ROCKY MOUNTAIN SETAC

Proceedings and Agenda for the 29th Annual Meeting of the Rocky Mountain Chapter of the Society of Environmental Toxicology and Chemistry

April 8th, 2016

U.S. Geological Survey, Fort Collins Science Center
2150 Centre Avenue, Bldg. C (Main Conference Room by front door)
Fort Collins, CO
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AGENDA – Friday, April 8th

Time	Activity
8:30-9:15	Registration, Refreshments, Breakfast Snacks
9:15-9:30	Opening Remarks
9:30-10:15	Plenary
10:15-10:30	Poster Review, Refreshments, Break
10:30-11:30	Oral Presentations
11:30-1:00	Lunch, BOD meeting
1:00-1:15	SETAC NA Update
1:15-1:30	NASAC Update
1:30-2:30	Oral Presentations
2:30-2:45	Poster Review, Refreshments, Break
2:45-4:00	Oral Presentations
3:30-4:00	Poster Review, Refreshments, Break
4:00-4:30	Student Judging Deliberations
4:30-5:00	Student Awards and Raffle

9:30 – 10:15 **Invited Plenary**

Dr. Annika Walters
U.S. Geological Survey
Wyoming Cooperative Fish and Wildlife Research Unit

Implications of oil and natural gas development for native fish populations

The rapid expansion of natural gas development has raised concerns about potential effects for aquatic ecosystems. Oil and gas production can alter streams due to surface disturbance, water use, habitat fragmentation, and pollution of air, water, and soil. We have conducted research on the sensitivity of stream habitats and native fishes to development in the La Barge oil and gas field in southwest Wyoming since 2012. We have found that habitat quality is altered in streams highly affected by oil and gas development. Likely mechanisms include spill history leading to elevated stream polycyclic aromatic hydrocarbon concentrations, and surface disturbance contributing to elevated suspended sediment concentrations and degraded riparian health. We have also examined fish stress physiology and population abundance. We found that fish species varied in their sensitivity to development, likely due to differing habitat and resource requirements. An improved understanding of the effects of oil and gas development will allow more explicit management and mitigation recommendations for the protection of native fish communities.

About our Plenary Speaker
Annika joined the Wyoming Cooperative Fish and Wildlife Research Unit in 2011. Annika was a post-doctoral researcher at the Northwest Fisheries Science Center and at the University of Washington in Seattle. She has a BA in ecology and evolutionary biology from Princeton University and a MS and PhD in ecology and evolutionary biology from Yale University. Annika's research has focused on aquatic ecosystems and how these ecosystems are altered by natural and anthropogenic disturbance.

ORAL PRESENTATIONS SCHEDULE – Friday, April 8th

10:30-10:45	**Duggan**, Sam. *Colorado State University*. Effects of petroleum hydrocarbons on stream communities in West Creek, Colorado
10:45-11:00	**McLaughlin**, Molly. *Colorado State University*. Environmental fate of hydraulic fracturing fluid additives after spillage on agricultural topsoil
11:00-11:15	**Firkus**, Tyler. *University of Wyoming*. Are water temperature standards adequately protecting fish species: A framework for development of water temperature standards?
11:15-11:30	**Hargis**, Lauren. *Colorado State University*. The influence of developmental stage on sensitivity of mayflies (Ephemeroptera) to major ions.
1:30-1:45	**Heiker**, Laura. *University of Northern Colorado*. A tale of two bats: differential mercury uptake in two Chinese species
1:45-2:00	**Bertolatus**, David. *University of Colorado Denver*. Transcriptome profiling in fathead minnow following exposure to complex chemical mixtures
2:00-2:15	**Kotali**k, Christopher. *Colorado State University*. Stream benthic and algal community responses to heavy metals: an evaluation of endpoint sensitivity
2:15-2:30	**Gensemer**, Bob. *GEI Consultants*. Challenges for application of Biotic Ligand Model-based water quality standards to permits and impaired waters designations
2:45-3:00	**Krueger**, Annie. *University of Wyoming*. Determining the realistic toxicity of imidacloprid for bumblebee (*Bombus impatiens)* workers
3:00-3:15	**Williamson**, Jacob. *Colorado School of Mines*. Examination of metal deposition and removal processes in an acid mine drainage contaminated stream using *in-situ* sediment samplers
3:15-3:30	**Wolff**, Brian. *Colorado State University*. Stream microbial community responses to metals exposure
3:30-3:45	**Morris**, Jeffrey. *Abt Associates*. Photo-Induced Toxicity of North West Shelf Crude Oil in Australia: Early Life Stage Tests with Yellowtail Amberjack (*Seriola lalandi*) and Black Bream (*Acanthopagrus butcheri*)
3:45-4:00	**Nichols**, Tracy. *United States Department of Agriculture*. Dietary magnesium and copper affect survival time and neuroinflammation in chronic wasting disease

