Water Pollution Studies

Federal Aid Project F243R24

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Federal Aid in Fish and Wildlife Restoration

Job Progress Report

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Aquatic Research Section

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State: Colorado **Project No. F-243-R23**

Project Title: Water Pollution Studies

Period Covered: July 1, 2016 to June 30, 2017

Project Objective: To develop quantitative chemical and toxicological data on the effects of pollutants and water quality on aquatic life, investigate water quality problems in the field, and provide expertise and method development in aquatic chemistry and aquatic ecotoxicology.

Job No. 1: Toxicology Research - experiments and biomonitoring to assess the risk of emerging toxicants, derive new water quality standards and improve existing water quality standards.

Job Objective: Gather quantitative data and conduct experiments to build water quality standards that are protective of sport fish and sport fish habitat.

Need

Water Pollution Studies: Over seven million recognized chemicals exist and 80,000 are in common use (GAO 1994). However, Colorado regulates surface water concentrations of only 63 organic and 15 inorganic chemicals (CDPHE 2013). Colorado's mining heritage has left a majority of watersheds in the Colorado Mineral Belt with elevated metal concentrations. Links between mining activity, metal pollution and degradation of aquatic communities in streams are well established in the literature (Clements *et al*. 2000). An estimated 20,000-50,000 mines in the western United States produce acid mine drainage (AMD) which seriously affects 5,000-10,000 miles of streams (USDA 1993) and has been described as the greatest water quality problem in the Rocky Mountain region (Mineral Policy Center 1997). Regulatory agencies such as the US Environmental Protection agency (EPA) and the Colorado Department of Public Health and Environment (CDPHE), including the Water Quality Control Commission, act as moderators when building or refining pollution standards. These agencies largely rely on research from external sources and alter standards after requests from industry or stakeholders. Colorado Parks and Wildlife (CPW) is the primary stakeholder advocating for sustainable fisheries in Colorado by producing scientific evidence that ensures water quality standards are protective of fisheries.

Indirect and direct toxicity of metals: The current USEPA chronic criterion for protection of aquatic life is 1.0 mg/L (total recoverable) iron (Fe) based largely on field observations not experiments (USEPA 1976). In Colorado 1,454 miles of streams and rivers are considered heavily affected by Fe (Colorado State Department of Public Health and Environment 2008). There has been an increased interest in establishing new water quality standards for contaminants such as Fe and aluminum (Al) in Colorado and other states. Industry efforts to revise the 2007 standards for Al in New Mexico resulted in a markedly relaxed hardness-based criterion value (New Mexico State Surface Water Quality Bureau 2009), and the Colorado Mining Association

recently suggested establishment of a dissolved standard for Fe. Such a standard might result in reduced protection for aquatic life in pH-neutral waters in Colorado. Metals that precipitate into metal-oxides pose an indirect threat to aquatic organisms by smothering habitat, reducing primary productivity, and possibly increasing the accumulation of aqueous toxicants (Cadmus *et al.* In Review A). Using laboratory studies, Colorado Parks and Wildlife has derived a new recommended chronic toxicity value of 60% of the current standard (Cadmus *et al.* in Review B). The current methodologies of researching and regulating toxicants are tailored for aqueous toxicants and consider only direct effects on organisms. Further research into the indirect toxicity of Fe, Al and manganese (Mn) is needed to ensure standards are protective of sport fish and sport fish habitat.

Emerging Toxicants of Concern: Endocrine disrupting chemical classes such as estradiols and statin drugs are known to have an adverse effect on fish populations. Statin drugs are marketed to control blood lipids by altering how the body stores and metabolizes fats. These drugs are often highly synergistic and are not removed in wastewater treatment. Fat regulation of fish largely affects fish survival and may be altered by exposure to statin pharmaceuticals.

Rates of hydrocarbon extraction have increased in Colorado over the last 10 years. This presents new risks from extraction processes and transport processes. Uptake and trophic transfer of hydrocarbons from benthos to fish in both acute and chronic (Lytle and Peckarsky 2001) exposure regimes is well documented (Neff 1979, Lamoureux and Brownawell 1999, Clements *et al.* 1994, Schuler *et al.* 2003, Giesy *et al.* 1983). Increased susceptibility to disease is often correlated with polycyclic aromatic hydrocarbon (PAH) exposure (Damasio *et al.* 2007, Bravo *et al.* 2011). Safe concentrations of these chemicals are unknown.

Disease and Toxicant Interactions: Heavy metals, including copper (Cu), have been shown to increase salmonid susceptibility to disease (Hetrick *et al.* 1997, Baker *et al.* 1983, Knittel *et al.* 1981). Whirling disease has been a major cause of trout population declines. Distributions of trout, minerals high in Cu and whirling disease co-occur in Colorado. Water quality standards do not consider multiple stressors simultaneously but special consideration is given to watersheds where toxicants, species and stressors co-exist. Research exploring synergistic effects of whirling disease and heavy metal pollution is needed.

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Objectives

1-Assess toxicity of emerging contaminants pertinent to Colorado surface waters by conducting toxicity trials on sport fish and food species important to sport fish populations.

2-Improve state and national water quality standards to ensure they are protective of sport fish important to Colorado. These standards include toxicants (e.g. iron (Fe), selenium (Se), copper (Cu), cadmium (Cd), zinc (Zn), aluminum (Al), Manganese (Mn), benzene, petrochemicals) and physical properties (e.g. total suspended solids, temperature, nutrients). Improved standards rely on improved experimentation that is published in a timely manner and is designed to inform triannual reevaluation of toxicant standards by EPA and CDPHE. Experiments should:

a) Include rare or sensitive sport fish species underrepresented in the literature.

b) When possible, expose rare or sensitive taxa not lab cultured organisms. Expose for long environmentally relevant durations not the standardized 96 hour and 30 day trials. Expose organisms during sensitive life stages (e.g. early life stages, egg survival, drift of sac fry, mating, winter survival).

c) Consider ecologically relevant sublethal endpoints as technology and infrastructure becomes available to CPW toxicology laboratory (e.g. predator avoidance, olfactory function, fecundity, thermal tolerance, apoptosis, protein carbonyl content, histopathology, blood chemistry).

d) Examine all routes of exposure and all toxic pathways (e.g. dietary vs aqueous exposure, indirect vs direct toxicity).

e) Have increased environmental realism by using natural habitat, natural assemblages, mesocosm, communities and food chains both in laboratory and field settings.

f) Consider multiple stressors simultaneously, not limited to interactions between numerous toxicants, interactions between toxicants and temperature or interactions between toxicants and disease (e.g. whirling disease).

3- Create new aquatic life criteria (“standards”) for toxicants pertinent to Colorado by conducting toxicity tests that meet the minimal requirements for derivation. Present these findings to regulatory agencies through professional society meetings and peer reviewed publications.

Approach

Action #1.1 – Assessment of emerging pollutants: Antilipidemic pharmaceuticals and/or pesticides and/or petroleum hydrocarbons

Sub Action #1.1.1 – Assessment of antilipidemic drugs and antilipidemic like pharmaceuticals

●Level 1 Action Category: Data Collection and Analysis
●Level 2 Action Category: Research

Expose fish to environmentally relevant levels and mixtures of statin drugs or other cholesterol lowering pharmaceuticals from larval to reproductive age. Control treatments will be compared to exposure treatments using the following endpoints: survival, mass, length, fat to protein ratio, blood chemistry, fecundity, metabolism.

