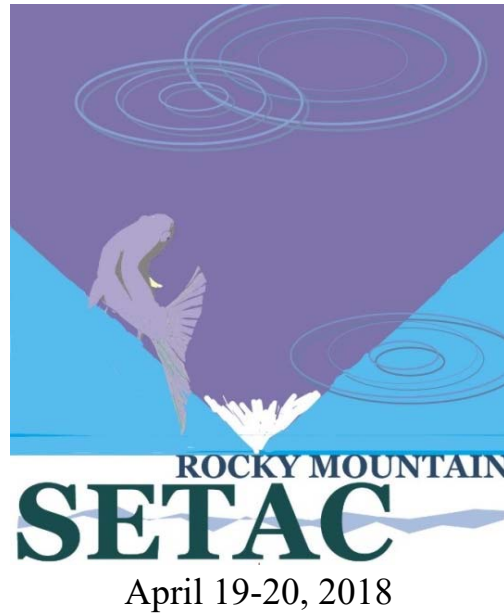


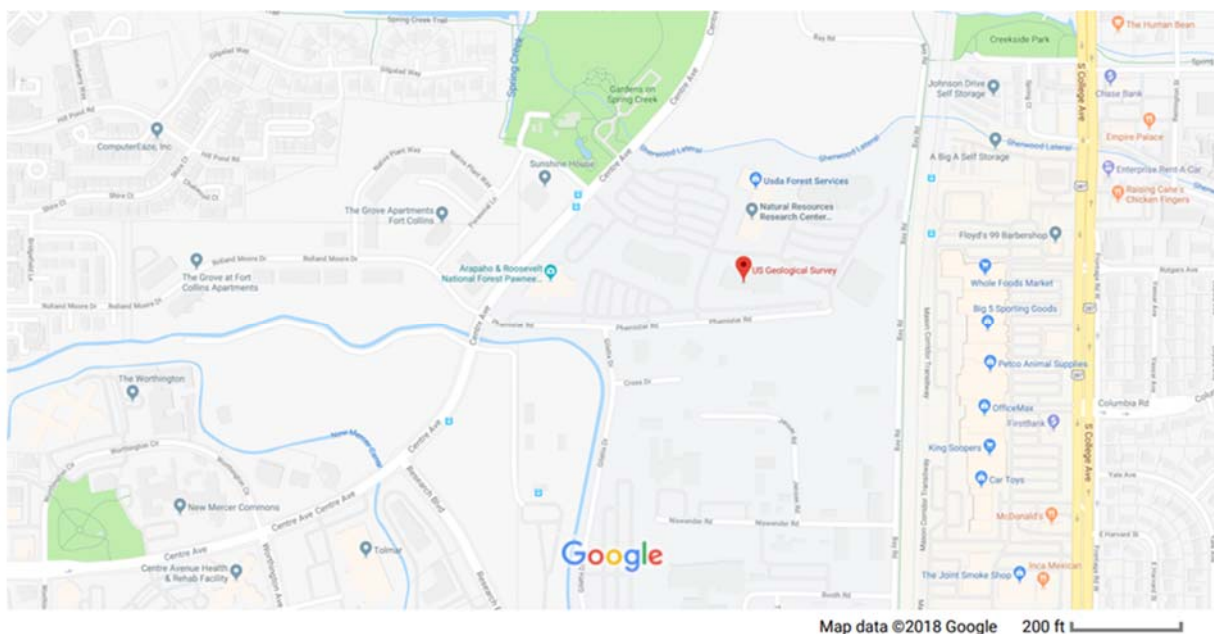
**31st Annual Meeting of the Rocky Mountain Chapter of the Society of
Environmental Toxicology and Chemistry**



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Meeting Agenda
USGS Fort Collins Science Center
Conference Room 2000

Day	Time	Activity
Thursday, April 19	10:00 – 3:00	Workshop: Unmanned Aircraft Systems technology in scientific research
Friday, April 20	8:30-9:15	Registration, Refreshments, Breakfast Snacks
	9:15-9:30	Opening Remarks
	9:30-10:15	Plenary Presentation
	10:15-10:45	Break/Posters
	10:45-11:30	Oral Presentations
	11:30-1:30	Lunch & BoD Meeting
	1:30-2:30	Oral Presentations
	2:30-3:00	Poster Review, Refreshments, Break
	3:00-4:00	Oral Presentations
	4:00-4:30	Raffle and Student Awards
	4:30	Meeting Adjourn