Abstracts for Oral Presentations

(Alphabetical by presenter)

Transcriptome profiling in fathead minnow following exposure to complex chemical mixtures

David W. **Bertolatus**, Department of Integrative Biology, University of Colorado Denver, Denver CO, david.bertolatus@ucdenver.edu; Chris Martyniuk, University of Florida, Gainesville, FL, cmartyn@ufl.edu; Larry B. Barber, National Research Program, U.S. Geological Survey, Boulder CO, lbbarber@usgs.gov; Alan M. Vajda, Department of Integrative Biology, University of Colorado Denver, Denver CO, alan.vajda@ucdenver.edu

Anthropogenic chemicals of emerging concern [CECs] are commonly detected in surface waters and have been shown to cause adverse effects in aquatic vertebrates, including reproductive endocrine disruption. To characterize effects caused by complex contaminant mixtures, we exposed adult fathead minnows (*Pimephales promelas*) to water from four different rivers within the Shenandoah River watershed (VA, USA) 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. RNA was extracted from livers, labeled, and hybridized to 8x60K microarrays, containing 22,010 unique, annotated genes. Differentially expressed genes and pathways were identified using ANOVA, Gene Set, and Sub-Network Enrichment Analyses. Transcript biomarkers of endocrine disruption, including *er1, er2, ar, vtg1,* and *vtg3,* showed little to no differential expression in exposed fish, suggesting these organisms did not experience estrogenic endocrine disruption. This is a surprising finding given that one exposure site was heavily impacted by municipal wastewater effluent, which is often estrogenic. At a highly agricultural site, IL6/STAT signaling and complement pathways were significantly downregulated. Both of these pathways are involved in pathogen resistance, and their down-regulation suggests these fish may be have altered immune function. Also at the agricultural site, pathways involving carbohydrate metabolism were significantly up-regulations, suggesting these fish had increased energetic demands. 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.

Effects of petroleum hydrocarbons on stream communities in West Creek, Colorado

Sam **Duggan**, Colorado State University, 1474 Campus Delivery
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Oil development has expanded dramatically in Colorado over the last decade. Associated with the rapid expansion has been a significant increase in the number of accidental releases into the environment. In 2014, over 300 petroleum spills were reported to the Colorado Department of Health. The Unaweep Canyon spill in January 2013 discharged 22,700 liters of gasoline and 7,300 liters of diesel fuel into West Creek, a tributary to the Dolores River, killing an estimated 1,206 brown trout and 8,172 mottled sculpin. Subsequent electrofishing surveys indicated that the fishery had not recovered, particularly with regard to abundance of sculpin populations. In June and October 2015, as part of ongoing efforts to determine long-term effects of this spill, we explored stream health indicators across multiple levels of biological organization. Histopathological abnormalities (e.g., ectopic neural tissue, cystic kidney, increased

melanomacrophage aggregates) were observed in sculpin collected from the spill site and nearby downstream sites. Significantly elevated white blood cell counts and an aberrant blood differential were detected in sculpin collected from the spill site. Altered benthic macroinvertebrate community structure was observed at the spill site compared with a reference site one kilometer upstream. Interestingly, a GC-MS finger-printing analysis of polycyclic aromatic hydrocarbons (PAH) in stream sediment, at all sites, revealed that PAH concentrations were typical of minimally impacted streams flowing adjacent to roads. These results suggest that effects of the spill were persisting after PAH concentrations had returned to 'normal'.

