30th Annual Meeting of the Rocky Mountain Chapter of the Society of Environmental Toxicology and Chemistry

April 13-14, 2017

American Mountaineering Center
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Golden, Colorado

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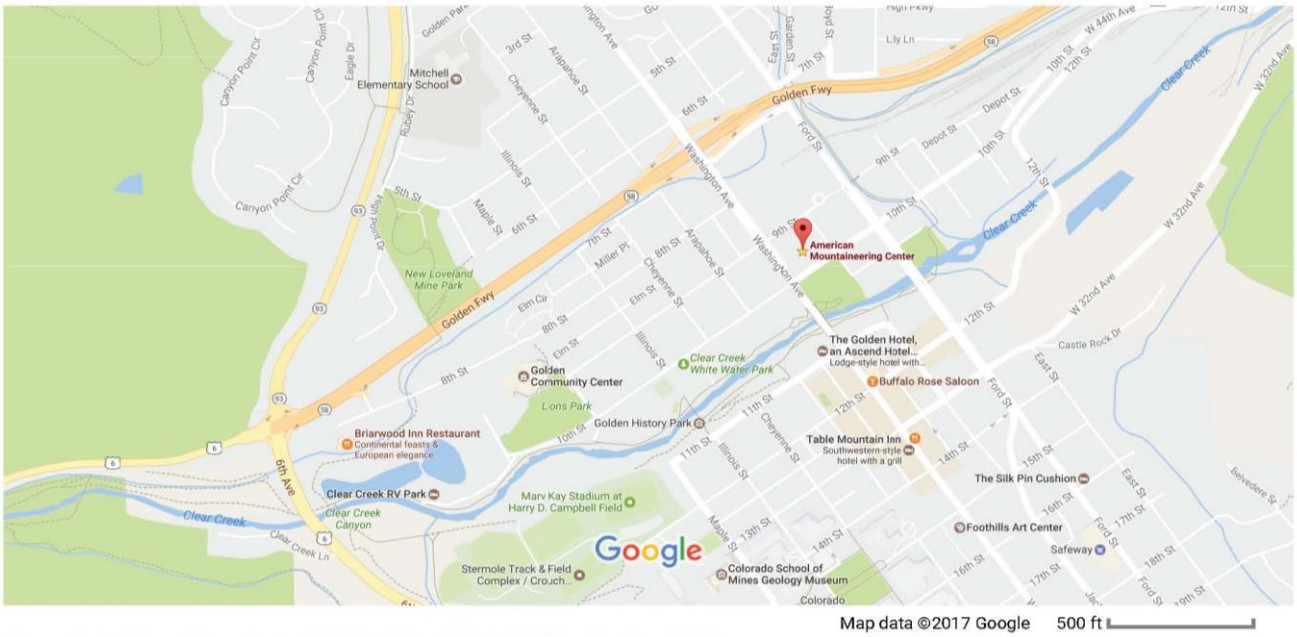

American Mountaineering Center
710 10th Street
Golden, Colorado

Meeting Agenda
American Mountaineering Center
Conference Room C & D

Day	Time	Activity
Thursday, April 13	8:30-4:00	Workshop: Information-theoretic methods in empirical science
Friday, April 14	8:30-9:15	Registration, Refreshments, Breakfast Snacks
	9:15-9:30	Opening Remarks
	9:30-10:15	Plenary Presentation
	10:15-10:30	Break/Posters
	10:30-11:30	Oral Presentations
	11:30-1:00	Lunch & BoD Meeting
	1:00-1:15	Brief Update from SETAC NA
	1:15-2:30	Oral Presentations
	2:30-3:00	Poster Review, Refreshments, Break
	3:00-4:15	Oral Presentations
	4:15-5:00	Raffle and Student Awards
	5:00	Meeting Adjourn

INFORMATION-THEORETIC METHODS IN EMPIRICAL SCIENCE

Thursday April 13

Conference Room D
American Mountaineering Center, Golden, Colorado

Dr. David R. Anderson
Emeritus Professor, Colorado State University
President, Applied Information Company

Overview of some modern methods for model selection and multimodel inference

This overview focuses on the assessment of alternative science hypotheses and models to represent them. Kullback-Leibler (K-L) information, Akaike's Information Criterion (AIC) and various extensions are a course focus. These extensions allow one to compute the likelihood of each model in an *a priori* set, the probability of each model and evidence ratios. One can easily rank the support for the alternative hypotheses. These quantities are generally trivial to compute. This class of methods is termed "information-theoretic" as they are based on K-L information theory. The afternoon session deals with effective ways to make formal statistical inference from all (or some subset) the models in the set (*multimodel inference*). Some comparisons with traditional approaches (e.g., null hypotheses testing and P-values) will be provided.

