2021 Carolinas and South East SETAC Joint Annual Meeting

Hosted by Dr. Mohammed Baalousha and

University of South Carolina

May 17-18, 2021
Virtual Meeting Information & Program

The CSETAC Board of Directors would like to thank the following sponsors:

Meeting Summary

Monday May 17, 2021

9:00am-12:00pm

Microplastics Workshop (previous registration required)

1:00pm-3:55pm
Welcome, Platform Sessions, with Coffee break (2:10-2:25pm)

4:10pm-5:20pm
Lightening Talks

5:20pm-6:00pm
Social "Hour"

Tuesday May 18, 2021

8:15-8:30am

Update on SETAC NA Initiatives

8:30am-9:30am

Lightening Talks

9:30-12:15pm
Platform Sessions, with Coffee break (10:45-11:00am)

12:15-12:30pm
Coffee Break

12:30pm-1:30 pm
Awards / Board Meeting

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CAROLINAS/SE SETAC ZOOM MEETING AGENDA, MONDAY, MAY 17, 2021

Time	Speaker	Title
9:00am-12:00pm		**Workshop (Microplastics) (previous registration required)**
12:00-1:00pm		**Lunch Break**
1:00-1:05pm 1:05-1:10pm	Dr. Tara Catron Dr. Joseph Bisesi	Welcome and Chapter Updates
1:10-2:10pm		**Platform Presentations (Session 1): Plastics and nanoparticles (11 min + questions)**
1:10-1:25	Stephanie LaPlaca	Comparison of Microplastics from Suburban and Rural Areas in South Carolina
1:25-1:40	Bonnie Ertel*	Prevalence of PPE in plastic litter before and during the pandemic
1:40-1:55	Jingjing Wang	Metallic Fingerprint in Nanoplastics from Real-life Plastic Products
1:55-2:10	Md Mahmudun Nabi*	Urban runoff is the key driver of titanium dioxide engineered particle concentrations in the Broad River
2:10-2:25		**Coffee Break/Social Interactions**
2:25-3:55pm		**Platform Presentations (Session 2): Metals and Organic Contaminants (11 min + questions)**
2:25-2:40	MaKayla Foster*f	Evaluating Per- and Polyfluoroalkyl Substances (PFAS) in Alligators and Assessing the Corresponding Lipid Alterations
2:40-2:55	Kaylie I. Kirkwood*	Utilizing Pine Needles and Untargeted Multidimensional Measurements to Monitor Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) in North Carolina
2:55-3:10	Paul G. Matson	Long-term monitoring of polychlorinated biphenyls (PCBs) in fish from eastern Tennessee
3:10-3:25	Emily C. Pisarski	Analysis of Floating Oil Under UV Light at Different Environmental Conditions
3:25-3:40	Lindsay Boone	A Case Study of PFAS in Wastewater Influent and Effluent
3:40-3:55	DH Love	Examining the potential for leaching and toxicity from mosquito net fishing
3:55-4:10		**Coffee Break/Social Interactions**
4:10-5:20pm		**Lightening Talks (3 min + questions)**
4:10-4:15	Rian Burris	Quantifying Microplastic Concentrations in the Sediment and Water of Three South Carolina Watersheds
4:15-4:20	Martina M Plafcan	Combined Effects of Microplastic Ingestion and Elevated Temperature on *Acropora cervicornis*
4:20-4:25	Adriana Apintiloaiei*	"Hot or Not? Evaluation of the Hot Needle Test for Identifying Microplastic
4:25-4:30	Kayli Paterson*	Adapting microplastic methodology for the improved enumeration of tire wear particles in environmental samples
4:30-4:35	Marian C. Smith*	Microplastics and Tire Wear Particles in Nuisance Floodwater: Role of Adjace nt Marshes in Particle Capture
4:35-4:40	Courtney Garneau *	Determination of Uptake and Trophic Transfer of Microplastics by Caddisflies [Hydropsychidae] to Riparian Spiders [Tetragnathidae] in Southern Appalachian Headwater Streams.
4:40-4:45	Andrea Landaverde*	Plant selection for Floating Treatment Wetlands in Brackish Waters
4:45-4:50	DJ Gabrielli	Disruption of the Gastrointestinal Lipidome After Exposure to Single-Walled Carbon Nanotubes in a Piscivorous Fish
4:50-4:55	Hannah Woodburn*	Assessment of Elemental Contamination from Multiple Point Sources on the Broad River, NC
4:55-5:00	Alexis Wormington	Exposure to organochlorine pesticides results in systemic alterations of liver lipids in largemouth bass
5:00-5:05	Daniel Short	Choice of Source Apportionment Model Impacts Emission Rates of Volatile Organic Compounds in the Bakken Shale Oil and Natural Gas Region
5:05-5:10	Jack Dekle*	Nutrient Dynamics in a Floating Treatment Wetland System after Overwintering in Eutrophic Conditions
5:10-5:15	Ariel A. c	Atmospheric Dry Deposition of Legacy and Emerging Per- and Polyfluoro--alkyl Substances (PFAS) in North Carolina
5:15-5:20	Christina Ek*	Enterococci Contamination on Edisto Island, SC: Frequency, Sources of Contamination and Prospects on How to Improve Water Quality
5:20-6:00pm		**Social "Hour"**

***Indicates CSETAC student competing in the presentation awards**

CAROLINAS/SE SETAC ZOOM MEETING AGENDA, TUESDAY, MAY 18, 2021

Time	Speaker	Title
8:15-8:30am	Dr. Lisa Ortego	SETAC NA BoD Representative, update on SETAC NA initiatives
8.30-9:30am		**Lightening Talks (3 min + questions)**
8:30-8:35	Emma Ivantsova	Investigating the developmental toxicity of the anti-neoplastic cyclophosphamide in embryo/larval zebrafish (Danio rerio
8:35-8:40	Jennifer Harfmann	Biodegradation of emerging per- and polyfluoroalkyl substances in sediments: Forever no more?
8:40-8:45	Karli A. Gaffrey*	Preliminary Scoping Experiment in Enantioselectivity of Polychlorinated Biphenyls on Silica Surfaces
8:45-8:50	A. O'Connor	How Azo Dyes and Microfibers may Negatively Impact Lung Cells and Contribute to Susceptibility to Influenza A Virus
8:50-8:55	Lauren Bradley	Elucidating Molecular Mechanisms of Harmful Algal Blooms on Respiratory Effects
8:55-9:00	JK Gwinn	Exploring in vitro Phase I biotransformation of brevetoxin (PbTX-2) in northern Gulf of Mexico fish microsomes
9:00-9:05	Nima J Madani	Investigating Impacts of Irbesartan on PPARγ in Aquatic Species via Non-Target Analysis and In Vitro Bioassay Testing
9:05-9:10	Sarah Thompson*	Evaluation of air mass back trajectory utility for determining ONG emission influence
9:10-9:15	Morgan Lowery*	Automated Morphology Assessments for Improved Larval Zebrafish Screening
9:15-9:20	Sarah Orr*	Ontogenetic differences of major ion toxicity in the mayfly, *Neocloeon trian- - gulifer*
9:20-9:25	Jonathan Stewart*	The Impact of Select PFAS-free Aqueous Film Forming (AFFF) on the Eastern Oyster, *Crassostrea virginica*
9:25-9:30	Catherine P. Sumner *	Prospective Research in Enantioselectivity of Reductive Dehalogenase Exposed to Metals
9:30-10:45am		**Platform Presentations (Session 3): Uptake and Toxicity (11 min + questions)**
9:30-9:45	Louise M. Stevenson	Biokinetic modeling of mercury accumulation in fishes: a case study in an Eastern TN river and review of the literature
9:45-10:00	CT Bennett	Depuration kinetics of Caribbean ciguatoxin in *Lagodon rhomboides* (pinfish)
10:00-10:15	Cassidy Dauer*	Post Remediation Assessment of Fish Assemblages in an Acid Mine Drainage Impacted Headwater Stream
10:15-10:30	Chicas-Mosier	Prolonged Exposure to Pesticides in Honeybees: Circadian Rhythms, Motility, and Lifespan
10:30-10:45	Christopher L. Souders II	Developmental toxicity of the dinitroaniline herbicide pendimethalin in zebrafish embryos/larvae (*Danio rerio*)
10:45-11:00am		**Coffee Break/Social Interactions**
11:00am-12:15pm		**Platform Presentations (Session 4): Uptake and Toxicity (11 min + questions)**
11:00-11:15	Deja Best*	Impacts of Temperature and Salinity Changes Associated with Climate Change on Shellfish Disease and Health in SC Oysters
11:15-11:30	Madison Suttman*	Environmental Justice in Sampson County: Can You Trust the Water?
11:30-11:45	Peter van den Hurk	Toxicity of passive sampler extracts from a coal tar contaminated section of the Congaree River, Columbia, SC.
11:45-12:00	Molly M. Miller	Coastal cyanobacterial harmful algal bloom dynamics in Mississippi and Alabama
12:00-12:15	Xuedong Chen	Laboratory testing of a new strain of the insect-pathogenic fungus Metarhizium anisopliae 'S54' as a potential control agent for *Lygus keltoni* (Hemiptera: Miridae), a pest of canola and alfalfa in western Canada
12:15 -12:30pm		**Student Awards**
12:30-1:30pm		**Breakout** **CSETAC Business Meeting** **SESETAC Business Meeting**