WORKSHOP: UNMANNED AIRCRAFT SYSTEMS IN SCIENTIFIC RESEARCH

Thursday April 19, 2018

Conference Room 2000
Fort Collins Science Center, Fort Collins, Colorado

Jeff Sloan
Project Leader, USGS – National UAS Project Office

Overview of Unmanned Aircraft Systems technology applications for scientific research and operational geospatial data collection

Cutting edge UAS technology receives much attention in the media, within universities, in public agencies and in the commercial industry. However, the collection and analysis techniques of aerial imagery from sensors on-board UAS are actually not significantly different than that of traditional data historically captured by satellites, manned aircraft and many other aerial remote sensing platforms for the past several decades by USGS scientists. The advent of UAS however, does provide exciting new capabilities by increasing data collection mobility, resolution, and safety, as well as reducing operational data collection costs. Results from the previous 10 years of UAS research by the USGS has shown great potential for the technology, which is now providing a new tool in the toolbox for researchers, applied science and land managers.

Background Required: No background is required for exploring the uses of UAS in remote sensing for scientific research. Knowledge of aviation rules, basic sensor capabilities and fundamentals of geospatial data is advantageous, but many studies have suggested that the most successful UAS programs do not require specialized staffing or long-term specific experience in order to benefit their programs and contribute to their new geospatial data acquisition capabilities.

Key Outcomes: Attendees will have a good overview of what UAS platforms and sensors are available in the public and commercial industry for use in research and operational activities. Basic knowledge of computer vision (structure-from-motion) photogrammetry will be explained so the three major pieces of UAS technology (platform, sensor and data processing) for data acquisition and mapping will be understood.

ORAL PRESENTATIONS SCHEDULE – Friday, April 20, 2018 Conference Room 2000

- 9:30-10:15 **Schmidt, Travis.** *U.S. Geological Survey.* Plenary presentation: Real life: understanding responses to contaminant mixtures in complex communities
- 10:15-10:45 Poster Review, Refreshments, Break
- 10:45-11:00 **Winkelman, Dana.** *U.S. Geological Survey.* Evaluating wastewater effluent exposure in wild fishes using insights from laboratory and wild populations.
- 11:00-11:15 ****Rupprecht, Meaghan.** *Colorado State University.* Spatial and temporal analysis of tissue contaminants in fish along the Ohio River.

- 11:15-11:30 ****Townsend, Alex.** *Colorado State University.* The effect of maternally-transferred selenium on the survival, hatch success and performance of Brown Trout fry.
- 11:30-1:30 Lunch & BoD Meeting & Posters
- 1:30-1:45 **Young, Liu.** *University of Colorado Denver.* Kinetics study of heterogeneous reactions of n-butylamine with succinic acid using an ATR-IR flow reactor.
- 1:45-2:00 **Richardson, Kristy.** *Colorado Department of Public Health and Environment.* Perfluorinated compounds in the Widefield Aquifer.
- 2:00-2:15 **Larson, Emily.** *Geosyntec Consultants.* Modeling avian exposures to perfluoroalkyl substances in aquatic habitats impacted by historical aqueous film forming foam releases.
- 2:15-2:30 ****Wolff, Brian.** *Colorado State University.* Responses of mountain stream microbial communities along a gradient of metals contamination.
- 2:30-3:00 Poster Review, Refreshments, Break
- 3:00-3:15 **Cadmus, Pete.** *Colorado Parks and Wildlife.* Tools for predicting recovery of aquatic ecosystems after mine reclamation: Biomonitoring and in-stream experiments on the North Fork of Clear Creek, Colorado.
- 3:15-3:30 ****Murphy, Jill.** *Colorado School of Mines.* Dietborne bioavailability of copper in acid mine drainage-related particles to freshwater snails (*Lymnaea stagnalis*), and the importance of organism size in a reverse-labeling stable-isotopic method.
- 3:30-3:45 ****Challis, Katie.** *Colorado School of Mines.* Toxicity of a mixture of copper and dissolved organic carbon leached from micronized copper azole (MCA)-treated lumber to *Daphnia magna*.
- 3:45-4:00 **Wildeman, Thomas.** *Colorado School of Mines.* Arsenic and thallium in foothills domestic wells.
- 4:30 Meeting Adjourn