Action #1.1.1 Accomplishments

Development of sublethal endpoints related to antilipidemic exposures

Antilipidemics are a commonly prescribed class of drugs that lower cholesterol and triglycerides in humans and are detected in waterways downstream of wastewater treatment plants. Use of antilipidemics have increased substantially since the early 2000s (Gu *et al.* 2014) and antilipidemics include some of the most prescribed pharmaceuticals in North America (Medscape 2014). Environmentally relevant concentrations of antilipidemics have been shown to have sublethal effects in goldfish (Mimeault *et al.* 2005). Fibrates and statins, both subclasses of antilipidemics, are highly synergistic in humans. It is hypothesized that antilipidemics may affect the ability of sport fish to regulate fat stores and growth needed for reproduction and winter survival.

Fathead Minnows (*Pimephales promelas*) are an ideal model for sport fish due to their ease of culture, use in standard toxicity testing, short life cycle and prevalence in nature. Colorado Parks and Wildlife Aquatic Toxicology Laboratory and Colorado State University are developing tools to examine the direct effects of antilipidemics on sport fish survival, physiology (sublethal endpoints) and reproduction. In addition to direct effects on target species, indirect effect of habitat loss, food species reduction or bioaccumulation in food species must be considered in order to sustain healthy populations and facilitate natural reproduction. To meet this need, end points considered for Fathead Minnows were also explored for lower trophic levels as modeled by planaria and zooplankton. Sublethal endpoints under development include measures of triglycerides, cholesterol and growth potential.

The importance of developing these endpoints is underlined by the limited number of blood and tissue-derived biochemical endpoints available to small or immature fish species. For example, blood draws allowing complete blood analysis are not possible on fish of a small size. Younger stages of development may be more sensitive when exposed to emerging contaminants.

Nucleic acid and protein indices are regularly employed in ecology and toxicology as a measure of growth potential and nutritional condition at the organismal level (Chicharo and Chicharo 2008). Methods suitable for small or immature fish, as well as macroinvertebrates, aquatic insects, algae and other food species are underdeveloped. Nucleic acid indices are indicative of short term growth potential while protein concentrations can be indicative of long term growth potential. Ratios of these measures give insight into the fitness of fish and aquatic life (Weber *et al*. 2003).

Cholesterol and triglyceride concentrations are important physiological endpoints that are directly affected by antilipidemic drugs. Triglycerides are the primary energy storage (via fat) for many animals including sport fish and aquatic invertebrates. Colorado State University and Colorado Parks and Wildlife Aquatic Toxicology Laboratory are adapting multiple methods for assessment of triglycerides from scientific literature for use with small fish and macroinvertebrates, aquatic insects, algae and other food species. Expansion of these sublethal endpoints for use in Colorado Parks and Wildlife Aquatic Toxicology Laboratory experiments may inform fisheries management and water standards.

Methods

Method development is primarily taking place using Fathead Minnows that were maintained in laboratory cultures or from tissue of other donated fish. Planaria (*Dugesia dorotocephala*) were acquired from established laboratory cultures. Zooplankton (*Ceriodaphnia dubia* and *Daphnia magna*) were donated by an established toxicology lab.

Nucleic Acids

Extraction of DNA, RNA and proteins allows for assessment of potential growth of the organism (Buckley 1984) and allows for further exploration of changes on the molecular level. One method investigated included extracting DNA, RNA and proteins from a variety of organisms with the use of a reagent (TRI® Reagent LS, Molecular Research Center, Cincinnati, OH). Purity of nucleic acids was easily measured with an extremely small volume on a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). Using easily accessible chemicals and instruments such as isopropanol, chloroform, ethanol and centrifugation at 4°C, the TRI® reagent method provided satisfactory extraction allowing measurement of DNA, RNA, and protein in zooplankton, planarian, and fish.

Table 1 summarizes the preliminary results (qualitative) of several species. The spectrophotometer provides absorbances at several wavelengths and automatically calculates critical ratios for analysis. Spectra also allow the researcher to quickly assess purity and interferences. The calculated ratios and visual analysis from samples of these varying trophic levels indicate that nucleic acids were successfully extracted but additional steps will be needed to better purify the nucleic acids. Purity is an important aspect to consider because further use of these isolated nucleic acids can be used in assessing genetic expression but contamination (from an impure sample) would reduce the reliability of those studies.

Species	DNA (µg/g)	RNA (µg/g)
Ceriodaphnia dubia	14.2	984
Daphnia magna	15.9	279
Dugesia dorotocephala	63.6	97
Pimephales promelas	46.9	54

Table 1. Preliminary results of nucleic acids isolated from several trophic levels. Treat as qualitative results because of poor purity obtained. The first three species are all representative of composite samples with many organisms obtained from laboratory cultures. Subsequent method development with only fish tissue was performed (data not shown) and purity improved. Follow up method development with invertebrate species will be established after optimization with fish samples.

Successive studies deviated from the manufacturer's instructions in attempts to optimize purity. Deviations included prolonged and increased number of washes, varying centrifugation steps and consideration of alternative wash solutions. RNA purity improved in fish tissue samples to levels that are acceptable for follow up gene expression studies. DNA purity improved but further method development is required if DNA will be used in other assays or analyses.

TRI® reagent usage is beneficial but has drawbacks. Samples can be weighed and placed directly into the reagent, even in the field, and will prevent RNA degradation without the use of other reagents. Determination of DNA, RNA and proteins can be made from one sample. Measurements are almost instantaneous (after preparation) and require very small volumes (leaving more of the sample available for other assays or analyses). Drawbacks of this reagent include lack of reproducibility and preparation steps are laborious and time intensive (several hours). Many steps involve pipetting which risks potential sample loss, bias or variability between lab technicians. Based on these findings TRI® reagent is optimal for obtaining purified samples for genetic studies but this reagent should be avoided for total DNA or RNA determination due to the loss of nucleic acids inherent to the protocol.

A method for preparing a crude homogenate is also being investigated for quantitative assessment of RNA and DNA. The crude-homogenate method adapted from Weber *et al*. (2003) is convenient because their method allows for nucleic acids, proteins and lipid endpoints to be determined from one fish sample. Methods are actively being explored now to adapt this procedure for smaller fish samples with improved recovery.

Proteins

Protein isolation can be achieved using TRI® reagent with the same single tissue sample used for DNA, RNA and proteins. Isolated protein was achieved with this reagent but the pellet was difficult to dissolve in the recommended solution (sodium dodecyl sulfate). Multiple methods (shaking, vortexing, sonication, warm water baths) and other solutions (for example, TritonX100) are currently being explored to improve pellet dissolution.

The Bradford assay and the bicinchoninic assay (BCA) are used for protein quantification. Depending on how the homogenate is prepared for these two assays, varying substances are used in the process. Some of these substances will interfere with either or both of

these protein assays. Thus, both assays are being considered based on how the homogenate has been prepared.

Lipids

A non-solvent approach to lipid extraction (Bennett *et al.* 2007, Weber *et al.* 2003) is being optimized through varied homogenization techniques and times. Composite samples will be necessary for lower trophic levels (i.e. zooplankton and planaria) but have been avoided thus far in fish studies. Preliminary results using minced fish tissue are compared in Table 2.

NO CENTRIFUGE	
Homogenization time (sec)	**Concentration (mg glycerol/g)**
60	0.016
90	0.009
120	0.004
240	0.002
360*	Not conclusive

CENTRIFUGE	
Homogenization time (sec)	**Concentration (mg glycerol/g)**
60	0.008
90	0.009
120	0.007
240	0.004
360*	0.013

Table 2. Preliminary results using modifications of Weber *et al.*'s (2003) approach and whether samples were centrifuged (right) or not centrifuged (left). First four samples were all using the homogenate from one fish (excluded head). Homogenate from a separate fish was prepared for the fifth observation (*, included head). Note: Aliquots were removed after each homogenization time. Implications of lipid removal at different points in homogenization and/or lipid peroxidation are being evaluated and their effects on final concentrations in this preliminary data cannot be distinguished. Spike recoveries have not been performed yet.