***Indicates CSETAC student competing in the presentation awards**

Day 1: Monday 05/17/2021

Platform Session 1: Plastic and Nanoparticle Pollution

(*indicates presenting author)

Comparison of Microplastics from Suburban and Rural Areas in South Carolina

Jeannette Fantone and Stephanie LaPlaca, Clemson University*

Plastic pollution is a worldwide problem, affecting both the marine and freshwater environment, threatening the health of the aquatic ecosystems, and largely contributing to climate change. Microplastics are small pieces of plastic that are < 5 mm in size - their small size makes it difficult to measure how much damage they can cause in the ecosystem. To gain a better understanding of which microplastics are primarily found locally, sediment samples were assessed from suburban and rural locations in South Carolina. The total amounts of microplastics were compared to determine if there is a relationship between the location of collection, the amount of microplastics, and the types of microplastics found in the sample. Sediment samples were collected from various locations including storm drains, streams, and creeks in suburban and rural areas. The samples underwent four rounds of density separation in a NaCl solution, utilizing 500 µm and 53 µm sieves to separate and isolate the microplastics. The microplastics were visually counted under a light microscope and identified as tire particles, fibers, or fragments. Total microplastics and microplastic types encountered will be discussed and will include comparisons between the locations sampled.

Prevalence of PPE in plastic litter before and during the pandemic

Bonnie Ertel, The Citadel*

The ongoing COVID-19 pandemic has affected our lives in profound ways including social distancing protocols, local lockdowns, and a marked global increase in the use of personal protective equipment (PPE) such as face masks and gloves. While certainly necessary to prevent viral spread, these plastic PPE items are likely to contribute to our plastic litter problem if not properly disposed or reused. The objective of this study was to document how the ongoing COVID-19 pandemic has affected volunteer efforts and PPE abundance reported in both national (U.S.) and local (SC) litter cleanups. By utilizing public litter cleanup databases maintained by the South Carolina Aquarium and the Ocean Conservancy, we tracked temporal trends in number of volunteers, number of cleanups, plastic litter composition, and PPE abundance throughout the pandemic. Compared to data from 2016-2019, in 2020 there were fewer total volunteers but more total cleanups, suggesting that volunteers continued to collect litter but did so as individuals or small groups in order to maintain social distancing. The number of PPE items collected in U.S. cleanups (n= 23,599) and SC cleanups (n=793) was higher than previous years (by a factor of 1966x and 40x, respectively) but this type of litter remains a small portion (0.671% and 0.213%, respectively) of the total plastic litter collected in cleanups. As the pandemic persists, it is likely that we will continue to see an increase in PPE items collected by volunteers during cleanups. At both the national and local level, 37% of cleanups reported finding PPE litter by December of 2020. Quantifying and tracking trends in litter cleanup databases will help us to better understand society's behavior during the pandemic and will ultimately help inform policy makers and regulators on waste management.

Metallic Fingerprint in Nanoplastics from Real-life Plastic Products

Jingjing Wang[1], Phillip M. Potter[2], Souhail Al-abed[2], Mohammed Baalousha[1]*
[1]Center for Environmental Nanoscience and Risk, Arnold School if Public Health, University of South Carolina, Columbia, SC 29208, USA; [2]Oak Ridge Institute for Science and Education (ORISE), U.S, EPA, Cincinnati, OH 45268, USA.

Micro- and nanoplastics (MNPs) are a serious global environmental problem and are receiving considerable attention from government agencies, research communities, and the general public. The global production of plastics exceeds 250-300 million tons per year and is expected to double in the next two decades. Due to improper disposal and transport via wind and surface runoff, large quantities of plastics enter aquatic ecosystems. These plastics breakdown in the environment into increasingly smaller pieces and gradually form micro- and nano-sized plastic particles (> 1 µm and 1-1000 nm, respectively), known as secondary (or real-life) MNPs. Most MNPs studies to date have focused on investigating commercially available synthetic MNPs and on the polymer content of MNPs. However, real-life MNPs are a complex mixture of polymers and additives, including metallic additives. Therefore, characterizing the metallic content and nature in MNPs is critical to to improve the understanding of the environmental fate, behavior, and risks of MNPs. This presentation will discuss the analysis of metallic fingerprint in real-life plastic products using laser ablation- and single particle-inductively coupled plasma-time of flight-mass spectrometer (LA-ICP-TOF-MS and SP-ICP-TOF-MS). ICP-TOF-MS is a powerful tool for the analysis of metals as it measures all elements and all isotopes in transient signals such as laser ablation and single particles. Our findings indicate that all plastics contain some combination of metals, providing MNPs a unique elemental fingerprint that could be used to track MNPs in environmental studies.

Urban runoff is the key driver of titanium dioxide engineered particle concentrations in the Broad River

Md Mahmudun Nabi[1], Jingjing Wang[1], Mohammed Baalousha[1]*
[1]Center for Environmental Nanoscience and Risk, Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, South Carolina, United States

This presentation will discuss the quantification, concentrations, and potential major sources of titanium dioxide engineered particles in the Broad River, Columbia, South Carolina, United States. Water samples were collected from the Broad River during a range of hydrologic conditions, i.e., following hurricane Florence (total daily precipitation of 42 mm), a major precipitation event of 33 mm, and a low intensity precipitation of 0 to 3.5 mm. The total metal concentrations in the water samples were determined by ICP-MS following total digestion using 2 mL of HF: HNO3 (3:1) mixture. TiO2 concentration was determined based on mass balance calculation, total Ti concentrations, and increases in Ti/Nb ratios above the natural background ratios. The total concentrations of Ti, Fe, Al, Nb, Ce, and La in the Broad River followed the same trend of rise and fall as the discharge/runoff. The Ti/Nb elemental ratios varied in the range from 330 to 565, which were higher than the natural background elemental ratio estimated in surface waters (e.g., 266.4 ± 8.9) in Columbia, SC and the average crustal values (e.g., 320), suggesting contamination with TiO2 engineered particles. The TiO2 engineered particles concentrations varied between 20 and 140 µg TiO2 L-1 during the sampling campaigns in the Broad river with highest concentrations occurring at/near the peak of storm events. These results, together with the absence of sewage discharges as indicated by the absence of gadolinium anomalies, suggest that urban runoff is the driver of TiO2 concentrations in the broad river during storm events.

Platform Session 2: Metal Organic Contamination

Evaluating Per- and Polyfluoroalkyl Substances (PFAS) in Alligators and Assessing the Corresponding Lipid Alt erations

MaKayla Foster[1], Scott M. Belcher[2], Erin S. Baker[1]*
[1]North Carolina State University, Department of Chemistry; [2]North Carolina State University, Department of Toxicology

Per- and polyfluoroalkyl substances (PFAS) are manmade chemicals utilized in household and industrial applications due to their chemically inert, thermally stable, and hydrophobic properties. These characteristics however are detrimental and cause PFAS to be extremely stable, leading to bioaccumulation, biomagnification and bioconcentration. Therefore, with each environmental release or spill, public health concerns rise about the safety of their drinking water and the species living in the water. Alligators are apex predators, which offer an important resource for PFAS exposure analyses as they are subject to both bioaccumulation and biomagnification. Over their approximately 30-year life span, alligators can provide insight into how long-term exposure that humans may experience affects health. Additionally, since alligators have robust immune systems, the immunotoxicant effects of PFAS can be further analyzed. To study PFAS levels in alligators, we analyzed serum from 15 different alligators from Florida and 45 different alligators from North Carolina using untargeted liquid chromatography (LC), ion mobility spectrometry and mass spectrometry measurements. PFAS identifications were first performed in a targeted manner with Skyline by matching the LC-IMS-MS features to a library containing over 100 PFAS standards. Machine learning and Kendrick mass defects were then utilized for the untargeted identification of potential PFAS. Since PFAS exposure has also been associated with lipidomic changes, lipid analyses were also performed on the alligator serum. The PFAS and lipid associations were then compared to provide a better understanding of if lipid alterations were occurring in alligators with differing amounts of PFAS in their serum.