This overview is as much about science philosophy as it is about "statistics." I will focus on concepts such as alternative science hypotheses (not just a sterile "null" hypothesis) and cover ways to both quantify and qualify the strength of evidence for these alternative hypotheses. The notion of *evidence* seems so central in empirical science; however, if you look for this word in your applied or theoretical statistics books, you are unlikely to find it! Instead, there has been focus on P-values and "statistical significance." A variety of examples will be given and discussed. People are often surprised to learn that these information-theoretic methods are replacing *t*-tests and ANOVAs for the analysis of experimental data. Finally, I will present a clear science strategy for efficient learning in the empirical sciences.

In general, the material will follow the 2008 textbook, "*Model based inference in the life sciences: a primer on evidence.*" Copies of this text will be supplied to each participant as part of their registration fee. The underlying theory for these new approaches can be found in the 2002 book with Kenneth P. Burnham, "*Model selection and multimodel inference: a practical information-theoretic approach.*" However, the overview will focus on application with only occasional reference to the deeper theory.

Background Required:
Reasonable exposure to basic statistics is expected (e.g., standard errors, residual sums of squares, coefficients of variation, bias, variance, multiple regression, ANOVAs, and test statistics such as *t, F,* and *z*). Ideally participants would have *some* exposure to likelihood functions, probability distributions, expectations, and logistic regression. Participants weak in some of these areas will likely to get a lot out of the overview because the focus will not be on statistical theory.

Key Outcomes: Attendees will have a good overview of these new approaches and most people will be able to perform analyses with their own data. The computations required are quite simple once the parameter estimates have been obtained for each model. A laminated reference sheet will lessen the need for note taking. Discussion is invited and these intensive learning sessions are actually fun.

ORAL PRESENTATIONS SCHEDULE – Friday, April 14
Conference Rooms C and D

9:30-10:15 **Kraus, Johanna**. *U.S. Geological Survey*. Plenary presentation: When fish eat trees and other tales of contaminant impacts at the land-water interface.

10:15-10:30 Poster Review, Refreshments, Break

10:30-10:45 **Heiker, Laura**. *University of Northern Colorado*. Bat activity in relation to heavy metal contamination of high-elevation streams in the Colorado Rockies.

10:45-11:00 **Guth, Dan**. *GEI Consultants, Inc*. Options for derivation of site-specific selenium standards in Colorado.

11:00-11:15 **Gerlock, Kimberly**. *GEI Consultants, Inc*. An evaluation of the field-based aquatic benchmark for specific conductance for use in northeastern Minnesota.

11:15-11:30 **Richardson, Kristy**. *Colorado Dept. of Public Health and Environment*. The state of the state: water quality standards development and other issues relevant Colorado's clean water regulations.

11:30-1:00 Lunch & RM SETAC Board of Directors Meeting

1:00-1:15 **James Lazorchak**. *U.S. Environmental Protection Agency*. Update from SETAC North America.

1:15-1:30 ****Kotalik, Christopher**. *Colorado State University*. Stream community responses to direct and indirect metals stressors using stream mesocosms.

1:30-1:45 **Adams, William**. *Red Cap Consulting*. Evaluation of multiple linear regression (mlr) models for predicting chronic aluminum and iron toxicity values.

1:45-2:00 **Traudt, Elizabeth**. *Colorado School of Mines*. Ion activity does not reconcile apparent non-additive toxicity in Cd-Cu-Ni and Cd-Ni-Zn mixtures.

2:00-2:15 ****Challis, Katie**. *Colorado School of Mines*. Application of single particle ICPMS to detect and characterize TiO2 in rice (Oryza sativa L.) plants exposed to nanoparticle and bulk particle TiO2.

2:15-2:30 ****Rand, Logan**. *Colorado School of Mines*. Diurnal cycling of Ti from recreational bathing activity in Clear Creek, CO.

2:30-3:00 Poster Review, Refreshments, Break

3:00-3:15 **Norris, David**. *University of Colorado*. Are intersex fishes in North American rivers a recent phenomenon?

3:15-3:30 ****Anderson, Jordan**. *Colorado State University*. Evaluating the distribution of estrogenic effects below wastewater treatment plants: estrogen persistence and fish movement.

3:30-3:45 ****David Bertolatus**, *University of Colorado-Denver*. Integrated chemical, organismal, and transcriptomic analyses reveal landscape-specific effects of exposure to complex chemical mixtures.

3:45-4:00 ****Lupardus, Randi**. *University of Northern Colorado*. Potential environmental impact of oil and natural gas emissions on a shortgrass steppe.

4:00-4:15 **Duggan, Sam**. *Colorado State University*. An ecotoxicology approach to researching petroleum spills through mesocosms, field observations and bioassays.