Lipid peroxidation must be considered in lipid analysis. Lipid peroxidation occurs when free radicals oxidize lipids, which destabilizes them. This destabilization will result in electron-deficient lipids oxidizing neighboring lipids. This cycle can lead to cell membrane instability, deterioration and tissue damage (Mylonas and Kouretas 1999). This can lead to decreased fish health, survival and decreased concentrations of omega-3 fatty acids. Depleted fish condition may also lead to decreased fish appeal. Non-enzymatic hydrolysis (breakdown of lipids with the addition of water) also breaks down triglycerides to provide glycerol and fatty acids. This may result in a rancid flavor (Ekezie 2015) of the fish and decreased consumption of sport fish.

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Action #1.2.1 – Toxicity experiments to inform water quality standards and policy

●Level 1 Action Category: Data Collection and Analysis
●Level 2 Action Category: Research

1- Method development from the 2014-2016 fiscal years has enabled the Colorado Parks and Wildlife Aquatic Toxicology Laboratory to include new sublethal toxicological endpoints and enabled trials to be conducted on sensitive life stages previously unstudied. When possible, method development in new tools and endpoints will continue.

2- Toxicity of contaminants common in Colorado (e.g.: Cu, Cd, Zn, Fe, Se, Mg, Al, benzene, PAHs, agrochemicals and pharmaceuticals) will be evaluated using egg, larval and early life stages of sport fish in the form of acute and chronic laboratory trials and field experiments. Results from such studies will be analyzed and published. When required, trials will include exposure of sport fish food such as aquatic insects.

3- These data will be presented in triannual recalculations of existing standards or will be used to inform creation of new policies to protect Colorado's sport fisheries.

Action #1.2.1 Accomplishments

Development of tools to measure olfactory function in salmonids after exposure to toxicants

Salmonids and other sport fish use olfaction to regulate critical behaviors like feeding, mating, migration, socialization and predator avoidance (Tierney *et al.* 2010). Exposure to metals, pesticides and other toxicants has been shown to reduce olfactory function in salmonids (Baldwin *et al.*2011, Tierney *et al.* 2010). Limited olfactory function could manifest into a reduced ability to find food, avoid predators and locate breeding grounds. Even a minor reduction of olfaction would result in less fit individuals or populations and would eventually manifest into reduced survival and reproduction (Baldwin *et al.*2011, Tierney *et al.* 2010).

Historically olfactory response testing in fish uses an electro-olfactrogram (EOG) to measure changes in neural excitation with administration of olfactory stimuli (Winberg 1992, Ottoson 1971). An absence of response relative to unexposed controls indicates insensitivity to olfactory stimuli (Winberg 1992, Tierney *et al.* 2010). While this methodology is considered the golden standard of olfactory experimentation for its sensitivity and direct observation of olfactory response, it requires specialized equipment to immobilize and maintain life support for the fish while electrodes are secured on the exposed olfactory epithelium and nearby skin (Winberg 1992). Measured amounts of olfactory cue are administered through the life support system in a controlled manner and summated generator potential is recorded (Winberg 1992). While this is practical for large fish, it presents challenges in assessing smaller fish. It is less suited to repeated measures of the same fish due to the exposure of olfactory epithelium and increased handling-associated stress. From 2005 to 2012, the Colorado Parks and Wildlife Aquatic Toxicology Laboratory invested in methods to assess olfactory function in small trout after exposure to aqueous metal pollution. Organisms were exposed to the scent of predators or

lacerated epidermis of sacrificed Rainbow Trout (*Oncorhynchus mykiss*) and a response behavior was graded. This method was time consuming and inherently subjective.

As an alternative to the EOG and predator response methods, Colorado Parks and Wildlife Aquatic Toxicology Laboratory has developed protocols using classical conditioning to train fish to respond to a conditioned stimulus (the smell of food) with a conditioned response ("feeding frenzy"). A feeding frenzy is observed in hatcheries when trout compete for food (unconditioned stimulus). We quantified response behavior by using an accelerometer that measured tilt and/or acceleration in three axes at the surface of the water. Thus, we are able to record a quantifiable physical disturbance in response to an olfactory stimulus. This method reduces handling stress on fish and does not require use of anesthesia, surgery or highly specialized equipment. It also avoids experimenter interpretation of complex and subtle behaviors.

We conducted a preliminary trial examining if HOBO® Accelerometer Data Loggers can discern an increase in water surface movement after the scent of trout chow is injected into the water. We compared fish that had been trained to fish that had not been trained to respond to the scent. If this method is found to be affordable and objective it will be employed to inform safe surface water standards that are protective of fish.

Methods

Rainbow Trout (*Oncorhynchus mykiss*) "jumper" eggs received from Trout Lodge (Sumner, WA) on June 15, 2016 were hatched from June 20 to June 23, 2016 and raised at 11°C at Colorado Parks and Wildlife Bellvue fish hatchery using standard procedures. A portion of these fish were transferred to Colorado Parks and Wildlife Aquatic Toxicology Laboratory in November 2016. Trout were held in two communal tanks supplied by flow-through dechlorinated Fort Collins municipal water at approximately 11-13°C. Spray bars were used to aerate and deliver fresh water and olfactory cue evenly. Fish were fed 1.59 mm Rangen trout chow at 3% body weight per day over five feeding sessions daily on weekdays and one daily feeding on weekends.

Prior to each feeding, the tank containing "trained" organisms received an olfactory cue. The cue (60 ml) was delivered via Luer-Lok disposable syringes plumbed into water lines leading to spray bars. The cue circulated for 30 seconds before food was administered. "Untrained" organisms were fed on the same schedule and raised in identical conditions in a separate tank out of sight of the trained organisms. To avoid association between experimenter and feeding, aquaria and covers were made of opaque materials and food was delivered through long powder funnels. Although scientists observed the health of the fish regularly, scientists were never observed during feeding.

Olfactory food cues were made daily by stirring a solution of 1.59 mm Rangen trout chow in distilled water (25-27 g/L) for ten minutes and settling for an additional ten minutes. Solutions were then decanted and filtered (#1 Whatman). Filtrate was collected and delivered to fish within 8 hours.

Testing chambers were constructed of 15.14 L lidded, white opaque high-density polyethylene (HDPE) buckets and lids (Affordable Buckets, L.L.C. Victor, IA, USA). Splitter boxes distributed 80 ml/min of dechlorinated Fort Collins municipal water at 11°C to each chamber. The water line to each bucket had a Y-joint connector above the lid which allowed for a Luer-Lok syringe to attach and deliver the olfactory cue. Wooden aerators were used to reduce water surface disturbance and were placed in a corner of the buckets to create a gentle cycling current that helped mix the cue. HOBO® Acceleration Data Loggers (Onset Computer Corp., Bourne, MA) were fastened to a hinge on an arm that could be lowered through each bucket lid to the water's surface such that the hinge was held at water level. To keep the Z-axis measurements consistently positive HOBO® loggers were floated so the front (blinking LED) side consistently faced up. Testing chambers were kept in a chilled recirculating water bath to maintain temperature. Standpipes allowed water level (volume) to be modified to adjust the fish/volume density and to adjust the concentration of cue. Treatment tanks had a maximum fill of 13 L but trials were conducted at 9.5 L.