Are water temperature standards adequately protecting fish species: A framework for development of water temperature standards

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The Environmental Protection Agency created the Clean Water Act in 1972 to mandate the protection of the nation's water resources. To comply with the Clean Water Act, state agencies are required to regulate water temperatures. States are challenged with establishing regulations which are detailed enough to sufficiently protect a wide variety of aquatic life, but are also simple enough to be effectively implemented with the available budget, technology, and knowledge. Because of the potential negative effects of altered water temperatures on fish species, most agencies have developed water temperature standards with the goal that water temperatures do not produce conditions that may be harmful to fish. Despite this common goal, there is a great variety of regulatory approaches to water temperature across the nation. While some of this discrepancy results from differences in ecological conditions and fish community assemblages, the diversity in temperature standards among states can be seen as an indication that state standards fail to provide consistently high levels of protection for fish. Despite the considerable variability in state water temperature standards, it is possible to identify protections that must be present in standards to result in adequate protection for fish species. We identify five considerations that are essential to developing effective temperature standards: physiological requirements, reproductive requirements, trophic requirements, disease and contaminant susceptibility, and thermal heterogeneity. Here we assess the extent to which these components are considered in current temperature standards, highlight examples of standards which meet these requirements, and suggest areas where standards could be improved.

Beyond standards: Challenges for application of Biotic Ligand Model-based water quality standards to permits and impaired waters designations

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Since publication of the National Recommended freshwater ambient water quality criteria (AWQC) for copper by the U.S. Environmental Protection Agency in 2007, substantial progress is being made in adopting the copper Biotic Ligand Model (BLM) for development of regulatory water quality standards. However, adopting standards is only the first step in regulating copper discharges, with these standards also being needed to develop effluent limitations in facility discharge permits, and for making waterbody impairment decisions under the 303(d) program. Concerns have been noted that the additional temporal

and spatial complexity of the 10-parameter BLM (vs. older single-parameter hardness-based criteria) makes application of the BLM into discharge permits and 303(d) programs very challenging. Such challenges include the types and amounts of data available in water quality monitoring programs, concerns over downstream protection of aquatic life in water bodies using a mix of both BLM- and hardness-based standards, and the longevity of these standards if and when water quality conditions change over time. We argue that the simplest place to start building regulatory frameworks for permits and 303(d) impairment programs is to realize that the BLM is simply a new model replacing an older model (i.e., hardness-based criteria equations). Using water quality standards and regulatory frameworks from Colorado as an example, this presentation explores the kinds of data and decision making processes available for use in applying BLM-derived standards in such a way that maintains existing application frameworks and decision criteria.

The influence of developmental stage on sensitivity of mayflies (Ephemeroptera) to major ions.

Lauren **Hargis**, Department of Fish, Wildlife, and Conservation Biology, Colorado State University, 1474 Campus Delivery, Fort Collins, CO 80523-1474. laurenhargis@yahoo.com; William Clements, Department of Fish, Wildlife, and Conservation Biology, Colorado State University, 1474 Campus Delivery, Fort Collins, CO 80523-1474 william.clements@colostate.edu; Janet Miller, US Geological Survey, Fort Collins Science Center, 2150 Centre Ave. Bldg. C, Fort Collins, CO 80526, jmiller@usgs.gov

Elevated concentrations of major ions (e.g., HCO_3^-, Cl^-, SO_4^{2-}, Mg^{2+}, Na^+ and Ca^{2+}) are an emerging issue in stream ecosystems. Anthropogenic disturbances, including those from developing and extracting energy resources are often the cause of elevated major ions. Mayflies (Order Ephemeroptera) have been identified to be some of the more sensitive taxa to high concentrations of major ions. Water quality criteria are designed to protect most aquatic life (95%) and are derived from short term single species aquatic toxicity tests using the model organism such as *Daphnia*. *Daphnia* are a small planktonic crustacean, which may not be representative of aquatic insect communities due to their differences in physiological traits and the fact that they don't usually exist in lotic environments. Also, the short term exposures typically used in laboratory experiments may not represent sensitive life stages of aquatic insects. To test these hypotheses, I used the parthenogenetic mayfly, *Neocloeon triangulifer*, a more suitable test species thought to better represent the sensitivity of stream taxa, to investigate the effects of various life-stage exposures and concentrations of NaCl on mayfly survival, mass, development time, rate of emergence, and fecundity. Results showed that the timing of exposure to NaCl determined effects and that early instars were consistently more sensitive than older individuals.