5:00 Meeting Adjourn

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

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Plenary Presentation: Dr. Johanna Kraus (jkraus@usgs.gov)
U.S. Geological Survey, Fort Collins Science Center, 2150 Centre Ave, Fort Collins, CO

When fish eat trees and other tales of contaminant impacts at the land-water interface
Freshwaters both receive and export resources from/to the terrestrial environment. As a result of this connectivity, stressors such as contaminants that impact aquatic food webs can reduce availability of resources such as adult aquatic insects in terrestrial food webs (e.g. spiders, birds, bats) and increase the use of terrestrial insect subsidies by aquatic consumers like trout. In addition to effects on resource production and consumption, contaminants can accumulate in prey tissues. Thus, animals with complex life cycles that leave the water as they metamorphose to terrestrial adults can serve as vectors of aquatic contaminants, expanding the circle of diet-related exposure to terrestrial consumers. Our field and laboratory work in metal-impacted streams has suggested that metamorphosis is a crucial determinant of both production and quality of adult aquatic insect prey. Metamorphosis seems to be a stressful endeavor that both increases the sensitivity of insects to toxic effects of some metals and acts as an adaptive step in regulating contaminant burdens through excretion of detoxified metals from insect bodies. We have also found through meta-analysis and empirical work in other systems that the effects of metamorphosis on contaminant burdens is contaminant-specific, and that certain chemical properties may predict differences in transfer from aquatic to terrestrial food webs. A main goal of my research has been to place our empirical findings within well-developed ecotoxicological and spatial food web paradigms to develop a predictive and spatially explicit framework for understanding how aquatic contaminants may impact linked aquatic-terrestrial food webs across the landscape.

Dr. Johanna Kraus is a Research Ecologist at the USGS Fort Collins Science Center. She got her B.A. (with honors) at Brown University and Ph.D. at University of Virginia with Prof. Henry Wilbur. Dr. Kraus viewed herself as a community ecologist studying aquatic-terrestrial linkages in invertebrate and amphibian food webs until she started working at the USGS as a Mendenhall Fellow in 2011, when she began her foray into all things contaminant.

Abstracts for Platform Presentations (Alphabetical by presenter)

[1]**William J. Adams**, [2]David K. DeForest, [3]Kevin V. Brix, [2]Lucinda M. Tear, [2]Robert C. Santore, [2]Adam C. Ryan. [1]Red Cap Consulting, [2]Windward Environmental LLC, [3]EcoTox.

Evaluation of Multiple Linear Regression (MLR) Models for Predicting Chronic Aluminum and Iron Toxicity Values

The bioavailability of both iron (Fe) and aluminum (Al) varies as a function of water chemistry parameters including pH, dissolved organic carbon (DOC) and hardness. Each of these parameters may individually affect Al or Fe bioavailability, or they may interact to affect the toxicological outcome of a given aquatic toxicity test. For example, the influence of DOC and hardness on toxicity is greatest at pH 6 and diminishes as pH increases. However, as pH increases from 6 to 8 the toxicity changes significantly due to speciation changes. We evaluated the ability of multiple linear regression (MLR) models to predict Al and Fe toxicity to green algae, *Ceriodaphnia*, and fathead minnows as a function of multiple combinations of pH, DOC and hardness. Both individual water chemistry terms (pH, ln[DOC], ln[hardness]) and interaction terms (pH×ln[DOC], pH×ln[hardness], ln[DOC]×ln[hardness], were included as independent variables in the analysis, either EC10s or EC20s were included as the dependent variable. The Akaike Information Criterion (AIC) was used to identify which combination of terms resulted in the best models for predicting Al EC10s and EC20s for the three-species tested. The MLR models were generally able to predict Al and Fe EC10/20s that were within a factor of two of the observed values for >90% of the toxicity tests. The MLR models were then used to normalize species sensitivity distributions (SSDs) such that Al and Fe HC5 (concentration hazardous to 5% of the species) values could be derived for site-specific pH, hardness and DOC conditions of interest.

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Evaluating the distribution of estrogenic effects below wastewater treatment plants: estrogen persistence and fish movement.

Estrogens are endocrine disrupting contaminants (EDCs), a large group of chemicals that can impair normal endocrine function. Our goal was to better understand the distribution of estrogenic effects downstream of wastewater treatment plants (WWTPs) in the South Platte River drainage. To do this, we estimated the downstream persistence of estrogenic exposure and evaluated differences in responses to estrogen between wild and laboratory Fathead Minnows. To evaluate the persistence of estrogenic effects downstream of WWTPs, male Fathead Minnows were caged at two WWTPs at the following intervals: one cage placed upstream of the effluent, one cage placed in the effluent, and four cages placed downstream at 400 m, 800 m, 1600 m, and 3200 m downstream of the effluent. Ten male Fathead Minnows were added to each cage and left for one week, at which point the cages were removed, the fish euthanized, and livers extracted for Vitellogenin (Vtg) analysis. The results of the caging were mixed with Vtg upregulation being quickly attenuated at one WWTP, and persisting for over two miles at the other. To evaluate previously observed Vtg differences between laboratory and wild fish exposed in streams, we conducted a laboratory study. We compared the Vtg response in laboratory and wild populations of Fathead Minnows exposed to 17α ethinylestradiol for seven days. Laboratory and wild populations expressed similar high levels of Vtg compared to controls.