After a minimum 60 days of training or holding, ten fish from either the trained or untrained treatment groups were assigned to each of ten testing chambers, resulting in five replicates for each treatment. Fish were assessed after acclimating to treatment tanks for four hours. For each assessment, airlines were turned off to reduce noise, accelerometers were removed, activated and returned and cue administered to all tanks within one minute of each other. The HOBO® loggers were programmed to take ten observations per second in the X, Y and Z axes. Ten seconds of recorded data from just prior to the cue injections was retained as the "before" condition. Based on previous observations it took approximately 30 seconds to deliver and mix the cue in the treatment tanks. Ten seconds of recorded data starting 30 seconds after cue administration was analyzed as the "after" condition. The average deviations from the mean (AVEDEV formula in MS EXCEL 2010) was calculated for each 10 second observation. The difference of the average deviations from each treatment chamber ("after" - "before") was calculated and the five trained groups were compared to five untrained groups using a t-test assuming equal variance. HOBO® loggers were returned to the testing chambers immediately after retrieving data to maintain a relatively constant environment for fish.

Results

Trained fish responded significantly more than untrained fish to olfactory cue (Table 3) as recorded by the HOBO® loggers in X ($p=0.0049$), Y ($p=0.0017$) and Z axis ($p=0.01739$). Our results show that training is advantageous. However, it was possible to observe increased agitation of the accelerometer after a cue is injected into tanks containing untrained fish.

	X axis		**Y axis**		**Z axis**	
	Trained	*Untrained*	*Trained*	*Untrained*	*Trained*	*Untrained*
Mean	0.00770	0.00098	0.00928	-0.00414	0.01185	0.00186
Variance	1.54E-05	4.40E-06	2.90E-05	2.42E-05	6.32E-05	1.41E-05
Observations	5	5	5	5	5	5
***t* Stat**	3.3695		4.1089		2.5386	
***p* value** **one-tail**	0.00490		0.00170		0.01739	

Table 3. Results from each axis reading using a HOBO® logger showing the difference between surface disturbance after and before the cue was injected is far greater in trained fish. Mean is expressed as the mean of the average deviation for the 10 seconds after the cue was injected minus the average deviation of the 10 seconds before the cues were injected. *t*-critical=1.85.

Discussion

The measurements of water agitation obtained from the use of HOBO® loggers were more objective than the methods we explored when characterizing predator avoidance and more cost effective than use of EOG. This tool will be used to assess the effects of copper and other toxicants on olfactory function of Colorado trout species.

A limitation of this method and predatory response methods previously explored is inability to distinguish between physical damage to olfactory apparatus and interference with olfaction regulated behavior or learned behavior. A population of fish may have diminished conditioned response due to non-olfactory neuronal damage as a result of toxicant exposure. Alternately, fish failing to exhibit the conditioned response may be suffering from lethargy or have other physical limitations as part of a global response to toxicant. By comparison, EOG can detect damage to olfactory nerves at the interface with environment and the point of measurement. While EOG answers a specific question regarding olfactory function when presented with olfactory stimulus, the presented method and predatory response methods are well suited to inform management decisions that center on the question of whether harm is done.

Throughout this study, we observed numerous ways the experimental equipment or design could be altered to increase the sensitivity and decrease the variance during toxicology trials.

- The number of fish could be increased to encourage competition and increase the feeding frenzy behavior. We found the relationship between water disturbance and number of fish is not linear and therefore we were unable to normalize results to account for the number of fish in each tank. As a result, size and number of fish must be the same in each tank. Feeding frenzy behavior increases with competition.
- Volume of treatment tanks could be reduced by trimming standpipes to increase the density of fish. This produces a larger signal.

- Tank size needs to be tailored to the size of the fish and density of fish. High density fish or large fish will be stressed. Low density fish or small will result in little to no feeding frenzy.
- Fish need a minimum of a one hour acclimation period when acclimating to the treatment tanks. If very acute effects of olfactory toxicants are to be measured, EOG is more appropriate.
- When conducting assessment of feeding frenzy the fish must be hungry.
- When assessing feeding frenzy numerous times during a toxicant exposure we have prevented loss of the conditioned response by delivering food 45 seconds after injection of the cue. This provides for the 30 second mixing time and 10 seconds for observations.
- Preliminary studies should be done to determine the minimal volume or concentration of cue needed to illicit the condition response. In the case of a copper toxicity test, use of too much cue might arrive at falsely high EC_{50} values.

Follow up studies should explore if the learned behavior is lost during acute toxicity trials or other experiments where organisms are not fed. Use of accelerometers to measure feeding frenzy behavior is considerably more objective and affordable than existing methods used to quantify olfactory function. Extensions of this technique will examine the effects of copper on trout.

Personnel: Pete Cadmus, James Pickert and Abbie Jefferson

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Action #1.2.2 – Investigation of direct and indirect effects of metal toxicity

●Level 1 Action Category: Data Collection and Analysis
●Level 2 Action Category: Research

Action #1.2.2 Accomplishments

Instream experiments to predict fish response to mine restoration

The areas surrounding Blackhawk and Central City Colorado host some of the highest densities of legacy mine shafts and waste piles in the country. A large number of these shafts drain 13 miles south via the ARGO tunnel to Idaho Springs. The ARGO water treatment facility was installed in 1998 and markedly improved water quality in the main stem of Clear Creek. Mine shafts too shallow to reach the ARGO complex drain into the National Tunnel and the Gregory Incline adits. Until 2017, these adits delivered untreated mine drainage to two locations in the North Fork of Clear Creek (NFCC) in Blackhawk, Colorado (Figure 1). Water quality below these points is significantly impaired. Aquatic insects, algae and fish are largely absent. Upstream of these point sources we have measured rich diversity of insects, high abundance of insects, healthy algal function and self-reproducing trout populations. In mid-2017 a treatment plant will go online to treat National Tunnel and Gregory Incline effluent. This plant is projected to remove the vast majority of metals from the river. This plant cost $16.6 million and will have an annual operating cost of $1 million.

For over 20 years Colorado State University and Colorado School of Mines has studied the chemistry and aquatic macroinvertebrates of North Fork of Clear Creek. Over the last 17 years CPW has regular quantitative sampling events in both reference and polluted reaches. During the past seven years numerous sampling sites have been added and observations taken seasonally regularly. Instream experiments, laboratory experiments and mesocosm experiments using NFCC organisms and water characteristics have been conducted (Cadmus *et al*, 2016). This consortium of researchers and coordinated studies has aimed to achieve the following:

- Produce detailed biomonitoring that will document the improvement in water chemistry, stream productivity, aquatic life diversity and density, sport fish populations and angler use.
- Create novel tools that predict what fish and insect species will survive after restoration efforts.
- Create useful tools to model toxicity of metal mixtures for both the regulatory and restoration field of environmental toxicology.
- Develop tools that help managers prioritize what potential mine restoration sites are most likely to improve fish habitat
- Develop tools that help managers estimate the proportion of aqueous toxicants that must be removed from an ecosystem for sport fish and other aquatic life to survive.

In the spring of 2016 the Gregory Incline adit became clogged and a small trickle of mine drainage consistently overflowed into relatively pristine habitat. This allowed for placement of fish cages and algal colonization tiles upstream and downstream of this overflow. The natural mixing of the overflowing drainage allowed fish cages to be exposed to a gradient of concentrations. Existing models of trout sensitivity to heavy metal mixtures is of limited value since most models consider only one toxicant at a time. This instream experiment allowed us to predict minimal dilution of mine drainage needed to support specific trout species and ages

classes using site specific water and metal mixtures. These data will ultimately be used to inform toxicity models for metal mixtures.