Utilizing Pine Needles and Untargeted Multidimensional Measurements to Monitor Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) in North Carolina

Kaylie I. Kirkwood, Scott M. Belcher, Erin S. Baker, North Carolina State University

Per- and polyfluoroalkyl substances (PFAS) are a class of manmade organofluorine chemicals used in a variety of household and industrial applications. PFAS have become a global concern due to their environmental persistence, bioaccumulative nature, and associations with adverse health effects, thus monitoring both spatial and temporal PFAS presence is crucial. PFAS concentration levels are commonly monitored in surface and groundwater, soil, and wildlife, however the assessment of atmospheric PFAS is less common due to the expensive equipment required which can only be placed at a limited number of sites. Recently, the passive sampling capabilities of trees have been investigated to address this challenge. For example, pine needles possess highly adsorptive wax cuticles which have been effective passive samplers for a wide variety of environmental contaminants, including legacy PFAS or PFAS compounds with long aliphatic chains. To understand PFAS contamination in North Carolina from point sources such as a fluorochemical manufacturer, military bases, and airports, we optimized the current pine needle extraction methods by adjusting the liquid and solid-phase extraction parameters. The needles were then analyzed using an untargeted platform coupling liquid

chromatography, ion mobility spectrometry and mass spectrometry (LC-IMS-MS) separations, allowing for the identification of PFAS species based on hydrophobicity, size, and mass. This new method resulted in the identification of over 50 PFAS compounds in the pine needles. Furthermore, the detected species covered all of the legacy compounds identified in previous studies, as well as multiple classes of emerging PFAS with shorter aliphatic chains and structural modifications such as ether linkages and branched or cyclic aliphatic chains, not previously detected in pine needles. This method was also successful in identifying multiple PFAS point sources and longitudinally monitoring the levels of the diverse PFAS compounds across NC to address environmental concentration changes due to remediation and introduction.

Long-term monitoring of polychlorinated biphenyls (PCBs) in fish from eastern Tennessee

Paul G. Matson[1], Louise M. Stevenson[1], Rebecca A. Efroymson[1], Mark J. Peterson[1], Teresa J. Mathews[1]
[1]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

Environmental contamination due to human activities is a major concern, particularly for persistent chemicals. Within catchments, there is great potential for persistent contaminants to be transported, either through adsorption or biological uptake, with downstream locations acting as sinks for accumulation. Polychlorinated biphenyls (PCBs) are a widespread legacy pollutant from industrial activities that have been linked to numerous negative health outcomes for wildlife and humans. Here we present long-term observations of PCB body burdens within fish found in lower-order tributaries on the Oak Ridge Reservation, an impacted US Department of Energy property in eastern Tennessee, and a large reservoir system adjacent to it composed of parts of the Clinch and Tennessee Rivers. Given that the reservoir system has experienced no direct PCB mitigation activities, this contamination offers an opportunity to explore potential natural attenuation of PCBs within a large lotic ecosystem. Temporal and spatial patterns in PCB concentrations were compared between multiple stream and reservoir sites, with a focus on gamefish species (sunfish and catfish) with potential to directly affect human health. Trends reported in this study are compared to similar efforts from other regions to contextualize and evaluate consistency in responses since the banning of PCB production in 1979.

Analysis of Floating Oil Under UV Light at Different Environmental Conditions

Emily C. Pisarski[1], Edward F. Wirth[1]
[1]NOAA/NOS/NCCOS; Stressor Detection and Impacts Division, Charleston, SC

Oil spills are subject to many environmental weathering processes that alter the chemical and physical properties of oil. Photo-oxidation has been previously thought to be a minor, long-term component in oil weathering, but after the Deepwater Horizon oil spill, it is now recognized as a more important and shorter-term weathering process. Understanding how oil weathers under solar irradiation can improve models that forecast the fate and transport of oil spills. This study examined chemical and physical changes that occur in floating oil exposed to ultraviolet light (UV-A) at different temperatures. Laboratory exposures of floating oil (Louisiana Sweet Crude) were prepared in beakers of seawater on an orbital shaker platform, with manipulations of light (UV-A or fluorescent light) and temperature (10, 21 and 30℃). Samples were collected (6h, 24h, 48h, and 7d) and the oil was photographed to examine physical changes. Changes in chemical composition were analyzed by gas chromatography mass spectrometry to examine differences in hopanes, steranes, polycyclic aromatic hydrocarbons (PAHs) and total extractable hydrocarbons (TEH). Chemical analysis of oil samples showed that there was a greater loss of certain PAHs in oil exposed to UV light when compared to oil without UV exposure. There were no differences between light treatments for hopanes, steranes and TEH. Physical changes were observed between light treatments at all three temperatures; oil exposed to UV light became more viscous and formed "tar-ball" like substances whereas oil exposed to fluorescent light remained less viscous and more sheen-like. Results from this study will be used to improve models that forecast the fate of floating oil in the environment and inform future directions for additional research.

A Case Study of PFAS in Wastewater Influent and Effluent

Lindsay Boone, Enthalpy Analytical

A case study will be discussed that highlights two topics concerning PFAS in wastewater. First, does the amount of particulate present in an influent sample cause PFAS to partition within the matrix? We examine how solubility and chain length of these compounds effect partitioning behaviors. Second, we examine how to properly measure both known and unknown PFAS precursor compounds in both influent and effluent. The amount of total PFAS that these compounds contribute is measured.

Examining the potential for leaching and toxicity from mosquito net fishing

Love, D.H.[1], Welsh, R.[2]. Larsen, D.A.[2], Hinz, F[3],Wilson, P.C.[3], Bisesi, J.H.[1]

[1]Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, USA.

[2]Department of Public Health, Food Studies and Nutrition, Syracuse University, Syracuse, NY, USA.

[3]Department of Soil and Water Sciences, University of Florida, Gainesville, FL, USA.

The distribution of insecticide treated mosquito nets (ITNs) is a great public health success story, with studies suggesting these nets reduced the global burden of Malaria by approximately 40%. ITNs are typically are treated with pyrethroids, which are known to have a low toxicity to mammals and high toxicity to aquatic organisms. Recent studies have shown that ITNs are being used for alternative purposes, including fishing. For example, in a study from a waterside community located on Lake Tanganyika, 87.2% of people utilized their mosquito net for fishing as opposed to malaria protection. Coinciding with reports of off label fishing, there are reports of decrease in fish quality and quantity. The purpose of our study is to determine how much chemical is leaching from the nets, and whether prewashing the nets reduces toxicity and mortality. We conducted a series of exposure experiments using D. magna by introducing them to different sized ITNs. These nets ranged from 1cm2-20cm2. The nets were pre-washed for 30 minutes in 300mL of moderately hard water and dried for 30 minutes prior to exposure. Once the nets were placed in exposure beakers, they remained for 30 minutes and were then removed and discarded. After exposure We measured growth and mortality in the organisms. In order to quantify the leaching concentration, we conducted experiments using liquid-liquid hexane extractions at different time intervals. The time intervals tested were dip, 12,24,48 and 72 hours. The extracts were analyzed by gas chromatography with electron capture detection for mortality experiments, there was a reduction in toxicity when the nets were prewashed, when compared to non-prewashed nets from previous experiments. For our leaching experiments, we observed the highest concentration of pyrethroids after 12 hours and the lowest concentration leaching after 72 hours. Results from these studies indicate that the use of ITNs for fishing represents a significant potential hazard to aquatic organisms and that leaching from the nets is occurring. However, prewashing the nets may be useful to reduce toxicity. Educational strategies may need implementation alongside ITN distribution to decrease this practice and reduce risk to aquatic organisms.

Day 2: Tuesday 05/18/2021

Platform Sessions 3 and 4: Uptake and Toxicity

Biokinetic modeling of mercury accumulation in fishes: a case study in an Eastern TN river and review of the literature

Louise M. Stevenson, Simon Pouil, Amber Hills, Paul G. Matson, and Teresa J. Mathews, Oak Ridge National Lab*

Mercury (Hg) is a potent neurotoxicant that bioaccumulates in aquatic organisms and biomagnifies across food chains, causing widespread impacts. Hg-contaminated fish threaten human health, emphasizing the need to understand the dynamics of this bioaccumulation. Bioaccumulation can be described by models of varying complexity, from the simplified bioconcentration factor which describes the partitioning of contaminants between water and the organism, to more sophisticated models which take into consideration metal speciation, complexation, and/or bioavailability. Biokinetic models have been developed to understand and predict the body burden of metals in organisms depending on exposure and uptake route (e.g. Luoma and Rainbow 2005). However, these models have traditionally been applied in simplified lab settings over short time periods, where complicating factors such as diet composition and growth are not relevant. In this talk, we will present preliminary data using this model to understand and predict the patterns of Hg bioaccumulation in redbreast sunfish and rock bass over 10 years in East Fork Popular Creek (EFPC), a Hg-contaminated stream located on the Oak Ridge Reservation in East Tennessee, a federally owned property managed by the U.S. Department of Energy. These data span over a decade of sampling surveys across multiple locations along EFPC and include data from marked and recaptured fish, allowing for fine scale parameterization of the model. In developing this work, we also performed a literature review of toxicokinetic model parameters, such as assimilation efficiency and uptake and efflux rates, for various fishes across aquatic environments exposed to different forms of mercury (e.g, inorganic Hg and methylmercury, among others). We will also present preliminary findings on how these parameter values vary across Hg forms, fish species, and environments, and how these implications could scale up to population or community levels.