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Integrated chemical, organismal, and transcriptomic analyses reveal landscape-specific effects of exposure to complex chemical mixtures

Aquatic habitats are often contaminated with mixtures of legacy and emerging contaminants. Although a large body of research documents the effects of exposure to single chemicals in laboratories, less is known about the effects of exposure to the complex mixtures that occur in aquatic ecosystems. To characterize effects caused by these mixtures, we exposed adult fathead minnows (*Pimephales promelas*) to water from multiple locations within the Shenandoah River watershed using flow-through mobile laboratories. The exposure locations were chosen to capture unique and representative landuse classes, including agricultural, urban, mixed-use, and forested. Genome-wide transcription profiles were measured to investigate the molecular underpinnings of higher-level changes and to gain an unbiased observation of the physiological state of animals following exposure. Fish exposed at both agricultural and WWTP impacted sites had a reduced number of nuptial tubercles and decreased GSI. However, transcript biomarkers of endocrine disruption, including *er1, er2, ar, vtg1,* and *vtg3,* showed little to no differential expression in exposed fish, suggesting the changes in organismal endpoints were not caused by an estrogenic mechanism of action. Hierarchical clustering of total transcriptome profiles showed individuals generally clustered according to exposure location, demonstrating that exposure to water from sites with different landuse results in unique and site specific effects at the transcript level. These data provide insightful hypotheses regarding the specific effects of exposure to different types of complex mixtures and demonstrate the value of our complex mixture/landscape research approach.

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Application of single particle ICPMS to detect and characterize TiO_2 in rice (Oryza sativa L.) plants exposed to nanoparticle and bulk particle TiO_2

Titanium dioxide (TiO2) nanoparticles are one of the most common nanoparticles in consumer products. The particles are incorporated into products such as sunscreens, "self-cleaning" concrete, paints, toothpaste, and food products. The ubiquity of the TiO_2 in products is a cause for concern due to the inevitable release into the environment and eventual presence in agricultural areas. To investigate if TiO_2 particles could be taken up by crops, rice plants (*Oryza sativa L.*) were exposed to two different TiO_2 particles: nanoparticles (NIST SRM 1898) and bulk TiO2 (Acros Organics) at 50 ppm TiO_2 concentrations in hydroponic pots for one week. Plants were harvested and dried prior to digestion and sp-ICP-MS analysis. Two digestion methods were used to destroy plant material and free TiO_2 present in

the plants. The first method was an enzymatic digestion using a macrozyme enzyme to break down plant material. The enzyme did not achieve complete digestion. The second method was a microwave acid digestion method which successfully destroyed plant material. Single particle-ICPMS (sp-ICPMS) was used to analyze the digested suspensions to determine TiO_2 size and size distribution. In the enzymatic digestion, nanoparticles in plants were 44 ± 31nm and the bulk particles exposed plants did not release a sufficient number of nanoparticles to determine size and size distribution. In the acid digestion, the mode size of particles was 65 ± 53nm and 236±67nm in plants exposed to nanoparticle TiO_2 and bulk TiO_2, respectively. Particle size suggest that the TiO_2 was resistant to dissolution under extreme microwave conditions.

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An ecotoxicology approach to researching petroleum spills through mesocosms, field observations and bioassays

Oil development has expanded dramatically in Colorado over the last decade. Associated with the rapid expansion has been an increase in the number of accidental releases into the environment. Here, we explored a breadth of effects of petroleum spills on coldwater stream communities using data collected from a spill site, as well as data from spills simulated in a laboratory and in a mesocosm facility. First, we analyzed stream health indicators across multiple levels of biological organization to identify long-term ecological impacts associated with a petroleum spill field site. Histological pathologies in mottled sculpin, *Cottus bairdii*, and alterations in benthic macroinvertebrate communities were discovered at the spill location and at downstream locations more than two years after the spill occurred. Subsequently, we conducted two mesocosm experiments, using naturally colonized benthic macroinvertebrate communities. Exposure to simulated spill conditions caused concentration-dependent macroinvertebrate drift and mortality that occurred rapidly after the spills were initiated and at lower concentrations than expected. In addition, concentration-dependent lethal and sub-lethal effects were observed in rainbow trout, *Oncorhynchus mykiss*, subjected to simulated spill bioassays. We conclude that petroleum spills in coldwater streams risk adverse acute, chronic, lethal and sub-lethal effects to aquatic communities. Moreover, by utilizing field observations, mesocosms and bioassays we gained insights into consequences of petroleum spills using an ecotoxicological weight-of-evidence approach.