** Indicates presentations being considered for best platform presentation

Plenary Presentation: Dr. Travis Schmidt (tschmidt@usgs.gov)
U.S. Geological Survey, Colorado Water Science Center, Denver, CO

Real life: understanding responses to contaminant mixtures in complex communities

Multiple stressors impact aquatic environments at the species level, community level, and across ecosystem boundaries. To understand and predict environmental responses to multiple stressors, including contaminant mixtures in aquatic and linked riparian systems, I have employed a combination of field, laboratory and modeling approaches. Mesocosms, in particular, have proven to be a very useful tool for evaluating community level effects of stressors, allowing identification of causal relationships while maintaining more realistic environments (e.g., indirect community interactions, contaminant synergism) than typically found in standard laboratory toxicity tests. Results from these studies are helping managers to better evaluate water-quality criteria, to design and implement restoration plans for contaminated aquatic systems, and to assess the effectiveness of remedial actions.

Dr. Travis Schmidt is a Research Ecologist at the USGS Colorado Water Science Center. He received a B.S. in Ecology from Pennsylvania State University, M.S. in Aquatic Toxicology from Virginia Tech, and Ph.D. in Ecotoxicology from Colorado State University. Dr. Schmidt's career has shown him to be a renaissance ecotoxicologist who embraces the trifecta of community ecology: field surveys, laboratory experiments and modelling.

Abstracts for Platform Presentations
(Alphabetical by presenter)

Pete Cadmus, Colorado Parks and Wildlife. Aquatic Research Section. Fort Collins, Colorado 80526, pete.cadmus@state.co.us. 970-472-4332; **Abbie L. Jefferson**, Colorado Parks and Wildlife. Aquatic Research Section. Fort Collins, Colorado, 80526. abbie.jefferson@state.co.us; **Christopher J. Kotalik**, Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, Colorado, 80523. cjkotalik@gmail.com

Tools for predicting recovery of aquatic ecosystems after mine reclamation: Biomonitoring and in-stream experiments on the North Fork of Clear Creek, Colorado.

The construction of a mine effluent treatment plant in Blackhawk, Colorado, has potential to markedly improve water quality in the North Fork of Clear Creek (NFCC). Up-stream of Blackhawk, biomonitoring has shown fish, algae and macroinvertebrate populations to be healthy. However, immediately downstream the first mine adit aquatic life and function was largely limited by metal pollution. The restoration efforts in Blackhawk present a novel opportunity to develop tools to predict the recovery of aquatic life to the North Fork of Clear Creek. Several in-stream experiments were conducted to predict response of fish, aquatic insects and algae. Fish cages across a gradient of pollution were used to predict what species of trout could survive in NFCC after restoration. Metal concentrations were modeled using conductivity and depth data loggers to better capture natural fluctuations in metal concentrations that are often missed by daily or weekly sampling. Fish survival, fish drift behavior and pulse amplitude modulated fluorometry of periphyton were used across a gradient of metal mixtures to determine at what level or restoration these populations might survive. These tools can be used to a) prioritize possible mine

sites b) predict what species are best for reintroduction to restoration sites and c) study effects of metal mixtures and fluctuations in an environmentally relevant setting.

Katie Challis, Department of Chemistry, Colorado School of Mines, Golden, Colorado 80401, kchallis@mines.edu, (715) 862-0196; Grant Engberson, Colorado School of Mines; Joseph S. Meyer, Colorado School of Mines, and Applied Limnology Professionals LLC, Golden, Colorado 80401; James F. Ranville, Colorado School of Mines.

Toxicity of a mixture of copper and dissolved organic carbon leached from micronized copper azole (MCA)-treated lumber to Daphnia magna.