Depuration kinetics of Caribbean ciguatoxin in Lagodon rhomboides (pinfish)

*Bennett, CT [1,*2], Robertson, A [1,2]*
[1]School of Marine and Environmental Sciences, University of South Alabama, Mobile, AL, USA
[2]Dauphin Island Sea Lab, Dauphin Island, AL, USA

Ciguatoxins (CTXs) are considered persistent contaminants in marine fish and can cause ciguatera poisoning (CP), a prevalent algal derived neurotoxic illness. Evidence of persistence of CTXs in fish has been primarily from CTX measurements in wild-caught fish from CP-endemic regions without information on the exposure conditions, including the period between the last CTX meal and capture. Without understanding the kinetic response of CTX in fish during depuration, modeling CTX distribution in food webs remains a major gap in managing CP. To test the hypothesis that CTX concentrations in fish tissues decrease with time during a depuration state, we performed a two-phase, 17-week CTX feeding trial in Lagodon rhomboides. Fish were fed a known amount of Caribbean CTX-1 for up to 20 days and depurated for up to 99 days, randomly sampled at various intervals, and whole muscle, liver, and other pooled visceral contents (heart, spleen, gall bladder, intestine) were dissected separately for toxin analysis by an in vitro mouse neuroblastoma (N2a) assay and LC-MS/MS. The expected decrease in toxicity with depuration time only occurred in viscera extracts. Muscle extract toxicity shifted from a decreasing trend up to 20 days into depuration to an increasing trend from days 20 to 99, and maximum muscle toxicity (mean ± standard dev. = 0.09 ± 0.02 ppb) was measured on the final day. After 99 days in depuration, muscle, liver, and viscera samples had mean toxin concentrations 189%, 128%, and 42%, respectively, compared to fish sampled at the beginning of depuration. Further, we observed somatic growth in all fish and expected a dilution effect to decrease CTX concentrations in fish sampled later in the study, but growth dilution had no apparent effect on lowering toxicity. These data help to explain the potential persistent contamination of C-CTX-1 in the wild and suggests that a complex redistribution of CTX may occur during a depurative state.

Post Remediation Assessment of Fish Assemblages in an Acid Mine Drainage Impacted Headwater Stream

Cassidy Dauer[1], Brandon Little[1], Javier Del Toro Negron[1]; In Collaboration with Chelsea Blount[2], Kinnon Hodges[3], Thomas Johnson[3], and Shea Tuberty[1]*
[1]Appalachian State University, Dept of Biology
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Ore Knob Mine, a retired copper mine located in Ashe County, North Carolina has been out of use since 1962. When the use of the mine was discontinued in 1962, mine tailing piles were left above and below ground. These mine tailing piles contain high levels of metals such as copper, zinc, iron, arsenic and mercury. As the mine sat unoccupied, these contaminants have been leaching into the surrounding ground and surface waters as well as the sediment and soil. From 2010 to 2012 the EPA conducted sampling of 65 private wells and 15 springs. Due to the effect the contamination was having on the surrounding community, the EPA declared the mine a Superfund Site in 2009. In 2011, the EPA began an emergency response removal action. They removed 16,000 cubic yards of mine tailings from the sediment pond. They also refaced the dam and reconstructed the sediment pond embankment. In the years following 2011 water chemistry and macroinvertebrate sampling has shown improvements have taken place. Water chemistry in the Ore Knob Branch on April 8th, 2021 showed a pH of 3.2 and a specific conductivity of 478. Water chemistry in Peak Creek below the Ore Knob Branch confluence on the same day showed a pH of 6 and a specific conductivity of 32. Based on reported improvements to water chemistry and aquatic macroinvertebrate assemblages, the North Carolina Wildlife Resources Commission fish biologists are again interested in stocking this section of Peak Creek with trout. In order to assess the aquatic health of Peak Creek below the Ore Knob Branch confluence for stocking purposes, a 200-meter NC Fish Index of Biotic Integrity assessment was conducted as well as a Qual 4 macro-invertebrate assessment downstream of the Ore Knob Branch confluence in Peak Creek. Macroinvertebrate sampling produced an IBI Score of 2.85, which is considered excellent. However, fish sampling produced an IBI score of 21.6, which is considered poor. These differing IBI scores suggest that this section of Peak Creek is not yet suitable for stocking purposes.

Prolonged Exposure to Pesticides in Honey Bees: Circadian Rhythms, Motility, and Lifespan

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Honeybees are an economically valuable species because of their pollination services and honey production. These insects are negatively impacted by pesticide application in both agricultural and urban landscapes. The present study compared circadian rhythmicity and motility metrics following exposure to either an organophosphate insecticide (ethion: 80 mg L-1, 106 mg L-1, 160 mg L-1), a neonicotinoid insecticide (imidacloprid: 0.2 mg L-1, 0.4 mg L-1, 0.8 mg L-1), an herbicide, or an herbicide formulation (glyphosate and Ready-to-Use Round Up®, respectively: 0.12 mg L-1, 1.2 mg L-1, 6 mg L-1, 12 mg L-1, 24 mg L-1) as compared to distilled water controls. The lowest and mid-concentrations of imidacloprid and ethion were selected based on previously determined LD30 and LD50 for honeybees; glyphosate/Round Up® were based on published concentrations found in honey and water (0.12-20 mg L-1). The agrochemicals were delivered through the honeybee's water supply in an automated data-collection apparatus. This system records each honeybee's minute-by-minute movement inside a 15mL falcon tube for up to 14 days. The results showed Round Up® and its active ingredient, glyphosate, produced concentration-dependent responses, with the majority of concentrations decreasing motility and adherence to a circadian rhythm; however, the 1.2mg L-1 concentration increased these metrics as compared to controls. Imidacloprid decreased motility, circadian rhythmicity, and survival regardless of concentration, and ethion decreased circadian rhythmicity but not motility across all concentrations. Overall, the four tested pesticides have the potential to interrupt pollination services of honeybees by altering motility and circadian rhythmicity at these concentrations. Pesticide exposed foraging honeybees may not be able to collect enough resources to feed larvae and store for overwintering. These effects may be particularly pronounced in areas with longer winters that often struggle with annual colony loss rates.

Developmental toxicity of the dinitroaniline herbicide pendimethalin in zebrafish embryos/larvae (*Danio rerio*)

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Pendimethalin (3,4-Dimethyl-2,6-dinitro-N-pentan-3-yl-aniline) is a dinitroaniline herbicide currently used to control broadleaf weeds by inhibiting the formation of microtubules and subsequently cell division. Its use on a wide variety of crops leads to its potential entry into aquatic environments, but little is known about its sub-lethal toxicity to early developmental stages of aquatic vertebrates. To address this knowledge gap, we assessed acute toxicity of pendimethalin to zebrafish embryos and larvae by measuring mortality, developmental abnormalities, oxidative respiration, gene expression, and locomotor activity following continuous exposure to the herbicide throughout early development. Zebrafish embryos at ~6 hours post-fertilization (hpf) were exposed to either a solvent control (0.1% DMSO, v/v), embryo rearing medium (ERM), or one dose of either 1, 2.5, 5, or 25 μM pendimethalin for up to 7-days post fertilization depending on the bioassay conducted. Exposure to 25 μM pendimethalin resulted in high prevalence of spinal curvature, tail deformity, and yolk sac edema at 4 dpf, while exposure to 5 μM pendimethalin induced lordosis in the fish exposed for 7 dpf. Mortality was observed for all fish exposed to 25 μM pendimethalin after 4 dpf. We did not detect any changes in oxidative respiration in early staged embryos (30 hours post-fertilization) following a 24-hour exposure. Pendimethalin did not induce reactive oxygen species at 1 nor 2.5 μM exposure at 4 and 7 days. However, transcript levels related to oxidative respiration and oxidative damage response were altered in 7d-larvae; cox1 was increased in larvae exposed to 1 μM while cox5a1 and sod2 were decreased with 2.5 μM exposure. Four independent trials of a dark photokinesis assay were conducted assess pendimethalin-induced effects on locomotor activity. Activity in the dark period was decreased for zebrafish larvae exposed to >1 μM pendimethalin compared to both the ERM and DMSO solvent control groups. These data inform on the sub-lethal toxicity of pendimethalin to early stages of fish embryos and larvae.