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An evaluation of the field-based aquatic benchmark for specific conductance for use in northeastern Minnesota

We reviewed the application of the field-based aquatic benchmark for specific conductance, as originally developed for the central Appalachian Mountains, for use in northeastern Minnesota. We identified interesting issues that should be considered prior to more wide-scale application of the method. Comparing extirpation coefficients for genera common to both ecoregions showed substantially different limits for the same genera. This is an issue with the underlying premise of the conductivity benchmark that physiological limits to ion chemistry affect the distribution of benthic invertebrate taxa. Additionally, stressor-response profiles revealed different responses for common genera and highlighted the influence of low capture probability on the hazard concentration. The effects of low capture probability placed in the context of relative abundance for any one genera is not factored into the presence/absence approach

used in the benchmark. Single individuals in a sample are afforded the same weighting as multiple individuals in a sample. This issue should be further investigated, because extirpation of a genus is largely pinned on the presence/absence of a single individual and its likelihood of being collected in a stream. In fact, in the northeastern Minnesota case-study, approximately 35 percent of the data being used to develop extirpation coefficients and species sensitivity distributions was based on genera represented by a single organism in a sample. We conclude that the specific conductance benchmark approach would still benefit from further investigation of these issues prior to application to other ecoregions.

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Options for derivation of site-specific selenium standards in Colorado

In June 2016, the U.S. EPA released the final Aquatic Life Ambient Water quality Criterion for Selenium. In addition to the updated chronic water column criteria, the 2016 selenium criteria document included criteria for fish tissue, consisting of egg/ovary, muscle, and whole body elements. Due to the bioaccumulative properties of selenium, the EPA set the fish tissue criteria to take precedence over the water column criteria. Additionally, because of the bioaccumulative nature of selenium, and the tendency of selenium to accumulate in the reproductive tissues, the EPA prioritized the egg/ovary element over the whole body or muscle criteria. The guidance document provides two modeling approaches to develop a site-specific water column criterion element based on fish tissue concentrations, use of a mechanistic model or use of bioaccumulation factors. While we agree with the concept of tracking each step in the bioaccumulative process for selenium, we have concerns with the variability and validation of model results, especially when using site-specific data. As a result of the naturally elevated selenium concentrations in basins throughout Colorado, which is further complicated by seasonally elevated flows originating from snowmelt, trying to model a site-specific water column standard based on fish tissue has proven problematic. In this presentation we provide one potential solution: using measured data to directly calculate a protective standard.

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Bat activity in relation to heavy metal contamination of high-elevation streams in the Colorado Rockies

Metal contamination in freshwater ecosystems is a widespread consequence of mining activities, but effects on nearby riparian insectivores are poorly understood. In this study, we hypothesized that more contaminated stream reaches (>3,000 m) in the Colorado Mineral Belt would have lower proximate bat activity due to reduced densities of adult insect prey emerging from water. From August to October 2014, we used a paired design to sample twelve sites for bat activity, stream heavy metal concentrations, and aquatic emergent insect densities. There was no relationship between bat activity and aqueous metal concentration, despite a confirmed negative correlation between metal concentration and aquatic emergent insect biomass. However, there was a trend toward streams with lower metal concentrations having more bat prey capture attempts (as measured by feeding buzzes) than streams with higher metal concentrations. These data suggest that although bats are passing over streams of all contamination levels at a similar rate, they may be encountering prey more often over cleaner streams. While this study

highlights difficulties in determining sub-lethal effects on bats, the design may be a useful tool for assessing risk in higher-productivity habitats.

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Stream community responses to direct and indirect metals stressors using stream mesocosms

The ecological effects of trace metals to streams and rivers are well documented through laboratory, field, and mesocosm approaches. While most water quality criteria (WQC) are developed using traditional laboratory toxicity tests, mesocosm experiments offer a valuable middle ground between laboratory methods and field surveys. We evaluated the direct and indirect effects of metals by exposing benthic communities to Cu and Zn mixtures and Fe oxide for 14 d. Measured responses included the timing and abundance of emerging adult taxa, algal colonization, community metabolism, and community composition. Results for both stressors show differences in larval and adult responses within the same taxonomic groups, particularly among midges (Chironomidae) and mayflies (Ephemeroptera); however, algal colonization and community metabolism displayed the greatest sensitivity to exposure. In general, significant reductions in endpoint responses were observed at concentrations below the WQC for Fe, whereas observed effects for Cu and Zn occurred at or above their cumulative criterion value. This research demonstrates the adaptability of mesocosms to differing environmental stressors, while highlighting the need to comprehensively assess contaminant effects with ecologically meaningful endpoints.