Micronized copper azole (MCA) is a mixture of ball-milled (micronized) CuCO_3 and tebuconazole ($\text{C}_{16}\text{H}_{22}\text{ClN}_3\text{O}$) that is pressure-infused into outdoor residential-use lumber as a preservative. Copper leaches from the treated lumber during outdoor weathering experiments, but the toxicity of the resulting mixture of copper and wood leachate has not previously been tested. We leached MCA-wood sawdust in MilliQ water, resulting in a dissolved copper concentration of 5.65 mg Cu/L and a dissolved organic carbon (DOC) concentration of 173 mg C/L. Then we conducted standard 48-h lethality tests with *Daphnia magna* exposed to copper in moderately hard reconstituted water containing four different types of DOC: (1) Suwanee River fulvic acid (SRFA), (2) DOC leached from untreated wood, (3) DOC leached from MCA-treated wood, and (4) no DOC. Copper concentrations ranged from 0 to 100 $\mu\text{g/L}$ in the exposures, and DOC concentrations were 3 mg C/L in the first three waters. Median effect concentrations ($\text{EC}_{50\text{s}}$) were 60, 26, 29, and 17 $\mu\text{g Cu/L}$ in waters 1, 2, 3, and 4, respectively. Specific UV absorbances of the DOC at 254nm were 4.0 and 0.95 L/mg C·m for SRFA and leached DOC, respectively, suggesting leached DOC had lower aromaticity. We conclude that DOC leached from lumber is not as protective against copper toxicity as natural aquatic DOC (represented by SRFA), probably because of a lower affinity of DOC from lumber for copper. Thus, DOC concentration alone will be an inadequate predictor of DOC amelioration of copper toxicity in waters that receive MCA-treated-wood leachate.

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Modeling avian exposures to perfluoroalkyl substances in aquatic habitats impacted by historical aqueous film forming foam releases

Releases of Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) associated with Aqueous Film Forming Foams (AFFFs) have the potential to impact on-site and downgradient aquatic habitats. Dietary exposures of aquatic-dependent birds were modeled for seven PFASs (PFHxA, PFOA, PFNA, PFDA, PFHxS, PFOS, and PFDS) using five different scenarios based on measurements of PFASs obtained from five investigations of sites historically-impacted by AFFF. Exposure modeling was conducted for four avian receptors representing various avian feeding guilds: lesser scaup (*Aythya affinis*), spotted sandpiper (*Actitis macularia*), great blue heron (*Ardea herodias*), and osprey (*Pandion haliaetus*). For the receptor predicted to receive the highest PFAS exposure (spotted sandpiper), model-predicted exposure to PFOS exceeded a laboratory-based, No Observed Adverse Effect Level exposure benchmark in three of the five model scenarios, confirming that risks to aquatic-dependent avian wildlife should be considered for investigations of historic AFFF releases. Perfluoroalkyl sulfonic acids (PFHxS, PFOS, and PFDS) represented 94% (on average) of total PFAS exposures due to their prevalence in historical AFFF formulations, and increased bioaccumulation in aquatic prey items and partitioning to aquatic sediment relative to perfluoroalkyl carboxylic acids. Sediment-associated PFASs (rather than water-associated PFASs) were the source of the highest predicted PFAS exposures and are likely to be very important for understanding and managing AFFF site-specific ecological risks. Additional considerations for research

needs and site-specific ecological risk assessments are discussed with the goal of optimizing ecological risk-based decision making at AFFF sites and prioritizing research needs.

Jill Murphy, Colorado School of Mines, Golden, CO 80401, jkmurphy@mymail.mines.edu; Joseph S. Meyer, Applied Limnology Professionals, LLC, Golden, CO 80401, jsmeyer@alpscolorado.com; James F. Ranville, Colorado School of Mines, Golden, CO 80401, jranvill@mines.edu

*Dietborne bioavailability of copper in acid mine drainage-related particles to freshwater snails (*Lymnaea stagnalis*), and the importance of organism size in a reverse-labeling stable-isotopic method*

