailed)	.	.000	.000	.029	.001	.009	.095	.341	.088	.174	.033
	N	36	36	36	36	32	36	36	36	36	36	36
lnzntu_D2	Pearson Correlation	.572(**)	1	.317	.566(**)	.328	.737(**)	-.182	.240	.355(*)	.407(*)	-.476(**)
	Sig. (2-tailed)	.000	.	.059	.000	.067	.000	.287	.158	.034	.014	.003
	N	36	36	36	36	32	36	36	36	36	36	36
lncutu_D	Pearson Correlation	.616(**)	.317	1	.541(**)	.358(*)	.063	-.506(**)	.388(*)	-.025	-.243	-.306
	Sig. (2-tailed)	.000	.059	.	.001	.045	.717	.002	.019	.883	.153	.070
	N	36	36	36	36	32	36	36	36	36	36	36
lncutu_D2	Pearson Correlation	.364(*)	.566(**)	.541(**)	1	.070	.258	-.441(**)	.629(**)	-.234	-.016	-.142
	Sig. (2-tailed)	.029	.000	.001	.	.703	.128	.007	.000	.169	.927	.408
	N	36	36	36	36	32	36	36	36	36	36	36
lnbflow	Pearson Correlation	.573(**)	.328	.358(*)	.070	1	.507(**)	.246	.213	.651(**)	.390(*)	-.169
	Sig. (2-tailed)	.001	.067	.045	.703	.	.003	.174	.243	.000	.027	.356
	N	32	32	32	32	32	32	32	32	32	32	32
lnbflow_p	Pearson Correlation	.428(**)	.737(**)	.063	.258	.507(**)	1	.263	.067	.567(**)	.678(**)	-.378(*)
	Sig. (2-tailed)	.009	.000	.717	.128	.003	.	.121	.698	.000	.000	.023
	N	36	36	36	36	32	36	36	36	36	36	36
LNPH75A	Pearson Correlation	-.283	-.182	-.506(**)	-.441(**)	.246	.263	1	-.034	.557(**)	.582(**)	-.079
	Sig. (2-tailed)	.095	.287	.002	.007	.174	.121	.	.844	.000	.000	.646
	N	36	36	36	36	32	36	36	36	36	36	36
EPT_tr	Pearson Correlation	.163	.240	.388(*)	.629(**)	.213	.067	-.034	1	.057	.033	-.086
	Sig. (2-tailed)	.341	.158	.019	.000	.243	.698	.844	.	.743	.849	.616
	N	36	36	36	36	32	36	36	36	36	36	36
avgm_jcfs	Pearson	.289	.355(*)	-.025	-.234	.651(**)	.567(**)	.557(**)	.057	1	.396(*)	-.243

	Correlation											
	Sig. (2-tailed)	.088	.034	.883	.169	.000	.000	.000	.743	.	.017	.154
	N	36	36	36	36	32	36	36	36	36	36	36
avgm_jcfs_p	Pearson Correlation	.232	.407(*)	-.243	-.016	.390(*)	.678(**)	.582(**)	.033	.396(*)	1	-.405(*)
	Sig. (2-tailed)	.174	.014	.153	.927	.027	.000	.000	.849	.017	.	.014
	N	36	36	36	36	32	36	36	36	36	36	36
countyoy	Pearson Correlation	-.356(*)	-.476(**)	-.306	-.142	-.169	-.378(*)	-.079	-.086	-.243	-.405(*)	1
	Sig. (2-tailed)	.033	.003	.070	.408	.356	.023	.646	.616	.154	.014	.
	N	36	36	36	36	32	36	36	36	36	36	36

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

	Skewness	Std Error	Result	Kurtosis	Std Error	Result
lnzntu_D	0.092	0.393	0.234097	-1.141	0.768	-1.485677
lnzntu_D2	0.33	0.393	0.839695	-0.431	0.768	-0.561198
lncutu_D	-0.731	0.393	-1.860051	-0.45	0.768	-0.585938
lncutu_D2	-0.616	0.393	-1.56743	-0.718	0.768	-0.934896
LNPH75A	0.859	0.393	2.185751	-0.688	0.768	-0.895833
EPT_tr	-0.507	0.393	-1.290076	0.204	0.768	0.265625
avgm_jcfs	0.61	0.393	1.552163	0.241	0.768	0.313802
avgm_jcfs_p	0.344	0.393	0.875318	-0.34	0.768	-0.442708
lnbflow	0.457	0.414	1.103865	-0.514	0.809	-0.635352
lnbflow_p	0.625	0.393	1.590331	-0.547	0.768	-0.71224
countyoy	0.922	0.393	2.346056	-0.535	0.768	-0.696615