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Potential environmental impact of oil and natural gas emissions on a shortgrass steppe

Federal lands of the Pawnee National Grassland (PNG) lie on the Niobrara play and have potential to bring high profits to the State of Colorado; However, Oil and Natural Gas (O&NG) production can be a substantial source of air pollution on the shortgrass steppe. Four projects were undertaken to assess the potential impact of O&NG on the shortgrass steppe. I will focus on one of these projects and present results quantifying ambient levels of common fugitive emissions on typical PNG O&NG production sites. Twenty-four Volatile Organic Compounds (VOCs) were quantified in real time and used to determine the spacial and temporal windows of exposure for proximate flora and fauna. Eleven O&NG sites on the PNG in Northern Colorado were randomly selected and grouped according to production (i.e., wet pumping n=4, wet non-pumping n=3 , dry no pump jack n=3) along with 13 control sites. At each of sites, samples were collected 25 m from the wellhead or stack in NE, SE and W directions. In each direction, two samples were collected with a Gasmet DX4040 gas analyzer every hour from 8am-2pm (6 hours total), July-October, 2016 (N=864). Results indicated that (i) VOC concentrations were significantly different among Pump Groups (ii) VOCs were from O&NG and not from other sources, and (iii) VOC levels frequently exceeded reference standards and at biologically relevant levels for shortgrass steppe flora and fauna.

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Are intersex fishes in North American rivers a recent phenomenon?
We reported endocrine disruption in white sucker (*Catostomus commersoni*, WS) living downstream of municipal wastewater treatment facilities (WWTFs) in Colorado rivers and elsewhere as compared to upstream reference sites. Disruption of WS in Boulder Creek included intersex, skewed sex ratio toward females, feminization of juveniles and adult males (vitellogenin production), and lowered gonadosomatic indices in both sexes (Woodling et al., 2008; Vajda et al., 2008). Furthermore, treatment of male fathead minnows (*Pimephales promelas*, FHM) in reproductive condition with effluent from the WWTF diluted to reflect downstream levels was able to demasculinize and feminize FTMs within 7 days (Vajda et al., 2011). We histologically examined the gonads from WS collected between 1915 and 1974 from these same sites as well as FHMs collected between 1943 and 1955 that were maintained in the University of Colorado Museum of Natural History. We found no evidence of endocrine disruption in either species and conclude that the observed endocrine disruption we observed recently is a relatively new phenomenon linked to estrogenic chemicals present in wastewater effluent. Additionally we will report on a similar nationwide examination of endocrine disruption in museum specimens of large- and smallmouth bass (*Micropterus salmoides; M. dolomieui*).

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Diurnal cycling of Ti from recreational bathing activity in Clear Creek, CO
As engineered nanoparticles (ENPs) become more widespread in personal care products and industrial applications, there is a growing need to improve monitoring their release into the environment. We have observed that Clear Creek in Golden, CO has at times more than 400 recreational bathers on sunny summer afternoons. This activity could introduce Ti-containing nanoparticles from sunscreen into the water. In our study, Clear Creek was sampled at regular daytime and nighttime intervals over the Labor Day holiday weekend in 2015 and 2016. The water collected was analyzed for Ti using ICP-AES and single particle ICP-MS. Oxybenzone, an organic compound commonly used as a sunscreen active ingredient, was also measured using HPLC. The results show increases in oxybenzone and Ti, as well as shifts in the Ti:Al ratio that correspond with peak bathing periods. However, estimates of mass loading from bathers suggest that the elevated Ti levels cannot be attributed to sunscreen use alone. We propose that the bathing activity causes a resuspension of Ti-containing bed sediments, which compounds the analytical difficulty of observing ENP inputs above the natural mineralogic background. Future work in this project will focus on determining baseline concentrations and variation in the natural Ti content in order to define the minimum Ti ENP input required for ENPs to be detectable in this and other streams.

Kristy Richardson, Stephanie Baker, and Blake Beyea, Water Quality Control Division, Colorado Department of Public Health and Environment, Denver, CO 80246

The state of the state: water quality standards development and other issues relevant Colorado's clean water regulations

The state of Colorado frequently undertakes research projects to support development of water quality standards. Much of this work, including study design and implementation, is accomplished with the help of sister agencies, universities, and other organizations. We will share "hot topic" issues for Colorado, such as nutrients, benzene, and temperature, for which additional data collection is needed to support standards development and we are looking to build collaborations. These projects offer excellent opportunities for graduate students and other researchers interested in working on subject matter that can directly influence Colorado's water quality regulations. As an example of collaborations we are looking to build, we will discuss an ongoing project with CPW and CSU to support development of new selenium standards.

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Ion activity does not reconcile apparent non-additive toxicity in Cd-Cu-Ni and Cd-Ni-Zn mixtures

Multiple metals usually are present in surface waters, leading to toxicity that currently is difficult to predict due to potentially non-additive interactions among the metals. To address this complexity, we exposed *Daphnia magna* neonates to Cd-Cu-Ni and Cd-Ni-Zn combinations in standard 48-h lethality tests. In these mixtures, two metals were held constant while the third metal was varied from sublethal to lethal concentrations. We calculated the observed mortality/predicted mortality ratio in each mixture and used a Monte Carlo-type randomization procedure to statistically test for non-additive toxicity. Results were analyzed in a four-way matrix: assuming either the concentration-addition or the independent-action mixture-toxicity model, and using either the measured metal concentration or the calculated metal-ion activity to predict toxicity. In Cd-Ni-Zn mixtures, the toxicity was less-than-additive or approximately additive, depending on the concentration (or ion activity) of the varied metal but independent of the additivity model. In Cd-Cu-Ni mixtures, the toxicity was less-than-additive, additive, or more-than-additive, depending on the concentration (or ion activity) of the varied metal and depending on the assumed additivity model (i.e., concentration addition predicted the toxicity more accurately than response addition at intermediate to high concentrations of the varied metal). Contrary to our hypothesis, ion activities (which account for metal-metal competition for binding to dissolved organic matter and other ligands) did not reconcile the apparently more-than-additive toxicity in Cd-Cu-Ni mixtures. These results demonstrate complex interactions of potentially competing toxicity-controlling mechanisms can occur in ternary-metal mixtures, but the toxicity was qualitatively consistent with results in previous binary-metal toxicity tests.