Although the detrimental environmental impacts of acid mine drainage (AMD) have been extensively researched, little is known about the real-time responses of mining influenced systems to remediation efforts. To address this information gap, we examined the bioavailability of copper (Cu) from iron-oxyhydroxide (FeOOH) sediment coatings collected from the North Fork of Clear Creek (NFCC) in Black Hawk, Colorado. For more than a century, NFCC was contaminated with AMD until the completion of a high-density-sludge lime treatment plant in March 2017. Using an innovative reverse-labeling stable-isotope method developed by U.S. Geological Survey researchers, we calculated Cu assimilation efficiency (AE) in freshwater snails (*Lymnaea stagnalis*) that were fed sediment-coating particles collected from NFCC before and after remediation of the AMD. Although Cu bioavailability did not differ significantly between pre- and post-remediation FeOOH particles (~34% AE), high among-replicate variability resulted in low statistical power to infer significant differences. Our use of small organisms (<4 mg dry tissue weight) in the experiments may have contributed to the relatively high variability, especially related to (1) estimation of differences in tissue Cu concentrations between exposed and background snails, and (2) complete collection of snail feces after the dietborne exposures. These size constraints may have important implications for the planned adaptation of this method to smaller benthic organisms that are more ecologically relevant in high-gradient, montane streams (e.g., the midge *Chironomus dilutus*).

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Kinetics study of heterogeneous reactions of n-butylamine with succinic acid using an ATR-IR flow reactor

Kinetics of heterogeneous reaction of n-butylamine (BA) with succinic acid (SA) were studied via a flow system combined with attenuated total reflection infrared Fourier-transformed spectroscopy (ATR-FTIR) over wide ranges of BA concentrations (0.145-32.5 ppm), thin film thicknesses (0.05-0.15 μm), temperatures (263-295 K), and relative humidities (0-75% RH) under atmospheric pressure condition for the first time. Pseudo-first-order rate constants k_{app} and overall reactive uptake coefficients γ values were derived according to the changes in absorbance from peak located near 1634 cm^{-1} , which can be assigned to $-\text{COO}^-$ antisymmetric stretch ($\nu_{\text{as}}(-\text{COO}^-)$). The results show that the heterogeneous reaction of SA with BA follows Langmuir-Hinshelwood mechanism and k_{app} is largely dominated by surface reaction over bulk phase reaction. The studies of the influence of temperature and RH on the reaction of SA with BA show that both the k_{app} and γ values display very strong temperature and RH dependence. Low temperature promotes the reaction mainly due to the physisorption of BA predominated in the whole reaction. With an increasing RH, both the k_{app} and γ values increase indicating that the water vapor enhances the reaction. In addition, the water uptake results indicate that the hygroscopic behavior of the thin film is enhanced after BA exposure.

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Perfluorinated compounds in the Widefield Aquifer

Perfluorinated compounds (PFCs) are a family of organofluorine chemicals that have been used for several decades in various products including fire fighting foams, coating additives, and surface protection products for carpets and clothing because of their ability to resist heat, oil, stains, grease and water. EPA's third unregulated contaminant monitoring rule, published in 2012, required public drinking water systems to test for 6 PFCs (but did not regulate these chemicals). PFCs were detected in the Widefield Aquifer region, located south of Colorado Springs, in water and other environmental samples. Levels throughout the aquifer exceed the drinking water health advisory level designed to provide a margin of safety from the potential toxic effects of these chemicals. The local water suppliers had to alter their use of groundwater to reduce PFC levels in drinking water, and many smaller private wells and small water systems turned to alternate sources of drinking water. Treatment systems are being installed, so the use of the Widefield Aquifer as a drinking water source may again increase. CDPHE has also issued recommendations to allow a level of protection from PFCs for residents growing and eating food in the region. As there is no natural degradation process of PFCs, these chemicals will leave a lasting impact on the region. In order to drive clean-up of the area, a site-specific groundwater standard has been proposed for the area. The outcome of the scheduled April 2018 rulemaking hearing will also be discussed.