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
lnzntu_D2	.792	7.856	1	30	.009
avgm_jcfs_p	.847	5.418	1	30	.027
lnbflow_p	.882	4.017	1	30	.054
lnzntu_D	.931	2.223	1	30	.146
avgm_jcfs	.933	2.141	1	30	.154
EPT_tr	.973	.841	1	30	.366
lncutu_D2	.987	.386	1	30	.539
lnbflow	.990	.311	1	30	.581
LNPH75A	.999	.036	1	30	.851
countyoy	.561	23.435	1	30	.000

Incutu_D	1.000	.014	1	30	.908

Box's Test of Equality of Covariance Matrices

Log Determinants

crit_fsh	Rank	Log Determinant
0	11	.506
1	11	4.419
Pooled within-groups	11	7.077

The ranks and natural logarithms of determinants printed are those of the group covariance matrices.

Test Results

Box's M		134.521
F	Approx.	1.209
	df1	66
	df2	2769.426
	Sig.	.121

Tests null hypothesis of equal population covariance matrices.

Summary of Canonical Discriminant Functions

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	2.811(a)	100.0	100.0	.859

a First 1 canonical discriminant functions were used in the analysis.

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	.262	32.778	11	.001

Structure Matrix

	Function
	1
countyoy	.527
lnzntu_D2	-.305
avgm_jcfs_p	-.253
lnbflow_p	-.218
lnzntu_D	-.162
avgm_jcfs	-.159
EPT_tr	.100
lncutu_D2	.068
lnbflow	-.061
LNPH75A	.021
lncutu_D	-.013

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions Variables ordered by absolute size of correlation within function.

Canonical Discriminant Function Coefficients

	Function
	1
lnzntu_D2	-1.058
avgm_jcfs_p	-.004
lnbflow_p	-.029
lnzntu_D	-.423
avgm_jcfs	-.001
EPT_tr	-.009
lncutu_D2	1.804
lnbflow	.215
LNPH75A	4.618
countyoy	.057
lncutu_D	1.223
(Constant)	-9.670

Unstandardized coefficients

Functions at Group Centroids

crit_fsh	Function
	1
0	-1.728
1	1.525

Unstandardized canonical discriminant functions evaluated at group means

Classification Statistics

Prior Probabilities for Groups

crit_fsh	Prior	Cases Used in Analysis	
		Unweighted	Weighted
0	.500	15	15.000
1	.500	17	17.000
Total	1.000	32	32.000

Classification Function Coefficients

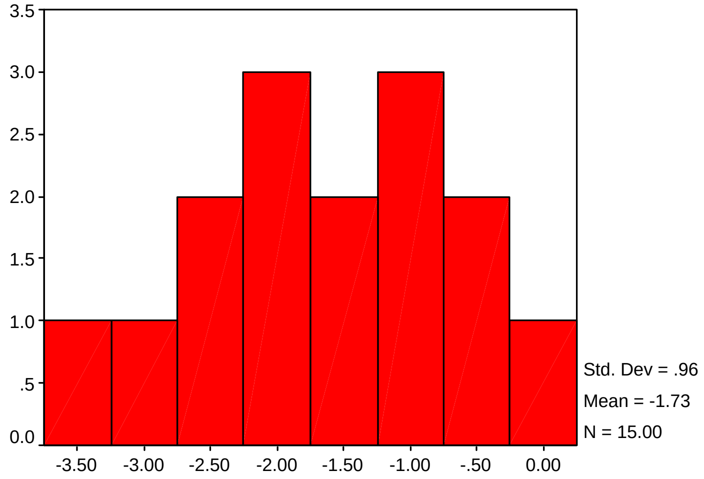
	crit_fsh	
	0	1
lnzntu_D2	48.927	45.485
avgm_jcfs_p	-.515	-.527
lnbflow_p	292.113	292.020
lnzntu_D	41.568	40.193
avgm_jcfs	-.616	-.618
EPT_tr	7.299	7.269
lncutu_D2	-86.822	-80.955
lnbflow	332.674	333.372
LNPH75A	290.649	305.670
countyoy	-.921	-.736
lncutu_D	-57.395	-53.418
(Constant)	-1729.077	-1760.203