A tale of two bats: differential mercury uptake in two Chinese species

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Mercury is a toxic heavy metal emitted by industrial practices, primarily metal smelting and coal burning. We conducted a study to test the degree to which bats, a sensitive and understudied taxon, accumulate mercury within and around the growing urban center of Chengdu, capital of Sichuan Province, China. We hypothesized that bats nearer the city center would accumulate greater concentrations of mercury in their fur and that mercury concentrations would differ significantly between bat species, as well as between juveniles and adults of the same species. Japanese pipistrelles (*Pipistrellus abramus*) (n=14) and Chinese

noctules (*Nyctalus plancyi*) (n=30) were captured using mist nets over water at four sites in July and August 2013. Fur was clipped from the mid-dorsal region, and mercury concentrations were quantified with a direct mercury analyzer. T-tests and regressions were run in R. Fur values did not differ significantly between bats from urban versus non-urban areas, although sample size was likely an issue. Adult pipistrelles had significantly greater mercury concentrations than adult noctules (unpaired t-test, $p<.001$), and adult noctules had significantly greater concentrations than juvenile noctules ($p<.001$). Maximum mercury concentrations (up to 33 ppm) were found in adult pipistrelles, and 64% of pipistrelles exceeded fur mercury values associated with reduced neurochemical homeostatic control. Results indicate that Japanese pipistrelles are more susceptible to mercury accumulation than Chinese noctules, which may be due, in part, to their smaller size. Akin to other studies, we confirmed that mercury can accumulate in bat fur with age.

Stream benthic and algal community responses to heavy metals: an evaluation of endpoint sensitivity

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Benthic macroinvertebrate community responses to heavy metals are often used to assess and predict ecological impairment, yet evaluation of aquatic insect adults and algal community responses have received less attention. I evaluated several macroinvertebrate and algal endpoints by exposing benthic communities to mixtures of Cu and Zn for 14 d using stream mesocosms. I compared two streams in Colorado with past and present EPA superfund designation, the Arkansas River (AR) and the North Fork Clear Creek (NFCC), respectively. Measured responses included the number of emerging adults, algal community composition and biomass, community metabolism, and benthic community composition. EC50 values, defined as the metal concentration that reduced the response variable by 50%, were estimated for each endpoint. Results show significant differences in larval and adult responses within the same taxonomic groups, particularly among midges (Chironomidae) and mayflies (Ephemeroptera); however, algal biomass and community metabolism displayed the greatest overall sensitivity to exposure. The relative sensitivity of the endpoints was also different among the two communities, with the AR displaying greater tolerance than the NFCC. This result is likely because of differences in their respective exposure histories and community composition. This research highlights the need to comprehensively assess exposure effects beyond larval macroinvertebrate life stages using additional endpoints that differ in sensitivity, while acknowledging context-dependent responses resulting from different stream assemblages.

Determining the realistic toxicity of imidacloprid for bumblebee (*Bombus impatiens*) workers

Annie **Krueger**, University of Wyoming, Department of Zoology and Physiology, 1000 E. University Ave, Laramie, WY, akruege1@uwyo.edu; Michael Dillon, University of Wyoming, Department of Zoology and Physiology, 1000 E. University Ave, Laramie, WY, Michael.Dillon@uwyo.edu

The agriculture industry relies on pesticides for crop production, but growing evidence suggests that sublethal effects of pesticides may contribute to the decline of insect pollinators. Neonicotinoids are a widely-used, advanced class of insecticides that are incredibly effective for crop protection but, at low levels, can have pronounced sublethal effects on bees. Our understanding of bee toxicology comes primarily from studies on honeybees, and the few studies on bumblebees (genus *Bombus*) have assessed

toxicity on a colony level. We investigated how imidacloprid, a first generation neonicotinoid, affects the diet consumption, long term survival, and activity of individual bumblebees (*Bombus impatiens)* under different exposure scenarios and at a range of concentrations. We saw no significant effects when continuously fed 10 ppb imidacloprid in nectar but at 32 ppb, we saw a significant effect on diet consumption, long term survival (χ^2=6.042, $p<0.05$), and activity (χ^2=13.89, $p<0.001$). Knowing that 32 ppb produced effects with all three metrics, we looked at whether access to clean nectar following each day of exposure to 20 ppb imidacloprid would diminish the effects seen with continuous exposure. We saw a slight recovery in diet consumption and activity after the first day of clean nectar, however, after a second day on 20 ppb imidacloprid, we no longer saw this recovery. Importantly, all of the effects we document would be overlooked with current Tier 1 testing protocols, suggesting that it will be critical to consider sublethal effects to better regulate agrochemicals and protect insect pollinators.