Methods

Throughout the summer of 2016 a small trickle of mine drainage constantly overflowed into the North Fork of Clear Creek just upstream of the Gregory Incline adit (Figure 1). Conductivity, depth and velocity were mapped across transects running 3 m upstream and 150 m downstream from a small trickle of overflow mine drainage in 1-3 m intervals depending on proximity to source. Dissolved and total water samples were taken periodically throughout mapping and metal concentrations were confirmed by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES). Reference conditions were observed above the overflow and complete mixing was observed 18 m below the overflow. An in-stream experiment was conducted throughout this mixing zone by placing twenty-one exposure chambers throughout this reach (1 m above to 18 m below pollution source). Positions in the stream were assigned for locations with similar flow rates and depths but a gradient of pollution concentrations from 80 µs (above the point source) to 375 µs (just below point source). Conductivity was inversely proportional to the dilution of the overflowing mine drainage. Relative proportions of major metals remained consistent regardless of dilution factor and were similar to relative proportions of metals downstream of both major mine adits.

A major shortcoming of conducting tests in this manner is that treatment level cannot be randomly assigned to each exposure chamber. To compensate three control chambers receiving reference water through polyvinyl chloride (PVC) piping, as described below, were located immediately next to three randomly selected treatment chambers. If mortality was caused by an effect other than water chemistry (e.g. positional effects) we would hope these randomly assigned controls would behave unlike upstream controls.

Chambers were constructed of lidded 18.93 L high density polyethylene (HDPE) buckets drilled with a 1 cm x1 cm grid of 3.18 mm holes. Floors of buckets were left intact. Buckets were orange to mimic average river bed coloration. Stainless steel D rings were affixed to two points on the outside of the bucket and fastened to a rebar stake pounded into the river substrate. Once in place, a weight was placed on top of the lid. This ensured the chamber sat upright in the river and could withstand high flows without tipping over. Rebar stakes were flagged for safety and identification of experimental unit.

Three modified exposure chambers were gridded only on the downstream side of the chamber. Bulkheads were installed on the upstream side and plumbed into the PVC piping running up the river to above the mine drainage overflow. The diameter of the PVC pipe and reduced grid on opposing sides of the bucket ensured positive pressure and outward flow and thus, maintenance of near control metal concentration despite surrounding water of a higher concentration.

Three HOBO® conductivity Data Loggers (Onset Computer Corp., Bourne, MA) were deployed in a high, low and moderate conductivity chamber to continuously monitor conductivity fluctuations with weather and other environmental changes. A HOBO® Pressure

Data Logger (Onset Computer Corp., Bourne, MA) was added to the deepest portion of the river as a continuous measure of depth. HOBO® Temperature and Light Data Loggers (Onset Computer Corp., Bourne, MA) were added to two chambers. These continuous measures were used to compare to our observations and to model parameters.

A control location upstream of the experimental site and three locations downstream were chosen to match long term biomonitoring data. At each site four chambers were deployed, designed and secured in the same way as those at the experimental site.

Rangen #1 trout chow (0.6-1.0 mm) was stirred into 7 g Knox unflavored gelatin powder (Gloversville, NY) in 400 ml dechlorinated Fort Collins municipal water. Trout chow was added until mixture became a thick slurry and set in the refrigerator at 3°C. Food was kept under refrigeration for up to a week. Finished food cubes provided ad lib feeding necessary for life support of young fry without presence of experimenter of deployment of automatic feeders that could be compromised by high flows or precipitation. Trout offered food cubes in laboratory settings accepted the food and maintained adequate weight at various life stages.

Survival of 30 day post-swim up trout

Thirty-day post swim up Greenback Cutthroat Trout (*Oncorhynchus clarkii stomias*) "GBN", hatched July 9, 2016 at 11°C were transported from Colorado Parks and Wildlife Bellvue fish hatchery on August 16, 2016 at 12.0°C. After acclimation to river temperature (14°C) they were distributed (15 /chamber) throughout exposure chambers at upstream, experimental and downstream sites. Food cubes were replaced every other day. Dissolved oxygen, pH, conductivity, temp, alkalinity and hardness were taken every other day. Fish were visually quantified and mortalities removed. At conclusion of the 10 day exposure, remaining fish were humanely euthanized with tricaine methanesulfonate.

After loss of several exposure chambers due to high flows during the 30 day post swim up assessment, the number of chambers deployed at the control location was increased to six.

Survival of 45 day post-swim up trout

Greenback Cutthroat Trout "GBN", hatched July 9, 2016 at 11°C were transported from Colorado Parks and Wildlife Bellvue fish hatchery (45 days post swim up) on September 1, 2016 at 12.3°C. After acclimation to river temperature (15°C) they were distributed (15 /chamber) throughout exposure chambers at upstream, experimental and downstream sites. Food cubes were replaced every other day. Dissolved oxygen, pH, conductivity, temp, alkalinity and hardness were taken every other day. Fish were visually quantified and mortalities removed. At conclusion of the 10 day exposure, remaining fish were euthanized with tricaine methanesulfonate.

Survival and drift of Sac fry

Artificial redds were constructed to assess tendency of sac fry to drift when in unfavorable conditions. Transparent materials were used to allow visual assessment of sac fry

presence or absence. Two hundred fifty-ml Tri-pour® (VWR, Radnor, PA, USA) disposable beakers were modified and used as artificial redds within each exposure chamber. Five 5 cm by 3.18 mm slots were cut into the sides of each 250 ml Tri-pour® to facilitate flow. Field testing found no difference in dissolved oxygen within redds as compared to outside redds or the exposure chamber as measured by optical dissolved oxygen instrument (YSI, Yellow Springs, OH). Artificial redds were anchored to the base of exposure chambers by paired plastic coated magnets on the exterior of the chamber and interior of the artificial redd. To reduce chance of sac fry re-entry into redds, we exploited the sac fry's limited ability or willingness to swim upwards. To replicate substrate of a natural redd with transparent material, the artificial redds were filled with 100 ml of 1 cm diameter glass marbles to provide interstitial space to shelter sac fry.

Rainbow Trout sac fry (hatched October 1, 2016) were transported from Colorado Parks and Wildlife Rifle Falls fish hatchery on October 5, 2016 at 12°C. After acclimation to river temperature 7.2°C they were distributed (10 /chamber) throughout artificial redds in exposure chambers at upstream, experimental and downstream sites. Great care was taken to ensure all fry entered the artificial redds. Dissolved oxygen, pH, conductivity, temperature, alkalinity and hardness were taken initially and every other day. Fish were visually quantified and mortalities removed. At conclusion of the four day exposure, remaining fish were euthanized with tricaine methanesulfonate.

Results

Data analysis is ongoing. Generalized linear Models predicting minute by minute metal concentrations and water quality were created for each treatment cage and the best models will be selected using AIC. The following are being used to inform these models.

- Stream height/flow (on sight Logger every 10 min, downstream at USGS gauging station)
- Conductivity (every 2 days and continuous with loggers)
- pH (every 2 days)
- Temperature (every 2 days and continuous with loggers)
- Air temperature (continuous)
- Water Temperature (every 2 days and continuous with loggers)
- Solar Radiation (continuous with loggers and weather station)
- Precipitation (continuous from weather station)
- Dissolved metal concentrations s (every 2 days and continuous with loggers)
- Total metal concentrations
- Hardness and alkalinity (every 2 days by titration and by ICP-OES for hardness)
- Sulfate (weekly)

Survival of fish will be compared to observed metal concentrations and those predicted by models. This information will provide a dilution factor or minimal metal concentrations that will support fish for ten days for NFCC. Survival of fish in the years following the treatment plant will be compared to predictions made in this experiment.

We observed differences in survival when rain events occurred outside of the sampling days. These were captured by HOBO® conductivity and pressure loggers. The added realism of conducting these whole effluent toxicity tests in nature allowed us to discover spikes of metal pollution coming from Chase Gulch. Whole effluent tests in the laboratory would have missed these events.