Impacts of Temperature and Salinity Changes Associated with Climate Change on Shellfish Disease and Health in SC Oysters

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Historical shellfish disease data from 1995-2004 in SC was obtained from the South Carolina Department of Natural Resources Marine Resources Research Institute relating to water quality, oyster disease, and oyster health along the SC Coast. This project aimed to identify the effects that changes in salinity and temperature may have on Perkinsus marinus infection intensity and prevalence and Crassostrea virginica sex ratios. The objectives of the study were to 1) identify significant associations between Perkinsus marinus prevalence and infection intensity in relation to the climate change variables, salinity and temperature; and 2) identify significant associations between Crassostrea virginica sex ratios and these same climate change variables. Multiple ordinal logistic regression procedures on SAS Studio® and Odds Ratios statistical analysis were performed to examine associations between salinity and temperature on each shellfish disease and oyster health metric. Murrell's Inlet (highly urbanized) and North Inlet (pristine) were selected as preliminary sampling sites for analysis along the South Carolina coast. Results indicated that a significant association between salinity and infection intensity was not detected for either North Inlet (NI) or Murrell's Inlet (MI). A significant association was also not detected between salinity and the odds of being female but a significant effect with temperature was observed for both MI and NI. Temperature and infection intensity were also found to have both a positive and negative significant ($P < 0.05$) association in both MI and NI, respectively. An interesting finding was that the odds of having an infection intensity above 0 on the Mackin's scale (0-6) was the same for both MI and NI.The interaction of temperature and salinity was also analyzed and was found to be significant for all significant interactions noted with temperature, but a noticeable finding was that odds ratios decreased the magnitude of each significant temperature interaction.

Environmental Justice in Sampson County: Can You Trust the Water?
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As the second largest county in the nation for hog production and home to a privately-owned regional landfill, residents of Sampson County, NC are concerned about the potential for animal waste runoff and landfill leachate to enter drinking water sources. Consequently, residents are hesitant to drink tap water and complain about their inability to enjoy the outdoors because of the smell and poor air quality. It's well understood that leachate can be toxic due to contamination by heavy metals and organic compounds. A recent incident at a concentrated animal feeding operation (CAFO) demonstrated the risk of animal waste when a lagoon breach, resulting from inadequate management and hurricane flooding, spilled three million gallons. Officials found high levels of fecal coliform bacteria, nitrogen, phosphorus, and ammonia, and reported that one thousand fish were killed as a result of the incident. To assess drinking water quality, 72 tap, well, and river water samples were collected from residents and analyzed for *E. coli* and total coliform bacterial colonies, nutrients, and toxic elements. Using a 3M Petrifilm EC Plate, samples were pipetted onto plates and incubated for 24 hours before colonies were counted. For nutrient concentrations, samples were transferred to syringeless filters and stored in the freezer for later analysis using ion chromatography (IC). The remaining samples were acid digested and analyzed for heavy metals using inductively coupled plasma-optical emission spectroscopy (ICP-OES). No *E. coli* or coliform bacterial colonies were detected in drinking water samples but a total of nine colonies were counted in 1mL river water samples, which exceeds the safe limits for recreation of 126 CFU/100mL. Instrumental results from the IC and ICP-OES are still being evaluated. This study aims to determine the quality of drinking water in Sampson County and help make informed decisions about how to use the water.

Toxicity of passive sampler extracts from a coal tar contaminated section of the Congaree River, Columbia, SC.
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A section of the Congaree River in Columbia, SC is heavily contaminated with coal tar as a result of effluent produced from a now-closed coal gasification plant. The polluted sediment in this river section is scheduled to be remediated because of human and environmental health concerns. Even though there are currently no measurable concentrations of coal tar associated pollutants in the water, possible release from the sediments during remediation has led to a study using passive samplers to monitor the location before, during and after the remediation. This study targets the polynuclear aromatic hydrocarbons (PAH) associated with coal tar, and their possible breakdown products. Passive sampler units were deployed upstream and downstream of the contaminated sediment, and at the outfall of the drainage ditch that discharged the coal tar effluent into the river. PAHs were extracted from two types of absorbing material in the passive samplers, and were analyzed by GC/MS. The extracts were also used for a fetal embryo toxicity (FET) test with zebrafish embryos. The results showed that the passive samplers were accumulating PAHs, with only slight differences between the upstream ad downstream locations, but significantly higher concentrations were observed in samplers deployed near the drainage ditch. The zebrafish FET test results followed the observed pattern of the PAH concentrations. Principal component analysis of the PAHs in the passive sampler extracts, combined with available sediment concentrations, indicated that the PAH spectrum in the passive sampler extracts do not match well with the PAHs in the coal tar. This suggests that the PAHs in the water column have different sources than the coal tar, possibly being related to PAHs associated with current urban runoff.

Coastal cyanobacterial harmful algal bloom dynamics in Mississippi and Alabama
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During the first half of 2019, the Bonnet Carré Spillway was opened in order to protect New Orleans from Mississippi River flooding and remained open for a record 118+ days. Throughout this period, low salinities persisted across coastal Mississippi for several months and resulted in blooms of Microcystis spp. and other potential HAB species along the Mississippi coastline. While these genera have been identified locally in freshwater, this event was the first time that cyanobacterial HABs and their associated toxins have been reported in northern Gulf of Mexico. The objective of this

work was to examine how environmental conditions such as water temperature, salinity, dissolved oxygen, total suspended solids, and nutrients, affected in-situ toxin production through time. A west – east salinity gradient persisted throughout the study as well as many zones of hypoxia that extended from the surface through the water column. Dense blooms of cyanobacteria were not observed during any sampling event, however, Microcystis aeruginosa was detected via 16S rRNA across the coastal Mississippi transect. Microcystins were detected by enzyme linked immunosorbent assay and liquid chromatography/mass spectrometry, and persisted at concentrations >1 ppb total microcystin even in the absence of visible colonies or mcyB/E. Water movement in these coastal waters was influenced by meteorological conditions and west winds persisted throughout the summer of 2019, likely promoting spatiotemporal movement of toxin. With climate disturbances predicted to increase over the next several decades, prolonged openings of spillways may also increase in frequency. These data have allowed a deeper understanding of cyanobacterial bloom dynamics in freshwater dominated coastal systems and will directly inform on response efforts for future events.

Laboratory testing of a new strain of the insect-pathogenic fungus Metarhizium anisopliae 'S54' as a potential control agent for *Lygus keltoni* (Hemiptera: Miridae), a pest of canola and alfalfa in western Canada

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An entomopathogenic fungus with biopesticide potential, Metarhizium anisopliae sensu lato "S54", also called strain "DJ1" by the USDA, was isolated from soil in southern Alberta, Canada. Laboratory and field tests had indicated high infection rates and mortality of grasshoppers (Orthoptera: Acrididae). In vitro conidial germination rate, mycelial growth and colony size were examined under different heat stress conditions. The susceptibility of *Lygus keltoni* (Hemiptera: Miridae) to this isolate was tested under laboratory conditions. Bioassays were performed to evaluate the lethal concentration (LC50) and average survival time (AST) of the isolate against *L. keltoni*. Dose-response mortality bioassays using several concentrations of *M. anisopliae* indicated LC50 values of 6.3×104 conidia/ml at 7 days after inoculation with L. keltoni. The AST was 4.32 to 6.52 days. The results demonstrate that strain S54 performed well in terms of radial growth, conidial yield, and rate of conidial germination at 35 °C. Furthermore, conidia continued to grow vigorously for 10 days after 8 hours exposure to 48 °C. The role of such a high temperature tolerant strain in crop protection is discussed for its potential to lessen our reliance on insecticides to reduce yield losses. A biopesticide based on *M. anisopliae* has significant potential to manage *L. keltoni* and other plant bugs in alfalfa and canola.

Lightening Talks

Quantifying Microplastic Concentrations in the Sediment and Water of Three South Carolina Watersheds
Rian Burris, John Weinstein, Brooke Blosser, Bonnie Ertel, Marian Smith, The Citadel*

Our laboratory has conducted extensive research on microplastics in Charleston Harbor surface water and sediments, however, less is known about microplastic contamination of other South Carolina waterways. The aim of this study is to quantify microplastic (MP) concentrations in the water (microplastics/liter) and sediment (microplastics/kg) of three South Carolina watersheds. Four locations were chosen from within the Peedee, Santee, and Salkehatchie watersheds and three sites were sampled from within each location during the fall of 2019 and the spring of 2020. Surface water and subtidal sediment were taken simultaneously. Sediment samples underwent NaCl density separations to isolate microplastics <1.2 g/mL, and both water and sediment samples were sieved through 63, 150 and 500µm sieves to separate microplastics by size. Microplastic concentrations were quantified by examining the processed samples under a dissecting light microscope. Preliminary data has shown that our samples captured a wide range of microplastic concentrations from 0-148 MP/L in water and 10-155 MP/kg in sediment. Further analysis will reveal if there is a difference in microplastic concentrations between the two mediums and between each of the three watersheds. These results will help us better understand the ultimate fate of these particles in the estuarine environment.