Meaghan Rupprecht, Undergraduate Student, Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, Colorado 80523, Meaghan.rupprecht@gmail.com

Spatial and temporal analysis of tissue contaminants in fish along the Ohio River

Contamination of aquatic ecosystems has become a great concern in recent years. Mercury and polychlorinated biphenyl (PCB) contamination especially have resulted in human health advisories across the United States. Previous studies conducted by the EPA have emphasized this concern for mercury and PCB contamination due to the prevalence of both contaminants in sampled fish. The Ohio River is considered one of the most contaminated aquatic ecosystems in the United States, and displays the contamination of both compounds in fish tissues. Data from the Ohio River Valley Sanitation Commission (ORSANCO) has monitored the levels of both contaminants in fish tissues along the river since 1983. Changes in the contamination of fish tissues over time and according to sampling location are analyzed in this paper, in addition to the differences of contamination between feeding guilds. Feeding guild results are also discussed in relation to both contaminants' ability to bioaccumulate in food chains. Urbanization and agricultural use are typically attributed to increased contamination by both chemicals, so the impact that land use change could be having on the contamination levels in fish tissues is considered as well.

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The effect of maternally-transferred selenium on the survival, hatch success and performance of Brown Trout fry

Colorado has geologic sources of selenium that are transported into aquatic systems via natural and anthropogenic activities such as weathering, sediment transport, irrigation of soils and mining. Dietary

selenium is maternally transferred to fry and can have a negative response on reproduction. These endpoints have been studied for several species, however few data are available on the effects of maternally-transferred selenium on sub-lethal fitness measures such as the ability to escape predation, thermal tolerance and oxygen tolerance. Furthermore, few studies have analyzed selenium concentrations in multiple trophic compartments simultaneously with fish samples. We are investigating the effects of maternally-transferred selenium on fish reproduction and the movement of selenium through various food web compartments. In the fall of 2016 and 2017 Brown Trout from 10 locations in Colorado were collected to assess their reproductive success and fitness of offspring. Eggs were fertilized streamside and transported to the CPW Aquatic Toxicology Laboratory in Fort Collins, CO where they were reared to the swim-up stage. Hatching success, survival to swim-up, fast-start response, critical dissolved oxygen minima and critical thermal maxima were evaluated for the eggs and fry. Muscle and egg tissue samples from each female fish were analyzed for total selenium. At each location samples of water, sediment, periphyton and invertebrates were collected and analyzed for total selenium. The environmental selenium concentrations are being used to evaluate the movement of selenium through a relatively simple aquatic food web.

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Arsenic and thallium in foothills domestic wells

An assessment study of domestic wells that penetrate Front Range sediments is being conducted. The wells are used by homes along Pine Ridge Rd. just north of Golden. The presence of As in the wells has been known for 20 years and many residences have a reverse osmosis system to eliminate the problem. The objective of this study is to determine what factors cause the presence of As in the wells and includes the question of seasonal variation in contaminant concentrations. Starting in mid-August of 2017, 5 wells have been sampled 5 times. The sampling times have roughly correlated with changes in precipitation. Highlighting the complexity of the aquifers, each well is different from the others. One well penetrating Precambrian metamorphics contains no contaminants. Another well has neutral pH and low ionic conductivity but yet contains As and Tl in concentrations that are 3 to 10 times above drinking water limits. A third well has a pH ~ 8 and ionic conductivity of 1700 $\mu\text{S}/\text{cm}$. It has minimal iron but yet has As that is 3 times drinking water limits. The fourth well appears to have penetrated an aquifer associated with mining. It has a pH of 4.7 and over 1 mg/L of Fe. Both As and Tl are 10 to 20 times limits. Finally the fifth well is neutral with an ionic conductivity of 270 $\mu\text{S}/\text{cm}$. Yet it has As concentrations that can be 50 times limits. Trends that might affect the concentrations of contaminants will be explored.

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Evaluating wastewater effluent exposure in wild fishes using insights from laboratory and wild populations.

Complex chemical mixtures in effluents below municipal wastewater treatment plants (WWTPs) are difficult to assess and their effects on organisms are poorly understood. In the South Platte River basin in Colorado, estrogenic impacts from treated municipal wastewater have been observed, but there is limited