Abstracts for Poster Presentations
(Alphabetical by presenter)

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Riverine microbial RNA degradation by anthropogenic chemical contamination

The South Platte River represents a significant conduit along the Colorado Front Range with varying land uses, including areas with major wastewater treatment facilities, land regions of heavy agricultural use, and areas inundated by animal feed lots. This variation in land use results in mixtures of contaminated effluent and runoff that affect riverine ecosystem services. The primary objective of this study is to address the functionality of ammonia-oxidizing microbial communities along the South Platte in response to anthropogenic pollution. We collected monthly water column and sediment samples at six points across the South Platte River for one year. Samples were analyzed for water chemistry, physicochemical parameters, and total coliformic counts. We measured seasonal variation in environmentally relevant concentrations of antibiotics and antibiotic degredates and found that sites with mixed urban and agricultural land use resulted in the largest seasonal variation of antibiotic concentrations. Multiple extraction kits were tested, along with multiple optimization strategies, with the intent to co-extract DNA and RNA from filtered water samples. Bioanalysis to test quantity and quality of extracted DNA and RNA revealed poor RNA extraction concentrations with likewise poor RIN values. Results also showed an additional peak to the right of 18S and 28S peaks, present across different extraction methods but not in blank samples. Current experiments are geared towards further exploring the effect of South Platte riverine water chemistry on the ability to extract microbial RNA from water column and sediment samples, as well as optimizing integrity for downstream applications.

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Quantification of Single Walled Carbon Nanotubes in Estuarine Sediment: Comparing State-of-the-art Nanometrology

The release of single-walled carbon nanotubes (SWCNTs) into the environment is an inevitable consequence of their growing use in consumer and industrial applications. However, the ability to detect and characterize CNTs upon release is challenging given both the ubiquity of carbon in the environment and the prevalence of naturally-occurring nanomaterials. To evaluate the limitations of detecting and quantifying SWCNTs in environmental matrices, four techniques (near-infrared fluorescence, UV-vis spectroscopy, single particle ICP-MS, and chemical-thermal oxidation) were used to quantify three different SWCNTs extracted from estuarine sediment. Each analytical technique exploits a different property or defect for the purpose of detecting SWCNTs amidst a background of naturally occurring interferences. Estuarine sediment was amended with increasing loadings of SWCNTs and extracted via chemical sonication prior to quantification. Near-infrared fluorescence spectroscopy was found to be the most sensitive and selective with detection limits in sediment extract approaching 10 μg kg^{-1}. However, this requires that the SWCNTs be fluorescent, pristine and individualized, criteria that may be difficult to achieve in environment systems. UV-vis and single particle ICP-MS were less sensitive with detection

limits approaching 1 mg kg^{-1}. The prevalence of soot carbon in the sediment presented a challenge to chemical thermal oxidation, and impeded the detection of SWCNTs even at the highest of SWCNT loadings. The results of this work demonstrate not only the potential for these techniques to be used for addressing SWCNT fate and behavior in the environment, but also the many limitations that may require the development of more sophisticated analytical techniques.

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Ultra Sensitive Salmonid Vitellogenin ELISA: A new tool allowing accurate determination of vitellogenin in male and juvenile fish

Recently we have introduced a new collection system allowing a non invasive, repeatable and effective mucus sampling for vitellogenin determination in fish. This new sampling method opens the possibility to study vitellogenin concentrations over time or over treatment periods even in small juvenile fish. Juvenile fish are useful in the effect assessment of wastewater effluents and therefore we specifically developed a very sensitive vitellogenin ELISA for Salmonids. This sandwich ELISA requires two immune incubations, first with the capture antibody for 2 hours and second with the detection antibody for 1 hours both at room temperature. At the end the colour reaction is measured at 450 nm and a 4-parameter curve fit should be used for automatic data reduction and calculation. The linear standard range is between 0.012 and 1.00 ng/ml (undiluted samples) covering an OD range between appr. 0.05 and 2.8 at 450 nm. The lower limit of detection (LLD) was calculated to be 0.002 ng/ml. The intra- and inter-assay CVs were between3.7-5.2% and 6.4-9.5%, respectively. This ultra sensitive vitellogenin ELISA can be used to measure vitellogenin in Atlantic salmon (Salmon salar), Brown trout (Salmo trutta), Chum salmon (Oncorhynchus keta); Pink salmon/humpback salmon (Oncorhynchus gorbuscha), Rainbow trout (Oncorhynchus mykiss), Brook trout (Salvelinus fontinalis), "Common whitefish, European whitefish" (Coregonus lavaretus), Maraena whitefish (Coregonus maraena). With this new assay, changes in vitellogenin level can be accurately determined even in male and juvenile salmonid fish.