Combined Effects of Microplastic Ingestion and Elevated Temperature on *Acropora cervicornis*
Plafcan, Martina M. and Stallings, Christopher D., College of Marine Science, University of South Florida, St. Petersburg, FL, USA.*

Microplastics are of growing concern to the marine environment and taxa, with corals being particularly sensitive to environmental stressors. Globally, corals are already threatened by numerous stressors, with a main focus on increasing sea surface temperatures (SST) caused by climate change. Elevated SST can cause coral bleaching and the frequency and severity of these events are worsening as temperatures continue to rise. Microplastics are being recognized as an additional threat to corals with many studies seeing negative effects to coral health, with a common route of exposure being ingestion. Yet, few studies have assessed the combined effects of microplastic ingestion and elevated temperatures on coral. My study looks to fill in this knowledge gap using an important species of Caribbean coral, *Acropora cervicornis*, to see if microplastic ingestion combined with thermal stress will worsen bleaching. I used two temperatures (28°C: ambient and 32°C: elevated) in combination with two microplastic concentrations (0 particles/L and 11.8 particles/L) to assess zooxanthellae densities of the coral fragments along with daily assessments of bleaching and necrosis to measure the bleaching response. The elevated temperature used is within the range predicted for future conditions and the microplastic concentration, 11.8 particles/L, is based off the global average. Polyethylene microplastic beads were used in two sizes: 212-250 µm (1.025 g/cc) and 300-355 µm (1.010 g/cc). No bleaching or necrosis was observed in corals exposed to the ambient temperature, including the microplastic treatment. Bleaching and necrosis was observed in all coral fragments after five days exposed to the elevated temperature. However, the results indicated that microplastics did not have an effect on the bleaching response of *A. cervicornis* in either temperature treatment. While this study found no effect of microplastics on the bleaching response of corals, more research is necessary to evaluate effects of other plastic conditions (type, size, concentration) on the combined effects of microplastics and elevated temperatures.

Hot or Not? Evaluation of the Hot Needle Test for Identifying Microplastic
Adriana Apintiloaiei and Dr. Barbara Beckingham, College of Charleston*

Microplastics are small (<5 mm) synthetic polymers that are ubiquitous in indoor and outdoor environments and may affect human and ecological health. The hot needle test (HNT) helps researchers identify suspected microplastics under the microscope by probing their melt behavior with a heated metal tool, and is a method considered accessible enough for widespread use. To our knowledge the accuracy of the HNT has not been fully evaluated. A literature review found that two articles De Witte, et al. 2014 and Devriese, et al. 2015 were referenced by 25% and 18% respectively of all the articles reviewed, yet neither had any evaluation of the accuracy of the HNT. In order to close this knowledge gap, the HNT response of 9 fibers, an assortment of synthetic, natural, or semi-synthetic materials, were observed and then a single-blind trial was prepared with these fibers randomly arranged on a grid to test the ability of researchers (N=8) to characterize them using their preferred HNT conditions (a red hot needle, a high heat soldering iron, or a low heat soldering iron). Synthetic and some natural fibers were identified correctly over 70% of the time. Cotton and semi-synthetic fiber results were less consistent (<55% correct). Future work will aim to update HNT conditions and to improve its application in microplastic research.

Adapting microplastic methodology for the improved enumeration of tire wear particles in environmental samples
Kayli Paterson and Barbara Beckingham, College of Charleston*

Tire wear particles, emitted due to the friction of tires on road surfaces during vehicle driving and breaking, are increasingly being recognized as a dominant fraction of microplastic in traffic-affected areas yet methods focused on their extraction and enumeration in environmental samples are still in need of development. Tire material has long been a subject of ecotoxicity assessment due to potential for toxic leachates, and analysis of levels in soils and sediments has mostly focused on mass-based measures using analysis of an indicator chemical, such as zinc, styrene butadiene, vinyl cyclohexene, or organic thiol. Researchers interested in harmonizing analysis with microplastic methods and evaluating particle-based physical toxicity require count-based measures. Properties of tire wear particles present unique challenges to this, including particle composition and density which affect matrix separation and spectroscopic identification. This presentation will outline the steps needed to isolate and enumerate tire wear particles in solid samples, including recommendations for reagents to use in processing samples, visual and hot needle test criteria used for differentiating tire road wear particles under microscopy, and a filter subsampling tool for dealing with highly loaded samples such as street dust. For instance, it would be recommended to use NaOH to digest samples instead of other common reagents for microplastics that are incompatible with tire rubber. Also, for highly loaded samples (e.g., >#100s/gram), a stratified random grid subsampling scheme would save analysts time and energy. We found that by understanding the pattern of particle deposition on filters, we can analyze 1/3 of the filter area and be within 15% of a true sample count. Coding the tool in Microsoft Excel Visual Basic allows this scheme to be applied in real-time for filter analysis. The use of these methods would allow broader application of tire wear particle studies alone or in parallel with microplastic analysis.

Microplastics and Tire Wear Particles in Nuisance Floodwater: Role of Adjacent Marshes in Particle Capture
Marian C. Smith, Bonnie M. Ertel, John E. Weinstein, The Citadel

Nuisance flooding of low-lying street surfaces is of concern in coastal cities such as Charleston, SC, which experiences more than 50 days of nuisance flooding annually due to heavy rainfall and encroaching high tides. Frequent street flooding may contribute to the movement of microplastics (MP), including tire wear particles (TWP), away from the street surface towards local waterways. TWP are produced by friction between tires and street surfaces, and previous studies in our laboratory have indicated that these particles make up a large component of microplastic debris found in street floodwater. However, adjacent tidal creeks did not have higher levels of MP or TWP following flooding events suggesting that the nearby marsh sediment may trap these particles before they reach the harbor. The aim of this research is to quantify MP and TWP levels in marsh sediment adjacent to a street surface which regularly experiences nuisance flooding. A preliminary location, Gadsden Creek marsh, was chosen based on previous nuisance floodwater studies. Marsh sediment (250mL) was sampled at three distances from the street surface, processed using NaCl density separations to retain particles $\leq$1.2 g/mL, and sieved at 63 and 500 micrometers. MP and TWP were quantified under a dissection microscope. Preliminary data shows close to 1700 TWP per sample. Based on this data, additional investigative studies will be conducted to document the pathway by which these particles travel from the street surface to adjacent coastal waters.

Determination of Uptake and Trophic Transfer of Microplastics by Caddisflies [Hydropsychidae] to Riparian Spiders [Tetragnathidae] in Southern Appalachian Headwater Streams
Courtney Garneau, Appalachian State University

Microplastic is a pollutant that is rapidly increasing in both terrestrial and aquatic environments, and the extent of its influences on soil and aquatic macroinvertebrates is still largely unknown. The integration of microplastics into freshwater ecosystems is escalated by the presence of industrial and municipal facilities that act as point sources to streams, like the Boone Wastewater Treatment Facility next to the South Fork New River in Watauga County, North Carolina. It is predicted that microplastics are entering the watershed from precipitation and point sources and affecting the biological processes of net spinning caddisfly larvae, a species that are bioindicators of the overall health of a river or stream. Orb-weaving riparian spiders are one of the natural predators to emerging adult caddisflies and have been shown to bioaccumulate toxins from contaminated stream sediments. The incorporation of the pollutant in the caddisfly diet represents the possibility of greater integration of pollutants (and associated toxins that bind to plastics) in the South Fork New River ecosystem. A study will be conducted determining the rate of microplastic uptake within the caddisfly life cycle as well as the existence of microplastic trophic transfer from caddisflies [Hydropsychidae] to riparian spiders [Tetragnathidae]. Once the microplastic is separated from the samples, it will be weighed and analyzed to produce data that will show the types of microplastic that were most abundant, the rate of microplastic uptake in caddisflies, whether the microplastic found within riparian spiders and their webs suggest trophic transfer or atmospheric deposition, and if samples contained more microplastic downstream of the Boone Wastewater Treatment Facility than upstream reference sites. This project will serve as a pilot study regarding the feasibility of tracking microplastics between aquatic and terrestrial ecosystems and will be the basis of an undergraduate honors thesis.