Environmental fate of hydraulic fracturing fluid additives after spillage on agricultural topsoil

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Hydraulic fracturing (HF) is a widely used technology that enhances oil and gas extraction from unconventional formations. The fluids used in HF contain about 90% water, 10% proppant and 0.5-1% chemical additives. Inadvertent fluid releases may occur during many different stages in the hydraulic fracturing process, with surface spills being the most commonly reported cause of contamination. Since hydraulic fracturing frequently occurs in the vicinity of agricultural lands, these releases may lead to complex soil and water contamination. To study the environmental fate of these chemicals in agricultural topsoil, aerobic batch studies were conducted using topsoil collected from Weld County, Colorado. Three widely used HF additives were studied: the biocide glutaraldehyde (GA), polyethylene glycol (PEG) surfactants, and a polyacrylamide-based friction reducer (PAM). In addition, the impact of salt was investigated because high salinities can occur in flowback and produced water. Results showed that PEG surfactants were completely biodegraded in agricultural topsoil within 71 days, but their transformation was impeded in the presence of GA and completely inhibited by salt at concentrations typical for oil and gas wastewater. At the same time, aqueous GA concentrations decreased due to sorption to soil, and were completely biodegraded within 33 to 57 days. The carbon backbone of PAM was not degraded, however, PAM cross-linked with GA, further lowering the biocide's aqueous concentration. The results from this study clearly show that co-contaminant interactions need to be considered in future fate, transport, and human health risk assessments of spills resulting from unconventional oil and gas extraction.

Photo-Induced Toxicity of North West Shelf Crude Oil in Australia: Early Life Stage Tests with Yellowtail Amberjack (*Seriola lalandi*) and Black Bream (*Acanthopagrus butcheri*)

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Photo-induced toxicity of oil occurs when ultraviolet light (UV) contacts certain photo-active polycyclic aromatic hydrocarbons (PAHs), which act as catalysts and produce reactive oxygen species (ROS). When this reaction occurs in the tissues of transparent organisms the ROS production causes cell/tissue damage and results in acute mortality. There are at least 14 PAHs in crude oil that are known to be photo-active and our recent work on the toxicity of *Deepwater Horizon* oil indicates that photo-activation increases toxicity by 10-100 times. In this study, we conducted several bioassays at the Indian Ocean Marine Research Centre (Perth, Western Australia) investigating the photo-induced toxicity of crude oil from Australia's North West Shelf (NWS) to embryos and larvae of indigenous yellowtail amberjack (*Seriola lalandi*) and black bream (*Acanthopagrus butcheri*). In addition to investigating photo-induced toxicity as a result of PAH activation in the tissues of these fish, we also investigated the apparent toxicity of photo-products that are produced in the water when UV light contacts PAHs in water accommodated fractions (WAFs) generated using NWS oil. Preliminary results demonstrate that exposure to NWS oil and UV results in photo-induced toxicity in both species tested. Additionally, UV exposure of WAFs produced using NWS oil generates toxic photo-products that increase the toxicity of these WAFs to our test species when they are exposed to the mixture in the absence of UV.

Dietary magnesium and copper affect survival time and neuroinflammation in chronic wasting disease

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Chronic wasting disease (CWD), is a prion disease of deer, elk and moose and continues to expand geographically in both wild and captive North American cervid populations. It is difficult to control in part due to the extreme environmental persistence of prions, which can transmit disease years after initial contamination. The role of exogenous factors in CWD transmission and progression is largely unexplored. We collected and analyzed water and soil samples from CWD-negative and positive captive cervid facilities, as well as from wild CWD-endozootic areas. We found that CWD-negative sites had a significantly higher concentration of magnesium, and a higher magnesium/copper (Mg/Cu) ratio in the water than that from CWD-positive sites. To explore the significance of this finding, we utilized cervidized transgenic mice as an animal model and fed a custom diet devoid of Mg and Cu and drinking water with varied Mg/Cu ratios, we found that higher Mg/Cu ratio resulted in significantly longer survival times after intracerebral CWD inoculation. We also detected reduced levels of inflammatory cytokine gene expression in mice fed a modified diet with a higher Mg/Cu ratio compared to those on a standard rodent diet. These findings indicate a role for dietary Mg and Cu in CWD pathogenesis through modulating inflammation in the brain.