data about the consequences of estrogen exposure for wild organisms. Our goal was to evaluate several species of wild fish for evidence of estrogen exposure and to determine if the effects observed in laboratory raised Fathead Minnow (FHM, *Pimephales promelas*) caged in wastewater were similar to those observed in wild fish populations. To accomplish this goal, we placed ten laboratory raised male FHM in cages both upstream and downstream of a WWTP effluent for one week, and then euthanized them, and extracted their livers for Vitellogenin (VTG) analyses. The presence of VTG is a common biomarker for estrogen exposure in male fish, and VTG presence was determined using qPCR. We also captured wild fish species (*Catostomus commersonii*, *Rhinichthys cataractae*, and FHM) at the WWTPs where we caged FHM, and measured VTG protein in wild fish blood plasma using ELISA. Finally, we caged wild FHM at the same locations in which we caged laboratory raised FHM and measured VTG production. Analysis for wild fish VTG data is ongoing, but our initial results indicate that wild FHM captured at the caging sites did not express similar levels of VTG as their caged laboratory raised counterparts. Additionally, caged wild FHM expressed more VTG than unconfined wild FHM, but less VTG than the laboratory raised FHM caged at the same spot downstream of the WWTP effluent. Our results indicate that the response of VTG production in laboratory raised FHM is more sensitive to estrogen exposure than VTG production in wild FHM, demonstrating that wild FHM seem to be acclimated or adapted to living in WWTP effluent compared to the naïve laboratory population. These results have consequences for the potential inferences being made between laboratory and wild populations and indicates that more research needs to be done to understand the differences between wild and laboratory raised populations.

Brian A. Wolff, Department of Fish, Wildlife and Conservation Biology, Colorado State University, 1474 Campus Delivery, Fort Collins, CO 80523; William H. Clements, Department of Fish, Wildlife and Conservation Biology, Colorado State University, 1474 Campus Delivery, Fort Collins, CO 80523; Ed K. Hall, Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523

Responses of mountain stream microbial communities along a gradient of metals contamination

The upper Arkansas River has been impaired by metal pollution due to historical mining in nearby Leadville, Colorado. Implementation of water treatment facilities and on-site treatment of floodplain mine tailings resulted in significant improvements in water quality. Despite improved water quality, macroinvertebrate community structure remains different between upstream reference sites and sites downstream of California Gulch, the inlet that contributes metal contamination. One hypothesis for this observation is that metal contamination alters the stream microbial communities, which may structure macroinvertebrate communities through altered microbial biomass (e.g. food quality). To address this, we sampled benthic sediments at multiple sites upstream and downstream of California Gulch, across multiple seasons (spring and fall), and years (2015 – 2017). Microbial community composition was measured using 16S rRNA gene amplicon sequencing. Our results show: (1) metals concentrations were elevated at downstream sites relative to upstream communities, (2) microbial communities were different between sites upstream and downstream of California Gulch, and (3) dietary metals exposure is likely an important driver of macroinvertebrate community structure.

Abstracts for Poster Presentations (Alphabetical by presenter)

****David Bertolatus**, Department of Integrative Biology, University of Colorado Denver
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Network analyses to quantify the association between individual contaminants and biological responses in environmental mixture studies

Aquatic habitats are often contaminated with complex mixtures of pollutants. Interpreting biological effects caused by mixture exposures is an ongoing challenge in ecotoxicology. Exposure assessments utilizing various omics technologies have been valuable for characterizing overall effects of environmental mixtures and establishing the ubiquity of impacts throughout aquatic habitats. However, quantitative approaches are needed to begin narrowing down the long list of potential causal agents and provide more specificity to risk assessors and managers. Here, we will leverage existing chemical and transcriptomics datasets to estimate the association between the occurrence and concentration of individual chemicals and transcriptomic changes following exposure. Both data-driven and hypothesis-driven analyses will be used to establish linkages between contaminants and molecular effects in organisms. Our hypothesis-driven approach will use existing databases of chemical:gene interactions to develop hypotheses regarding patterns of differential gene expression expected for an individual chemical and test these using Gene Set Enrichment Analysis. Significant enrichment of a gene set for a given chemical suggests a causal role for that chemical. The data-driven approach will model correlations between detected contaminants and individual genes. The resulting high-dimensionality correlation matrix will be used to build a bipartite network and identify the contaminants with the highest degree (significant correlations to gene expression). This will provide another line of evidence to identify chemicals with a potential causal role in the observed biological impacts. The intersection of these approaches will be used to identify a candidate list of contaminants that are likely contributing to exposure effects in complex environmental mixtures.