Plant selection for Floating Treatment Wetlands in Brackish Waters
Andrea Landaverde, Sarah White, Bill Strosnider, Clemson University

Brackish water bodies in coastal regions provide critical ecosystem services that support both human and environmental health. Increasingly, anthropogenic activities (e.g., agriculture, urban settlements, and industry) impact water quality in brackish ecosystems. Development activities change land use, often resulting in increased velocity and volume stormwater runoff paired with the increased presence of nutrient, metal, plastic, and organic-based contaminants that affect water quality. Mitigation of contaminants in brackish waters is critical. Floating treatment wetlands (FTWs) are a remediation technology used to improve water quality that have been proven effective and studied mainly in freshwater systems and could be an alternative to mitigate pollution in brackish water. However, FTWs application in systems with fluctuating salinity, such as brackish waters, still requires further investigation. Our goal is to determine the plant species suitable for use in FTWs deployed in brackish water ecosystems with fluctuating salinity. This experiment will evaluate the performance of four plant species (*Distichlis spicata, Juncus roemerianus*, *Spartina alterniflora*, and *Spartina patens*) grown under three different salinity levels (0.5 ppt, 5 ppt, and 18 ppt). The results will inform the selection of plant species to use in future contaminant remediation experiments with FTWs and provide background information for the application of FTWs as a remediation technology in brackish ecosystems.

Disruption of the Gastrointestinal Lipidome After Exposure to Single-Walled Carbon Nanotubes in a Piscivorous Fish
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Background: Carbon nanomaterials are organic compounds increasingly used in commercial, industrial, and medicinal applications. They are released into the environment through anthropogenic processes, where they enter into soils and sediments. Carbon nanotubes may enter the food chain through aquatic animals that inhabit, forage, or choose prey in the benthic zone. Due to their large surface and lipophilic quality, dietary exposure to carbon nanomaterials may alter the availability, metabolism, storage, and transport of lipids in the intestinal lumen or at the epithelial barrier, impacting the health of these aquatic animals.
Methods: A feeding study was performed to examine the associations between the gastrointestinal lipidome and foodborne exposure to SWNT. Adult largemouth bass were fed food mixed with SWNTs at 1.7 mg/kg daily for 7 weeks, with 10 animals per treatment. At the end of the exposure, SPLASH semi-targeted lipidomics was conducted in the intestine using liquid chromatography tandem mass spectrometry.
Results: Statistically significant exposure-associated alterations in gastrointestinal lipidomes were observed in the treatment group across many lipid classes. Specifically, hydoxyceramides, lysophospholipids, and phospholipids were most significantly altered in the guts of the SWNT-exposed fish. The above saw lipid abundance primarily increase in the treatment group, with the exception of the lysophospholipid, LPE, which decreased. These lipids are typically considered to be signaling lipids and these mostly increased levels may point to downstream effects on metabolism. To a lesser extent, a decrease in lipid abundance was observed in triacylglycerides (TAG) and diacylglycerides (DAG) of the treatment group. TAGs and DAGs are utilized for short-term energy storage and their decrease could impact the accumulation of fats required for healthy growth and survival.
Conclusion: The results indicate a treatment-related effect in either lipid uptake or processing in the gastrointestinal epithelium of exposed fish. This could have potentially deleterious effects on largemouth bass, due to the adverse impact on lipid metabolism and the storage of essential fats.

Assessment of Elemental Contamination from Multiple Point Sources on the Broad River, NC
Shea Tuberty, Hannah Woodburn, Cristina Sanders, Loren Rader, Appalachian State University*

The Broad River is a tributary of Congaree River and part of the Santee Watershed, flowing through North and South Carolina and drains into the Atlantic Ocean. Two operations along the river are known sources of heavy metal contamination: Rogers Energy Complex (REC) in Cleveland and Rutherford Co., NC and American Zinc Products (AZP) in Cleveland Co., NC. This study investigated the impacts of elemental contamination on the surrounding biota on the Broad River, NC. There have been no previous studies (e.g. US EPA or NC DEQ) quantifying the levels of elemental contamination in fish, sediment and water along ~30 miles the Broad River in order to determine risk and hazards to humans and wildlife. The byproducts and waste produced from coal combustion and the zinc recycling process are known to contain toxic elements and heavy metals (e.g. lead, cadmium, arsenic, selenium). Toxic elements can leach from unlined coal ash ponds and contaminate groundwater that feeds drinking wells and enter streams and rivers. In 2019, the NC DEQ

mandated the removal of coal ash from unlined ponds and REC has started to transfer ash to lined ponds offsite. This study will provide a baseline for toxic element levels before and during the removal process. Samples were stored and processed according to the SOP for Elemental Analysis of Biological, Water and Sediment Samples. All samples were analyzed and the elemental concentrations determined by ICP-OES. Results indicate that metal contamination is significant enough to issue fish consumption advisories for Se and As. Concentrations of lead in water samples exceeded the NC benchmark for aquatic life at every site downstream from REC. This study provides a multi-year assessment critical for understanding the acute and chronic environmental impacts of anthropogenic pollution.

Exposure to organochlorine pesticides results in systemic alterations of liver lipids in largemouth bass

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Organochlorine pesticides (OCPs) are legacy contaminants that persist in environmental matrices for several years, even decades, following their release into aquatic ecosystems. Despite being phased out almost 50 years ago, OCPs are still ubiquitous in US surface waters, sediment, and wildlife surrounding major agricultural areas, such as Florida. Many OCPs, such as DDT/DDE, can activate estrogenic hormone systems and cause endocrine disruption in exposed wildlife. Though OCPs accumulate in fat tissue, it is unclear how the biosynthesis and metabolism of lipids are impacted by exposure to these compounds. Lipid homeostasis is crucial for maintenance of ecological fitness and impacts on lipids can result in numerous deleterious effects including growth, immune, and reproductive responses. We conducted a dietary exposure using DDE, the principal metabolite of DDT, in largemouth bass (*Micropterus salmoides*). Largemouth bass are highly prevalent in Florida and occupy waters highly contaminated with OCPs, such as Lake Apopka. Three DDE doses were prepared using pelletized fish feed: 0, 1.25, and 125 mg/kg. Treatment animals were group-fed for 7 weeks before collection of liver tissue. Semi-targeted LC/MS-based lipidomics was conducted on extracted and purified liver lipids. There was widespread lipidomic disruption in the liver at both DDE exposure levels, especially among phosphatidylserines and cholesterol Metaboanalyst functional profiles revealed disruption to pathways involved in the metabolism of fatty acids and glycerophospholipids. These results further support the endocrine-disrupting potential of OCPs and highlight important lipid bioindicators during an estrogenic dietary exposure.

Choice of Source Apportionment Model Impacts Emission Rates of Volatile Organic Compounds in the Bakken Shale Oil and Natural Gas Region

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The Bakken Shale oil field is one of the major producers of oil and natural gas (ONG) in the United States. Due to the number of wells in the region, fugitive emissions of volatile organic compounds (VOCs) from these operations have the potential to affect downwind air quality. Previous work has suggested regionally and globally important emissions of VOCs from the Bakken region; however, this work is potentially sensitive to source apportionment methodology. To better understand the impact of different source apportionment models on emission estimates a two tracer model and a Positive Matrix Factorization (PMF) model were used to calculate source apportionment and emissions for the Bakken oil fields. The two tracer model used propane to calculate apportionment of alkanes to ONG emissions and ethyne to calculate combustion emissions apportionment. PMF models use a multivariate factor analysis algorithm to calculate source apportionment based on given chemical concentrations and uncertainties. The PMF model identified four source factors: ONG, combustion, photochemically aged VOCs, and background. Apportionment of alkanes to ONG sources was dominant in both models, but consistently lower in the PMF model. For example, the two tracer model gave an ONG apportionment for propane of 91% while the PMF model gave a value of 74%. Ethyne apportionment to combustion sources was similar with 60% and 63% in the two-tracer model and the PMF model, respectively. Apportionment of the hazardous air pollutants BTEX to ONG sources was 33% in both the two tracer and in the PMF model. Depending on the source apportionment model used, estimated yearly ONG C2-C6 alkane emissions ranged from 433 Gg/yr in the two tracer model to 425 Gg/yr in the PMF model. Differences in ONG alkane emissions estimates have implications for modeling downwind ozone production.

Nutrient Dynamics in a Floating Treatment Wetland System after Overwintering in Eutrophic Conditions

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Agricultural operations are considered major non-point source contributors of excess nitrogen and phosphorus into surface and ground waters. Effective removal of excess nutrients from irrigation return water is necessary to prevent diminished water quality and eutrophic conditions that result from nutrient contamination of surface waters. Floating treatment wetlands (FTWs) are modified constructed wetlands that use floating mats to maintain wetland plants at the water surface and can be installed within existing stormwater infrastructure without site modification. FTWs have been implemented in various geographic regions and environmental conditions; previous research demonstrated their effective remediation of nutrients from affected water bodies. FTW plants demonstrate peak growth and nutrient uptake periods in the summer months. When fall transitions to winter, the nutrient removal capacity of FTWs declines and may even exhibit nutrient release when plant tissues senesce. Active management of FTWs via plants harvest is debated in the literature, thus there is need to characterize nutrient release by plants in FTWs over winter to better inform year-round nutrient management of FTW systems. The goal of this study was to quantify the mass of nutrients released from senescing plant tissues and to characterize the nutrient removal efficiency of FTW plants following overwintering. Batch-fed mesocosms were planted with Juncus effusus and Pontederia cordata, and nutrient dynamics were monitored from winter of 2019 through spring 2020. A better understanding of winter nutrient dynamics in FTWs will help growers and stormwater managers better understand FTW management and support effective implementation and management of FTW-based nutrient mitigation efforts in water bodies sensitive to nutrient contamination.