Examination of metal deposition and removal processes in an acid mine drainage contaminated stream using in-situ sediment samplers

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Metal-mining processes can generate acidic mine drainage (AMD) that negatively impacts the water quality and aquatic ecosystem of receiving streams due to increased metal toxicity, decreased pH, and destruction of aquatic habitats. The drivers of metal deposition onto stream sediments are either the formation of insoluble colloids, adsorption to particles, or complexation with sediment surfaces. The North Fork of Clear Creek (NFCC) in central Colorado receives 2 point sources of AMD in the city of Black Hawk. In 2015, we deployed rock trays at a site downstream of the AMD inputs in order to accumulate metal-oxide particles and coatings on substrate surfaces in the trays for 4 weeks, and then we redeployed them at an upstream reference site to simulate recovery post-remediation for 8 weeks. Afterward, the tray substrates were extracted to dissolve the deposited metals and surface coatings without digesting the sediment substrate. After 4 weeks of AMD deposition, the average concentrations of extracted Cu and Fe significantly increased by ~1700% and ~95%, respectively; and after 8 weeks of recovery, the average concentrations of Cu and Fe decreased (but not significantly) by ~21% and ~7%, respectively. The calculated Cu:Fe ratio, varied significantly among the treated and recovery extracts, implying that metal loss from AMD-contaminated sediment isn't only dependent on physical processes. Examination of the processes of metal deposition and removal from sediment can lead to a better understanding of the fate of AMD-associated metals and better prediction of the time course and extent of post-remediation recovery from AMD contamination.

Stream microbial community responses to metals exposure

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The upper Arkansas River (near Leadville, Colorado) has been heavily impacted by metal pollution due to historical mining. In 1983, the U.S. EPA added California Gulch, the primary source of metals into the Upper Arkansas River, to the U.S. National Priorities ('Superfund') List. By the late 1990s, the completion of water treatment facilities, removal of waste tailings, and stabilization of eroding banks resulted in significant improvements in water quality. Metals pollution caused a shift in stream benthic invertebrate community composition–from 'metal-sensitive' taxa (e.g. mayflies) at reference upstream sites to 'metal-tolerant' taxa (e.g. caddisflies) at polluted sites downstream of California Gulch. Despite significant improvements in water quality over the past fifteen years, these differences in benthic invertebrate communities remain skewed between upstream and downstream sites. Although much research has been focused on benthic invertebrates at the Arkansas River, a significant knowledge gap remains related to microbial community responses along the gradient of metal exposure in the Arkansas River. It is increasingly recognized that microbes play essential roles in ecosystem functioning. However, the understanding of microbial biodiversity and how it contributes to ecosystem processes is currently very limited. I will present preliminary data characterizing microbial community responses to experimental metals exposures, and community composition at sites upstream and downstream of California Gulch. Our results indicate that metals exposure can create changes in microbial composition, and community structure remains different between sites upstream and downstream of California Gulch. Such changes in microbial community composition may have effects on stream ecosystem processes.

Abstracts for Poster Presentations

(Alphabetical by presenter)

Evidence of estrogenic endocrine disruption at several municipal wastewater treatment plants within the South Platte watershed, Colorado

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Endocrine disrupting compounds (EDCs) are a large group of chemicals that can impair normal endocrine function. Concern over EDCs has grown due to an increasing number of studies that have indicated that many of these contaminants are widespread and have induced measurable effects in both terrestrial and aquatic animals. Estrogens and xenoestrogens are common EDCs often found below wastewater treatment plants. The goal of our study was to gain a better understanding of the distribution of estrogenic EDCs in the South Platte watershed. Male fathead minnows, *Pimephales promelas*, were caged above and below 10 municipal wastewater treatment plants on streams and rivers of the South Platte watershed, Colorado. Caging efforts were focused at municipal wastewater treatment plants because little is known about the estrogenic potential of effluents coming from many of Colorado's treatment plants. Cages were left for one week at which point they were removed and fish were euthanized for liver and gonad extraction. Liver tissue was analyzed for the presence of RNA that codes for vitellogenin, an egg yolk precursor protein that can be used as a biomarker for estrogenic exposure. Initial results showed that treatment plants in Fort Collins and Longmont induced significant vitellogenin production, while three other treatment plants induced only a small effect, or no effect at all. Wild fish were also concurrently collected at one treatment plant and the vitellogenin production by those fish was significantly less than that of the caged fish. Our results indicate that estrogenic exposure varies across the landscape. Additionally, wild fish may be avoiding or adapted to exposure.