****Evan Lloyd**, Colorado School of Mines, MS Student, Department of Chemistry and Geochemistry, Golden CO 80401, evanhunterlloyd@mines.edu; James Ranville, Colorado School of Mines, Professor, Department of Chemistry and Geochemistry, Golden CO 80401, jranvill@mines.edu; Dana Portlock, Colorado School of Mine, Undergraduate Student, Department of Chemistry, Golden CO 80401, dportlock@mines.edu

Water chemistry of North Fork of Clear Creek pre- and post-remediation of acid mine drainage effluents. North Fork of Clear Creek (NFCC), located in Black Hawk, Colorado, has been affected by acid mine drainage (AMD). This is the result of the extensive hard rock mining that occurred in the area since the late 1800s. The Central City/Black Hawk area was placed on the list of EPA Superfund sites in 1983. In April 2017, a lime-based high density sludge (HDS) water treatment plant was brought online by the Colorado Department of Public Health and the Environment (CDPHE). The plant addresses Gregory Incline and National Tunnel, which are the two point sources of AMD. The water treatment plant removes metals from these two sources and reintroduces treated water back into North Fork of Clear Creek. We have monitored the water chemistry of the site on a monthly basis since the water treatment plant has been online. After one year of remediation, the plant has been successful at reducing the water column metal concentrations. At a sampling location, directly below the water treatment plant, levels of iron, copper, and zinc dropped from 19.3 mg/L to 0.193 mg/L, 0.111 mg/L to 0.0290 mg/L, and 1.94 mg/L to 0.518 mg/L, respectively. The resulting decreased metal concentrations are a positive sign for the aquatic health of NFCC. However, even after the treatment of the two point sources, the zinc concentration is still elevated, indicating that there are other potentially sources of from a non-point source affecting the water quality of NFCC.

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Culturing and feeding evaluation of the Daphnia magna four-day survival and growth test method
In 2009, EPA researchers James Lazorchak, Mark Smith, and Herman Haring published a new test method based on the growth endpoint for the *Daphnia magna* species. The *Daphnia magna* four-day survival and growth test method was developed to provide an additional option to evaluate the potential for sublethal toxicity in the aquatic environment (Lazorchak, 2009). Current methods for the species involve an acute exposure of only 24 to 96 hours, or long-term chronic exposures of 21 to 28 days. The proposed method would allow a shorter duration test while still incorporating the sublethal endpoint. Recently, many dischargers in the Western United States have been struggling to meet whole effluent toxicity (WET) sublethal limits for *C. dubia* due exclusively to interference from the ionic balance of total dissolved solids (TDS) components in their effluent. While the four-day *D. magna* method has not been formally approved by the United States Environmental Protection Agency for compliance testing, we have begun to evaluate concurrent chronic *C. dubia* and four-day *D. magna* tests in various waters in Colorado to determine whether the method could potentially isolate the TDS interference while still providing an estimation of true toxicity. At the 2016 and 2017 SETAC conferences, we presented several key observations and suggested modifications to the method focusing on control performance criteria requirements. This current study focuses on nutrition and culture practices to eliminate factors that may be affecting the reproducibility and test performance. The future of the method will also be discussed.

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Effect of benthic macroinvertebrate size on mortality due to trace metal contamination in Colorado streams.

Benthic macroinvertebrates are an important food source for fish and other species in stream ecosystems. Their ecological role in these systems, along with their short life cycles, high abundance, and their differential sensitivity to trace metals, makes them great indicators of river health. They are often employed in ecotoxicology studies; however, minimal research has evaluated how different instar sizes respond to contamination. The goal for our study was to assess the responses of different size classes of benthic macroinvertebrates to trace metals contamination using stream mesocosms. Benthic communities were collected from the Arkansas River, Colorado using artificial substrates and treated with different concentrations of Cu and Zn. Results showed a significant interaction effect between instar size and mortality, whereby smaller instars displayed greater mortality compared to more mature later instars individuals. These results are important because they demonstrate that the effect of metals contamination to aquatic insects is most sensitive at the smaller instar levels, a size class that is used infrequently in ecotoxicology assessments. Our study shows that by examining all instar size classes, including early instars, researchers can better predict population-level responses to metals contamination in streams ecosystems.

****** Indicates presentations being considered for best poster presentation