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The effects of selenium on fish reproductive success and building a predictive model for selenium in fish tissues based on selenium concentrations at multiple trophic levels

The 2016 EPA Aquatic Life Ambient Water Quality Criterion for selenium (Se) in freshwater is driven by limits on Se concentrations in fish ovaries. The lethal sampling techniques to assess selenium levels might put a strain on fish populations including Brown Trout (*Salmo trutta*), which was one of the more sensitive species considered in the 2016 update. Previous research has shown correlations between increased selenium concentration in maternal fish tissues and increased rates of larval deformity and decreased hatch success. Despite this Colorado's most productive Brown Trout fisheries are very high in selenium. CSU, CPW and CDPHE are collaborating to investigate the efficacy of adopting the national standard in Colorado. Brown Trout, Walleye (*Sander vitreus*) and White Sucker (*Catostomus commersonii*) were be spawned from surface waters across Colorado, inclusive of a range of habitats and Se levels. These eggs were reared in the laboratory at Colorado Parks and Wildlife in Fort Collins, Colorado. We looked for correlations in parental tissue concentrations of Se and hatch success, larval

deformities and numerous physical fitness measures of offspring. Additionally, Se concentrations of water, substrate, periphyton, and insects will be assessed to better model accumulation in fish species. These parameters will be used in a model to predict Se concentrations in fish tissues based on Se concentrations at multiple trophic levels. Ideally a model can be created that will better predict adverse effects of Se and base a standard of measurements other than the reproductive tissues of economically and ecological valuable fish.

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A landscape-based approach to assess endocrine disruption in fish in the Shenandoah River
The elucidation of cause and effect relationships between putative endocrine disrupting chemicals and adverse outcomes in wildlife is confounded by the complexity of mixtures and temporal variation present in ecosystems. We have developed a landscape-based model in an attempt to link complex chemical mixtures to adverse outcomes across multiple levels of biological organization. We hypothesize that landuse patterning in a watershed will correlate with the chemical profile of the water, which in turn will correlate with biological effects at the molecular, cellular and organismal level. The Potomac River watershed represents an ideal microcosm for testing this hypothesis. Beginning in 2002, widespread fish kills have occurred in the Potomac and its tributaries, including the Shenandoah River. The cause of these fish kill has yet to be established, although high rates of intersex fish in the area have lead to a focus on endocrine disruption as a contributor to mortality. In 2014, 2015, and 2016, we deployed in-situ, flow-through aquaria at locations in the Shenandoah Valley with distinct landuse patterning, including sites reflecting agricultural, wastewater, mixed-use and forest-dominated landscapes. Fathead minnows (*Pimephales promelas*) were exposed to native water sources and sampled at 7 and 21 days of exposure. Water was sampled every seven days for analysis of over 550 analytes. Results demonstrate site-specific impacts on gonadal histology, plasma vitellogenin, vtg mRNA, and liver transcriptomic and metabolomics response. Together, these data provide novel insight into the relationship between landuse, chemical contamination and biological effect.

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Primary resource responses to metals contamination: mechanisms of metals exposure and bioaccumulation in a stream food web.
Metals pollution into the Upper Arkansas River has caused shifts in stream macroinvertebrate community composition; however, despite significant improvements in water quality these differences between upstream and downstream communities remain. Interestingly, the most sensitive stream insects are also commonly consumers of benthic microbial biofilm (‘scrapers’), but other groups that feed on stream seston (‘filterers’) are typically more abundant in metals contaminated streams. We had two competing hypotheses to test this observation: (1) Metals are elevated in downstream microbial communities, and benthic biofilms contain higher metals than in the seston (i.e., greater direct exposure); and (2) Aqueous metals concentrations may be low enough at downstream sites not be directly toxic to the consumer, but high enough to alter biofilm community structure and resource quality (i.e., greater indirect effect in biofilms). These hypotheses were tested by examining biofilm composition, metals concentrations, and

resource dietary quality (C:N) from biofilm and seston at sites upstream and downstream of California Gulch. Our results show that metals accumulation in caddisflies did not change between reference and impacted sites, suggesting that they may have the capacity to regulate metals. Metals also likely created a shift in algal community composition and changes in resource quality at impacted sites. However, resource quality only changed between reference and impacted site biofilms, but not seston C:N. Therefore, metals, in combination with a shift in resource quality may be more stressful to insect scrapers than filterers.

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