Capping contaminated sediments at groundwater-surface water interfaces

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Contaminated sediments are emerging as a major cost liability for the petroleum industry. In response, Colorado State University (CSU) initiated a new research thrust in 2015 exploring solutions for managing sediment impacted by petroleum facilities through the primary object of assessing current remediation strategies. Current best practices for sediment remediation include dredging, capping, and sequestration. These technologies are appropriate for stabilizing persistent contaminants such as metals and polychlorinated biphenyls (PCBs). However, capping and sequestration may limit natural attenuation of degradable contaminants through oxygen preclusion and/or increased oxygen demand exerted by sorptive capping materials. Further, the aerobic and biologically diverse nature of groundwater-surface water interfaces, where many contaminated sediment sites are located, are not leveraged through the capping approach. Laboratory experiments will elucidate the impact of various capping materials and inform a one-dimensional, multi-component model for capped sediments. A dissolved phase column study

examining the impacts of four different capping materials is currently underway; contaminant concentration data are being collected and analyzed. Meanwhile, we are preparing to conduct a frozen core analysis on these columns. Additionally, a non-aqueous phase liquid (NAPL) column study is underway to determine the time-to-breakthrough of contaminants capped with the same selection of capping materials. A video of NAPL transport through the capped columns will be generated through this experiment. Collectively, the data will shed light on the perceived vulnerabilities associated with capping contaminated sediments.

Assessment of pharmaceuticals, personal care products, and pesticides in water resources on the San Luis Valley National Wildlife Refuge Complex using POCIS.

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The U.S. Fish and Wildlife Service evaluated the presence of pharmaceuticals and personal care products (PPCPs) and pesticides in water resources within the San Luis Valley National Wildlife Refuge Complex (Complex). The Complex is made up of the Baca, Monte Vista, and Alamosa National Wildlife Refuges (NWRs) and encompasses approximately 119,330 acres in southern Colorado. In addition to artesian well water, each NWR unit draws surface water from a source downstream of one or more wastewater treatment plants that may also receive runoff from agricultural operations throughout the area. As such, concern has been raised about how these inputs may affect the quality of water used by the Complex. To assess this, polar organic chemical integrative samplers (POCIS) were deployed in 2012 and 2013 on the Baca NWR, in 2014 on the Monte Vista NWR, and in 2015 on the Alamosa NWR. POCIS were deployed for approximately 30 days each and recovered extracts analyzed for 140 PPCPs in each year and 72 pesticides in 2015. Results indicate that 1) 65 and 86 PPCPs were detected on the Baca NWR in 2012 and 2013, respectively; 2) nine PPCPs were detected on the Monte Vista NWR in 2014; and 3) 15 PPCPs were detected on the Alamosa NWR in 2015. Eighteen pesticides were detected on the Alamosa NWR in 2015 and included 15 herbicides, two fungicides, and one neonicotinoid insecticide.

Tracing Cu uptake in *Lymnaea stagnalis* using reverse isotopic labeling

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We traced the flux of Cu in isotopically-labeled freshwater snails (*Lymnaea stagnalis*) that were fed flocculent material rich in Fe and other metals (e.g., Cu, Zn) collected from sediment in the North Fork of Clear Creek (NFCC) in Black Hawk, Colorado. This study is part of a project to evaluate contamination and potential remediation of acid mine drainage (AMD) in NFCC. In these dietborne-metal experiments, snails are used as an easily-cultured surrogate for other invertebrates. The isotopic signature of the snails is reversed by culturing them in moderately hard reconstituted water containing a sublethal concentration of isotopically enriched ^{65}Cu for five weeks before the dietary exposure begins. The metal-contaminated flocculent material is mixed with a diatom (*Nitzschia palea*) to produce a series of dietborne-metal concentrations, and each mixture is collected on a filter to create a mat the snails can eat. Using mass balances and isotopic-ratio conversions, the uptake and depuration of Cu were traced over a 48-h exposure in which the organisms consumed control or contaminated food. The food-ingestion rate was constant at ~0.6 g food/g snail/day, for contaminated food regardless of dietborne Cu concentration; the