Sac fry are believed to be more tolerant than 30 - 60 day post swim up age classes. Preliminary results show this to be true as sac fry survival was greater than that in 30 day post-swim up trout. Interestingly, sac fry drifted at lower concentrations (conductivity) than 30 day post-swim up trout survived. Drift removes organisms from an ecosystem in the same way mortality removes organisms from an ecosystem. However, traditional laboratory toxicity tests or fish cages without artificial redds would not have considered loss of sac fry to low levels of metals.

Statistical analysis of results and models is pending analysis.

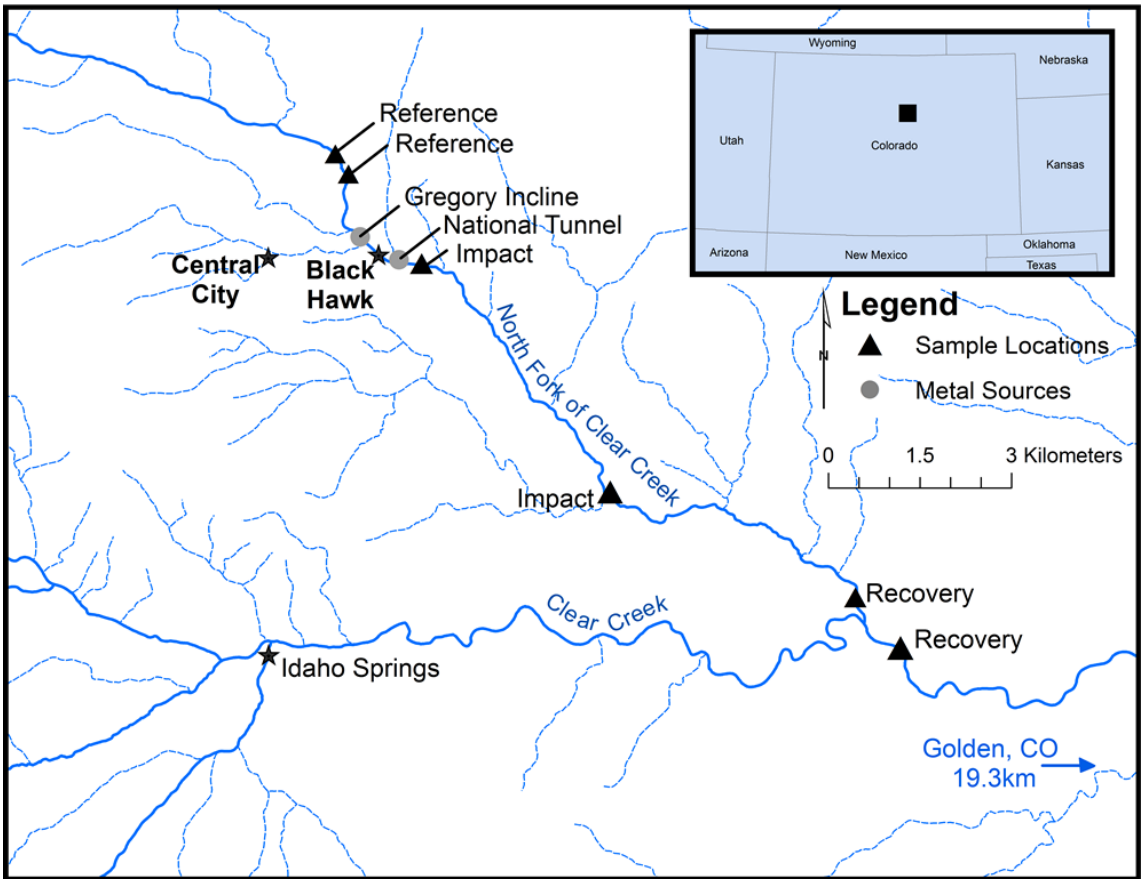

Figure 1: North Fork of Clear Creek Drainage.

Personnel: Pete Cadmus, Abbie Jefferson, James Pickert

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Action #1.2.3 – Assessment of whirling disease susceptibility in trout populations stressed by copper

●Level 1 Action Category: Data Collection and Analysis
●Level 2 Action Category: Research

1-Trout will be exposed to environmentally relevant concentrations of copper. This will be crossed with exposure to whirling disease per Fetherman *et al*. (2011, 2012)

2-Lethality and sublethal endpoints (*e.g.* fitness, growth, disease progression) will be assessed

Action #1.2.3 Accomplishments

Experiments exploring the effects of copper and whirling disease on trout

Heavy metals, including copper (Cu), have been shown to increase salmonid susceptibility to disease (Hetrick *et al*. 1997, Knittel 1981). Whirling disease, caused by the myxosporean parasite *Myxobolus cerebralis*, has been a major cause of trout population declines. Distributions of trout, minerals high in Cu and whirling disease co-occur in Colorado. Water quality standards do not consider multiple stressors simultaneously but special consideration is given to watersheds where toxicants, species and stressors co-exist.

Research exploring synergistic effects of whirling disease and heavy metal pollution is needed. Two experiments were performed: one to determine the viability of the parasite in varying Cu exposures, the other to measure the infection rate of whirling disease in the HxH (50:50), a cross between the Hofer and Harrison Lake Rainbow Trout strains, in three Cu concentrations.

Preliminary fish study

Experiments considering interactions of whirling disease and metal toxicity are complicated by numerous biological and toxicological constraints.

- Whirling disease infection is more severe in salmonids at a very young age (less than three months post swim up). Exposure should occur before approximately 970 degree days (Celsius) of development (Ryce *et al.* 2005).
- Sensitivity of salmonids to metals, especially Cu, is significantly greater at younger age classes (sac fry or just post swim-up juveniles). Lethal concentrations of Cu for a 30 day post-swim up trout would likely be insufficient to stress a trout that is 60 days post-swim up or greater.
- Myxospore counts used to characterize severity of whirling disease infections in salmonids can only be assessed after 1,820 degree days (Celsius) after exposure to triactinomyxon (TAM) life stage of the parasite. Deformities indicative of whirling disease occur when fry are infected with the parasite before cartilage has formed into bone. Malformed skulls, gills, bones and cartilage are common in infected adults. This requires that all experiments must be extended to a minimum of 20 weeks duration. This is far longer than the chronic limit of most toxicity trials (30 days).
- Strains of trout have varying resistance to whirling disease. A susceptible strain will have large mortality that will mask the effect of metal pollution. A strain deemed resistant to whirling disease might be less susceptible to infection (unable to show a Cu by whirling disease interaction) or might be more susceptible when exposed to aqueous metals.
- Cu and other heavy metal concentrations fluctuate in river systems depending on the climate, hydrology, parent material and anthropogenic disturbance. Although predicable on a site-specific basis, there is no one obvious level or fluctuation pattern for Cu in the laboratory studies.

Experiments examining Cu exposure and whirling disease interactions must have a start date (days of development) of toxicant exposure, a start date of whirling disease exposure and a study duration that is sufficient for infection at a non-lethal Cu concentration that addresses the constraints listed above.

A strain of Rainbow Trout that is moderately tolerant to whirling disease was exposed to three levels (plus control) of Cu starting at 697 degree days (Celsius) post-swim up, a developmental stage that is moderately sensitive to Cu. At nine weeks post-hatch, the age at which fish are still sensitive to whirling disease (Ryce *et al.* 2005), trout were exposed to TAMs.

Endpoints assessed included survival, growth rate, assimilation efficiency, myxospore count, splenosomatic index, viscerosomatic index, hepatosomatic index and whirling disease-related deformities.