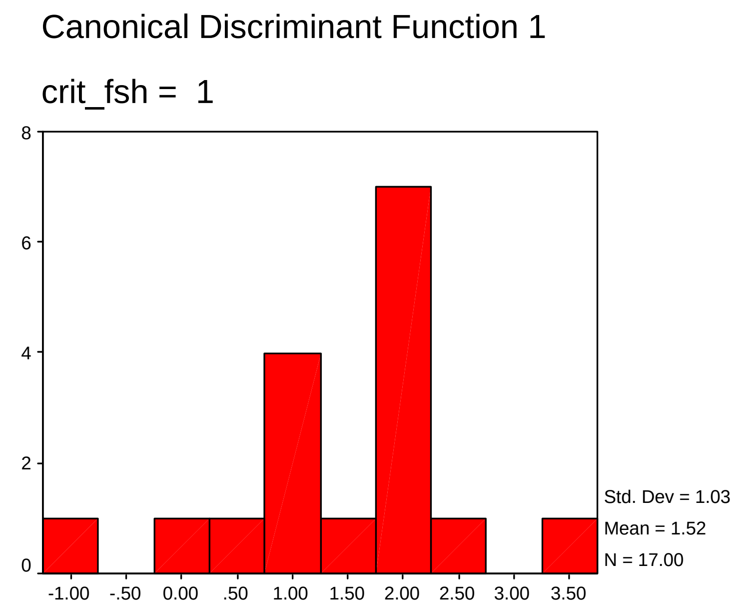
Fisher's linear discriminant functions

Separate-Groups Graphs

Canonical Discriminant Function 1

crit_fsh = 0





Canonical Discriminant Function 1

crit_fsh = 1

Classification Results(b,c)

			Predicted Group Membership		
		crit_fsh	0	1	Total
Original	Count	0	15	0	15
		1	1	16	17
	%	0	100.0	.0	100.0
		1	5.9	94.1	100.0
Cross-validated(a)	Count	0	11	4	15
		1	3	14	17
	%	0	73.3	26.7	100.0
		1	17.6	82.4	100.0

a Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
b 96.9% of original grouped cases correctly classified.
c 78.1% of cross-validated grouped cases correctly classified.

Appendix 4sa

DFA$_{YOY2}$ Equation Results

Correlations

		lnzntu_D2	lncutu_D2	lnbflow	LNPH75A	countyoy
lnzntu_D2	Pearson Correlation	1	.566(**)	.328	-.182	-.476(**)
	Sig. (2-tailed)	.	.000	.067	.287	.003
	N	36	36	32	36	36
lncutu_D2	Pearson Correlation	.566(**)	1	.070	-.441(**)	-.142
	Sig. (2-tailed)	.000	.	.703	.007	.408
	N	36	36	32	36	36
lnbflow	Pearson Correlation	.328	.070	1	.246	-.169
	Sig. (2-tailed)	.067	.703	.	.174	.356
	N	32	32	32	32	32
LNPH75A	Pearson Correlation	-.182	-.441(**)	.246	1	-.079
	Sig. (2-tailed)	.287	.007	.174	.	.646
	N	36	36	32	36	36
countyoy	Pearson Correlation	-.476(**)	-.142	-.169	-.079	1
	Sig. (2-tailed)	.003	.408	.356	.646	.
	N	36	36	32	36	36

** Correlation is significant at the 0.01 level (2-tailed).

	Skewness	Std Error	Result	Kurtosis	Std Error	Result
lnzntu_D2	0.33	0.393	0.839695	-0.431	0.768	-0.561198
lncutu_D2	-0.616	0.393	-1.56743	-0.718	0.768	-0.934896
LNPH75A	0.859	0.393	2.185751	-0.688	0.768	-0.895833
lnbflow	0.457	0.414	1.103865	-0.514	0.809	-0.635352
countyoy	0.922	0.393	2.346056	0.177	0.768	0.230469

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
lnzntu_D2	.792	7.856	1	30	.009
countyoy	.561	23.435	1	30	.000
lncutu_D2	.987	.386	1	30	.539
lnbflow	.990	.311	1	30	.581
LNPH75A	.999	.036	1	30	.851

Box's Test of Equality of Covariance Matrices

Log Determinants

crit_fsh	Rank	Log Determinant
0	5	-7.170
1	5	-3.732
Pooled within-groups	5	-3.981

The ranks and natural logarithms of determinants printed are those of the group covariance matrices.

Test Results

Box's M		40.662
F	Approx.	2.214
	df1	15
	df2	3489.901
	Sig.	.005

Tests null hypothesis of equal population covariance matrices.