Cu assimilation efficiency was ~50-60%; and the Cu uptake rate was 0.188 □g Cu/g snail/day food. The assimilation efficiency, which describes how well the particles are taken up by an organism, can be used to quantify the bioavailability of metals. These results will have direct implications to understanding and predicting potential instream toxicity of contaminated sediments before, during, and after remediation of AMD.

A landscape-based approach to assess endocrine disruption in fish in the Shenandoah River

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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 August 2014, we deployed in-situ, flow-through aquaria at four locations in the Shenandoah Valley with distinct landuse patterning, including an agriculturally dominated site, a WWTP effluent site, a downstream mixed-use site, and a pristine reference site. 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 chemical profiling. Gonads were prepared for histological examination and analyzed for cellular and tissue abnormalities. In males, serum concentrations of vitellogenin were measured by ELISA as a biomarker of exposure to estrogenic substances. To link these traditional biomarkers to molecular changes, RNA sequencing will be used to profile transcriptomic changes and identify differentially expressed genes [DEGs] following chemical exposure. Gene set enrichment analysis and sub-network enrichment analysis will identify biological processes and molecular pathways that are statistically over represented among DEGs. To link the transcriptomic profile back to landscape patterning, a principle components analysis will be preformed on the gene expression data. A significant clustering of samples by site will indicate that transcriptomic changes do indeed vary based on watershed. Together, these data will provide novel insight into the relationship between landuse, chemical contamination and biological effect.

Directions to Fort Collins Science Center

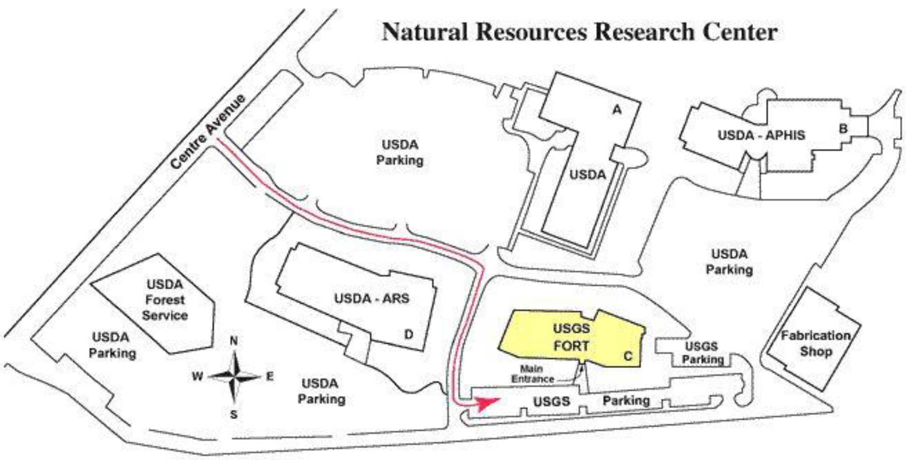
From I-25 Fort Collins Exits to the NRRC and the Fort Collins Science Center (FORT):

- Exit 268 (Prospect Road) off of I-25
- LEFT (WEST) on Prospect Road
- LEFT (SOUTH) on Centre Avenue
- LEFT (EAST) at NRRC stone sign
- Building C (USGS), *south entrance*

Click here for a printable version of this map and directions

Finding Building C

When you turn into the Natural Resources Research Center by the stone sign, you will be heading east on the main driveway. Our USGS building is ahead of you, the second one on the right. Take the second right and then the very next left into our parking area, which lines the south side of our building. Ahead of you and halfway down you will see the main entrance. You will need to park and check in with the receptionist, who will issue you a parking pass. (Vehicles with government plates do not need a pass.) For further information, contact the receptionist at our main number, 970-226-9100.



The Fort Collins Science Center address is:

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Fort Collins Science Center
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Tel. (970) 226-9100

Please consider carpooling or biking whenever possible as parking at the NRRC is limited.