TAM study

The tolerance of the TAM life stage of the whirling disease parasite to Cu is unknown. If TAMs are susceptible to Cu, exposure of fish to whirling disease in a Cu toxicity trial could produce spurious effects as Cu could have a protective effect against whirling disease infection. Additionally, if aqueous Cu had adverse effects on the TAM life stage natural ecosystems affected by Cu might have reduced whirling disease spread. To test the susceptibility of the triactinomyxon (TAM) life stage to Cu, we exposed TAMs to five levels of aqueous Cu (plus control) for 24 hours and then examined the viability of the TAMs per Fetherman *et al.* (2011,

2012). Cu concentrations straddled the Cu levels in the above preliminary fish study and those observed in Colorado rivers.

Methods

Preliminary fish study

Eight conditions (four replicates each) were assigned to thirty-two aquaria at Colorado Parks and Wildlife Aquatic Toxicology Laboratory (Fort Collins, CO) in a 4 x 2 factorial design. Control (0 µg/l), low (10 µg/l), medium (30 µg/l) and high (60 µg/l) levels of Cu were crossed with the presence or absence of whirling disease. Whirling disease treatments received exposure to whirling disease TAMs (Fetherman *et al.* 2011, 2012) at a density of 2,000 TAMs per fish. Each experimental unit had twenty-five fish for a total of 800 fish.

Experimental units consisted of 75 L aquaria that were held in recirculating water baths to maintain the desired testing temperature (12+/- 2°C). All units were held on two two-tiered bays that were custom built to allow for easy access and administration of appropriate conditions (control versus Cu exposures). Three stock solutions of copper sulfate ($CuSO_4$) were delivered by variable rate peristaltic pumps to mixing tanks that mixed stock and dechlorinated Fort Collins municipal water. Mixture was distributed to exposure tanks by food grade vinyl tubing via straw valves. Flow rates were set to 45 ml/min. Flow through conditions were maintained except for the duration of the TAM exposure.

Concentrations of Cu were assessed by inductively coupled plasma-optical emission spectrometry (ICP-OES) prior to the introduction of HxH fish. Subsequent confirmation of Cu concentrations was obtained from one of the four replicate blocks per week resulting in an assessment of all replicates within four weeks. Water quality parameters (pH, dissolved oxygen, hardness, alkalinity, ammonia, conductivity and temperature) were measured in a different replicate block each week on the same rotating schedule.

The HxH (50:-50) strain of Rainbow Trout was selected as a moderately tolerant strain of trout frequently stocked in Colorado. To make this hybrid, a resistant Rainbow Trout strain from a German hatchery (also known as the Hofer strain) was crossed with a Rainbow Trout strain from Harrison Lake, Montana (Fetherman and Schisler 2016). This strain has been regularly stocked in Colorado rivers for the past several years. The HxH eggs were spawned at and reared at the Bellevue Fish Research Hatchery (Bellvue, CO) at 12°C.

Post-swim up fry were initially fed live decapsulated brine shrimp (*Artemia nauplii*) and transitioned to Rangen brand trout feed when appropriate. Fish were fed in accordance with the Rangen feeding protocol for age and size class regarding size and rate. After transfer to Colorado Parks and Wildlife Aquatic Toxicology Laboratory, fish weight was assessed in each tank based on an initial subsample and on a bimonthly basis thereafter. Feed amount was adjusted weekly for each tank based on fish weight and the number of fish remaining. Feed per tank was readjusted if mortalities were observed. This ensured growth rate and assimilation efficiency could be assessed per tank. Fish were fed two times daily.

Swim up occurred on January 20, 2016. Beginning on March 17, 2016 at 697 degree days (Celsius) post-swim up, after holding in communal tanks at 12°C +/- 1°C, fish were transferred to experimental units (aquaria) and exposure to assigned Cu concentration (or control water) commenced for five days. Peristaltic pumps were turned off at the end of day five, ensuring that by day seven Cu had been removed from tanks. On day seven, water flow ceased and volume was reduced 50% in each experimental unit to ensure prolonged contact with TAMs in static conditions. TAMs were introduced into the appropriate tanks on March 24th, 2016 at 781 Degree days (Celsius). A combined total of 694,000 TAMs were administered to the corresponding experimental units. A maximum of 50,000 TAMs were placed in each unit based on the number of fish remaining after pre-exposure to Cu. Flow resumed in all tanks after one hour of TAM exposure in appropriate tanks. Cu exposure (if applicable) resumed the day following the TAM exposure. Blot dry weight were assessed in each experimental unit every two weeks from a subsample of fish to adjust feed amounts. Any mortalities that occurred throughout the study were collected and preserved.

The study concluded eight months after swim-up at 2,112 Degree days (Celsius). Fish were all terminated humanely with tricaine methanesulfonate. Common whirling disease related deformities were assessed by whirling disease experts. Standard length and total blot dry weight were measured. The liver, spleen and remaining viscera were removed and weighed for hepato-somatic index, spleno-somatic index and viscero-somatic index. The head was removed just posterior of the gill arches, frozen and sent to the Aquatic Animal Health Lab (Brush, CO) for myxospore enumeration. Confirmation of whirling disease infection was done by quantitative polymerase chain reaction (qPCR) assay testing for the presence/absence of *M. cerebralis* DNA by Pisces Molecular (Boulder, CO). Growth rate and assimilation efficiency were calculated from bimonthly assessments and final weights.

TAM Study

TAMs produced from freshwater oligochaetes (*Tubifex tubifex)* were collected from Parvin Lake Research Station (Red Feather Lakes, CO) and number of viable TAMs (ones showing clear, compact sporoplasms) were determined per Fetherman *et al*. (2011). Two 20 ml aliquots of TAM filtrate were delivered to 160 ml of copper sulfate stock solutions to create 0, 10, 19, 36, 72 and 135 µg/l exposure levels (n=3). Vessels were gently mixed and stored in an incubator at 12.0°C. After 24 hours, three subsamples of each experimental unit were taken and the proportion of viable TAMs were assessed per Fetherman (2011 and 2012). Dissolved (0.45 µm filter) and total Cu, and water quality was then sampled. The three subsample assessments of viability from each if the 18 experimental unit were averaged. Least Squares Means Tukey's multiple comparison were conducted using GLM procedure (SAS, version 9.3).

Results and Discussion

Infection of HxH fish in the preliminary study was unsuccessful based on myxospore counts and confirmed by qPCR. It may be that this strain of rainbow trout is more resistant to *M. cerebralis* in the age/size class exposed in this study or throughout their life history. If time and funding allow, this study will be repeated with more susceptible trout at a younger/smaller age class, but exploratory studies addressing changes in resistance with age/size should be completed

first. It is possible that residual Cu in exposure tanks affected survival, infection or development rates of *M. cerebralis*, but because there was no increased myxospore counts in control groups exposed to just whirling disease, this outcome seems unlikely. Results of the TAM study with Cu also support this conclusion. The viability of the TAMs was unaffected by Cu concentrations that were much higher than the highest Cu concentrations to which the fish were exposed ($p = 0.28$; Table 4; Figure 2).