Summary of Canonical Discriminant Functions

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	1.830(a)	100.0	100.0	.804

a First 1 canonical discriminant functions were used in the analysis.

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	.353	28.603	5	.000

Structure Matrix

	Function
	1
countyoy	.653
lnzntu_D2	-.378
lncutu_D2	.084
lnbflow	-.075
LNPH75A	.026

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions Variables ordered by absolute size of correlation within function.

Canonical Discriminant Function Coefficients

	Function
	1
lnzntu_D2	-2.232
countyoy	.044
lncutu_D2	2.068
lnbflow	.719
LNPH75A	1.316
(Constant)	-6.364

Unstandardized coefficients

Functions at Group Centroids

	Function
crit_fsh	1
0	-1.394
1	1.230

Unstandardized canonical discriminant functions evaluated at group means

Classification Statistics

Prior Probabilities for Groups

crit_fsh	Prior	Cases Used in Analysis	
		Unweighted	Weighted
0	.500	15	15.000
1	.500	17	17.000
Total	1.000	32	32.000

Classification Function Coefficients

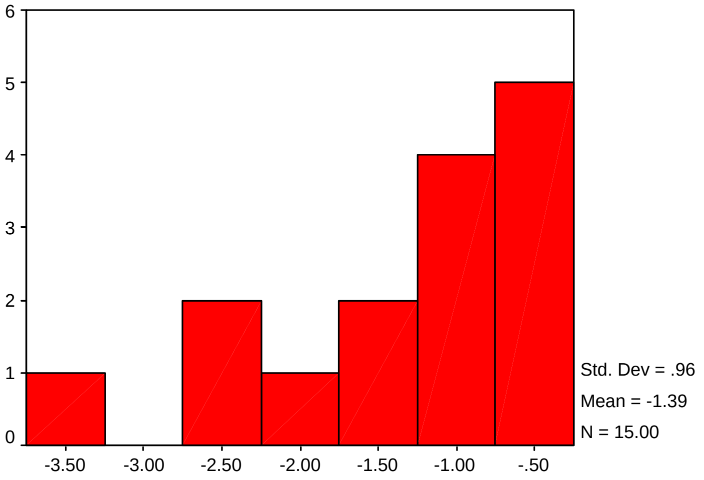
	crit_fsh	
	0	1
lnzntu_D2	-26.397	-32.255
countyoy	.121	.237
lncutu_D2	11.326	16.753
lnbflow	103.345	105.231
LNPH75A	25.360	28.814
(Constant)	-267.570	-284.057

Fisher's linear discriminant functions

Separate-Groups Graphs

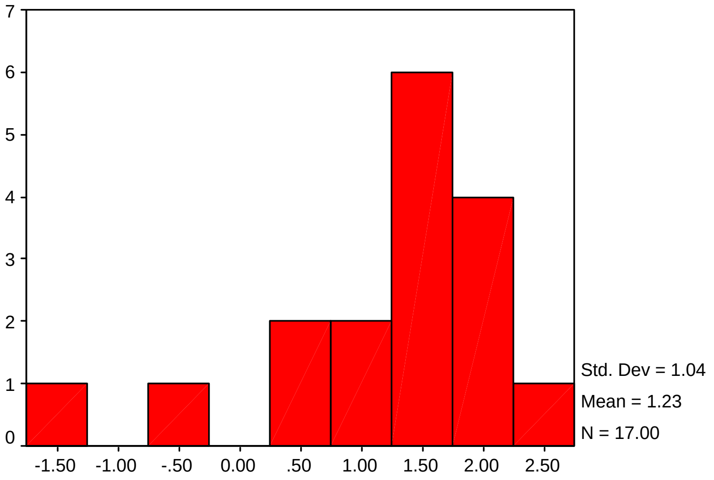
Canonical Discriminant Function 1

crit_fsh = 0



Canonical Discriminant Function 1

crit_fsh = 1



Classification Results(b,c)

		crit_fsh	Predicted Group Membership		Total
			0	1	
Original	Count	0	15	0	15
		1	2	15	17
	%	0	100.0	.0	100.0
		1	11.8	88.2	100.0
Cross-validated(a)	Count	0	15	0	15
		1	2	15	17
	%	0	100.0	.0	100.0
		1	11.8	88.2	100.0

a Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
b 93.8% of original grouped cases correctly classified.
c 93.8% of cross-validated grouped cases correctly classified.