Treatment	Viable TAMS Proportion	Target Cu (µg/L)	Dissolved Cu		Total Cu (µg/L)		Hardness (mg/L)	
			Ave	SE	Ave	SE	Ave	SE
Control	0.34	Control	1.25	0.08	1.77	0.35	26.20	0.54
Low	0.28	10	9.13	0.15	10.27	0.15	27.02	0.43
Medium	0.26	20	16.30	0.26	19.03	0.17	27.39	0.19
High	0.36	30	30.37	1.34	36.03	0.32	27.03	0.20
DoubleHi	0.33	70	63.47	2.84	72.00	0.51	27.10	0.44
TripleHi	0.32	120	96.93	5.75	134.67	2.33	25.83	0.57

Table 4. Water chemistry and proportion of viable tams. n=3 Hardness was estimated using the following equation. [Mg mg/L]*4.116) + ([Ca mg/L]*2.497) + ([Al mg/L]*5.564) + ([Fe mg/L]*1.729)+ ([Mn mg/L]*1.822) + ([Sr mg/L]*1.142) + ([Zn mg/L]*1.531). Ave = average, SE = standard error

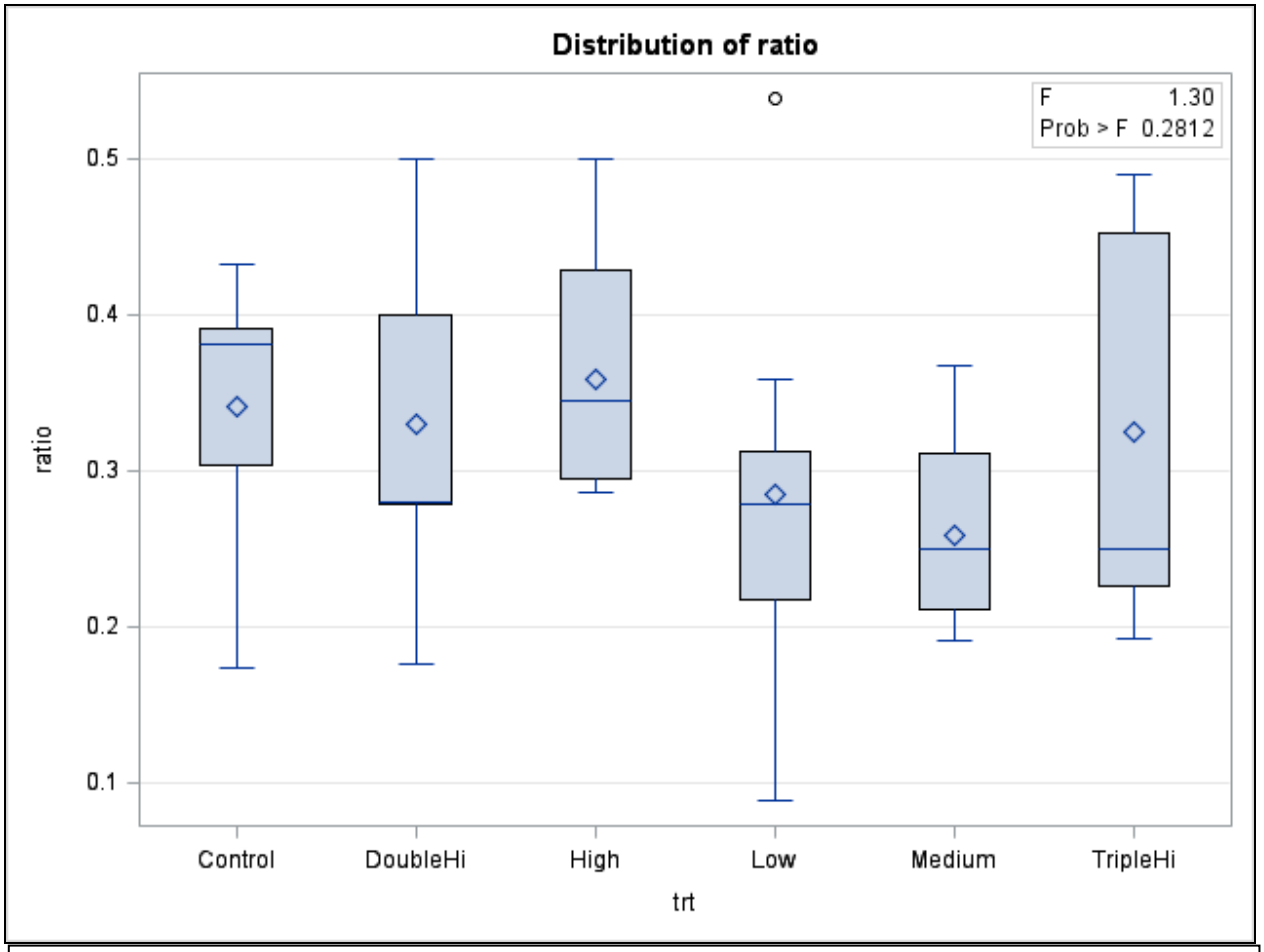

Figure 2. Boxplots of proportion of viable TAMs in each treatment group. There was no statistical significance

Personnel: Pete Cadmus, Eric Fetherman, Abbie Jefferson, Marta Hura and James Pickert

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Job No. 2: Water Quality Technical Assistance to Colorado Parks and Wildlife Personnel and Other State and Federal Agencies.

Job Objective: Provide technical support to CPW managers and other agencies.

Need

Water quality characteristics and pollution affect fish health and the viability of fisheries. Water chemistry and aquatic ecotoxicology demand specialized skill sets and unique instrumentation/infrastructure. Fisheries managers faced with chronic pollution issues, acute (accidental) spill events, fish kill events and other anthropogenic disturbances often need assistance with analysis of samples and characterization of toxicant effects before, during and after toxicological disturbance. Site specific and statewide water quality alterations risk compromising fisheries health in Colorado. Decision makers need to be informed of risks to Colorado's fisheries. Efforts to restore Colorado surface waters often require precise use of piscicides, which are difficult to assess in the field. However, the unique analytical capabilities of the CPW aquatic toxicology laboratory have recently been employed to provide this information on short turnaround using a mobile laboratory. Collaborators at state agencies and universities frequently approach research topics that complement the goals of CPW including those listed in Job A of this narrative. Providing these researchers with expertise and sharing equipment/infrastructure often produces better data that is useful to CPW.

Objectives

To provide technical assistance and expertise, consultation, evaluation and training in aquatic toxicology and aquatic chemistry to Colorado Parks and Wildlife and other state and federal personnel as requested. Assist in the investigation of fish kills. Conduct short or long term experiments to produce toxicity data, or develop site-specific field studies, when such data in the literature are lacking or inadequate. Collect and analyze water and/or fish tissues to assess water quality problems as requested. Analyze rotenone (and other piscicides) in water samples as part of Colorado Parks and Wildlife reclamation projects. Publish results of experiments and water quality investigations in peer-reviewed journals for consideration in policy making by other agencies.

Approach

Action #2.1 – Water Quality technical support to Colorado Parks and Wildlife managers and other agencies

●Level 1 Action Category: Data Collection and Analysis
●Level 2 Action Category: Research

As requested the following aid and support was provided by the Colorado Parks and Wildlife Aquatic Toxicology Laboratory:

1. Collected and analyzed water samples for rotenone as part of reclamation projects for Dry

gulch (September 27-29, 2016) and Hermosa Creek (August 2-7, 2016).

2. Continued to collect and analyze water samples and consult on biomonitoring and experimental design at mine restoration sites in Colorado including long term monitoring sites on the Arkansas River near Leadville, Animas River near Silverton and North Clear Creek near Blackhawk as needed.
3. Provided training for biologists on techniques to improve information gathering during spill events.
4. Provided experimental design support in investigations examining indirect or physical toxicity of Fe, Al and/or Mn in mine impacted watersheds as requested during mine restoration efforts and/or increases in mine pollution.
5. Collected, analyzed and interpreted water samples and biotic samples as part of fish kill investigations, pollution investigations and restoration efforts as requested.
6. Conducted biological monitoring and field experiments as part of reclamation projects that allow managers to better predict effects of rotenone on target and non-target fish as well as the insects and algal species that support sport fish populations as requested.
7. Provided the fisheries managers of Colorado and other states with milt extender as requested.

And

8. Provided ecotoxicological support and expertise to CPW managers, Colorado universities and fellow natural resource management agencies as requested.

Personnel: Pete Cadmus, Abbie Jefferson, Andrea Kingcade, James Pickert, Adam Augustine, Alex Townsend and Steven Brinkman.