Atmospheric Dry Deposition of Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) in North Car olina

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Per- and polyfluoroalkayl substances (PFAS) are ubiquitous anthropogenic pollutants but little is known about atmospheric dry deposition of these compounds. Fluxes of six PFAS were quantified at six locations across North Carolina from December 2018 to January 2021. Two legacy PFAS and four emerging (e.g. HFPO-DA) PFAS were quantified in each location. ΣPFAS fluxes for Wilmington ranged from 0.04-19 ng/m2/day for years 2019 and 2020, with PFOS and PFMOAA being the dominant compounds. In comparison, samples collected across North Carolina in 2018 and 2019 had ΣPFAS fluxes from below the method quantification limit to 50 ng/m2/day, with PFOS being the dominant compound. Annual dry deposition of PFAS in Wilmington for years 2019 (n = 23) and 2020 (n = 15) was 740 and 340 ng/m2 respectively. While PFOS and PFOA production in the United States was voluntarily phased out by 2002 and 2015 respectively, there are still sources of PFOS and PFOA to the atmosphere in the United States. This study is the first to show that dry deposition is a removal mechanism for emerging PFAS from the atmosphere and highlights the need for further investigation into their atmospheric lifetime and degradation pathway.

Enterococci Contamination on Edisto Island, SC: Frequency, Sources of Contamination and Prospects on How t o Improve Water Quality

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Beach monitoring samples were collected from 18 locations on Edisto Island, South Carolina from 2000 to 2016 and analyzed to assess patterns of water quality violations indicated by the presence of Enterococcus, a bacterium used to assess the health of surface waters for contact recreation. Statistical analyses were conducted comparing Enterococci levels and different environmental variables including location, tidal stages, wind direction and time. Specific focus was placed on temporal and spatial patterns for dates when the bacteria levels exceeded 104 MPN per 100 milliliters, the Enterococcus single sample maximum (SSM) water quality standard. Results indicated that 2.2% of the samples exceeded the Enterococcus SSM standard and that the majority of these SSM exceedances occurred in September, during periods without significant rainfall at or near the time of flooding associated with King Tides (flood tides higher than 7.5 feet). Statistical analysis indicated that wind direction and tidal stage (at or around high tide – ¾ flood to ¼ ebb) appeared to have more of an impact on bacterial levels than rainfall, per se. Microbial source tracking using quantitative polymerase chain reaction (qPCR) analysis was conducted and used to identify potential bacterial pollution sources. Results indicated that birds and dogs, rather than humans, were the major sources of bacterial pollution. These findings suggested that flooding during King Tides inundated a larger area of the beach face surface containing bird and dog feces. Changes in population growth on Edisto Island was also analyzed and indicated that permanent population has been increasing at a

relatively low rate, while high rates of tourism growth have been observed and may play a factor in observed increases in Enterococcus SSM exceedances. These low population densities at Edisto Island and microbial source tracking results further indicate that most pollution sources were from birds and dogs and the important role of coastal flooding associated with climate change. Better management of microbial pollution sources from pets and birds is essential to prevent further degradation and loss of ecosystem services.

Investigating the developmental toxicity of the anti-neoplastic cyclophosphamide in embryo/larval zebrafish (*Danio rerio*)

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Cancer is a leading cause of death worldwide. Antineoplastic drugs are medications used to treat cancers, and these like many other pharmaceuticals, can enter aquatic ecosystems through wastewater treatment facilities. Alkylating agents are anti-neoplastic pharmaceuticals that inhibit DNA transcription, acting to completely block the replication of DNA in the tumor cells. Cyclophosphamide is one example of an alkylating agent that is commonly used to treat leukemia, and one that has also been detected in relatively high abundance and frequency in surface water and in hospital effluent. However, the relative risk of exposure to aquatic organisms have not been adequately characterized. In this study, we exposed zebrafish embryos and larvae to cyclophosphamide to quantify both sublethal and lethal responses. Zebrafish exposed to 0.1-10 µM cyclophosphamide did not exhibit significant deformity nor mortality. To learn more about mechanisms of toxicity, we measured transcripts related to the immune system as cyclophosphamide can lead to immunosuppression. These data will be further discussed. We also measured the effect of cyclophosphamide exposure using a visual motor response test and determined cyclophosphamide did not affect total distance moved of the larvae. Our data suggests that environmentally relevant levels of the antineoplastic may not be toxic to early stages of fish. This is important as there are very little toxicity data available in aquatic organisms that investigate anticancer agents. With an aging population, it is anticipated that such drugs may be used more frequently and similar to what has been done with other pharmaceuticals such as antidepressants and blood pressure regulators, risk assessment studies are needed to evaluate potential impacts in aquatic ecosystems.

Biodegradation of emerging per- and polyfluoroalkyl substances in sediments: Forever no more?

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The biodegradation of long-lived ("forever") emerging environmental contaminants such as per- and polyfluoroalkyl substances (PFAS) is a potential mechanism to reduce environmental lifetimes and human toxicity impacts, in addition to more broadly understanding the behavior of these chemicals in long-term environmental sinks. Recent work has demonstrated that specific bacteria can degrade legacy PFAS compounds such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) causing transformations of forever chemical and subsequent shifts in sediment bacterial community composition. Yet these legacy PFAS are no longer in production and share no chemical traits to the replacement PFAS currently on the market, resulting in a paucity of knowledge on the biodegradability of compounds such as hexafluoropropylene oxide dimer acid (HFPO-DA; ammonium salt commonly known as GenX) that dominate PFAS production. This work will address the interplay between biological and chemical transformations in HFPO-DA-impaired sediments as a means of characterizing the biodegradability of HFPO-DA and other replacement PFAS compounds.

Preliminary Scoping Experiment in Enantioselectivity of Polychlorinated Biphenyls on Silica Surfaces

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The purpose of this preliminary scoping study is to determine the effect of silica surfaces on the enantioselectivity of polychlorinated biphenyls (PCBs). PCBs are persistent organic pollutants known to cause reproductive, neurological, and developmental disorders in animals and humans. A remediation process has not been extensively developed, and PCBs must be further understood to bring one into action. PCBs function as hydrogen acceptors given the pi bonds formed in the biphenyl rings, so it is imperative to see how these interactions could affect PCB adsorption onto an activated quartz surface. The common trace mineral, quartz, is being used as the silica surface because of its chirality. The quartz surface

was activated primarily using pH to promote hydrogen bonding between the PCB pi electrons and the oxygen molecules in the mineral. Additionally, previous studies have shown chiral amino acids adhering to activated quartz, leading to the use of quartz for PCBs in this study. This study uses chiral gas chromatography (GC) methods to analyze enantiomeric excess and achiral GC to compare the initial and final PCB concentrations. Thus far, the chiral PCB congeners 95 and 91 were tested separately as racemic mixtures. After experimentation and analysis, the chiral congeners remained racemic, meaning they have an equal concentration of the two enantiomers. Enantiomers are one of two stereoisomers that are non-superimposable mirror images of each other. Using the calibration curve created on the achiral GC, the concentration of both PCB congeners had significantly dropped, which could indicate a considerable amount of the PCBs adsorbed onto the quartz surface. Other factors, such as the unknown purity of the quartz and the methodology conducted, may have contributed to the loss of PCBs in the final extraction. Further experimentation with internal and recovery standards will assist in the understanding of the variable PCB concentrations.

How Azo Dyes and Microfibers may Negatively Impact Lung Cells and Contribute to Susceptibility to Influenza A Virus

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Synthetic azo dyes are widely used in industrial and consumer products, but some have been shown to be known allergens and carcinogens, negatively affecting human health. Inhalation exposure to azo dyes is a critical exposure route to examine since these dyes are present in house dust. Research has mainly focused on the effects of azo reduction in the human intestine, liver, and skin, while the effects on the pulmonary system remain less explored. To address this, we exposed small airway epithelial cells (SAECs) to two azo dyes, Disperse Orange 61 and Disperse Violet 93. A 48-hour exposure to Disperse Violet 93 resulted in a statistically significant decrease in cell viability in a dose-dependent manner, whereas exposure to Disperse Orange 61 did not. These results show there are varying levels of reactivity among different dye types. This could mean inhalation exposure to the more reactive dye, Disperse Violet 93, may increase host susceptibility to Influenza A virus (IAV) infection. To test this idea, we would expose SAECs to both Disperse Orange 61 and Disperse Violet 93, collect the supernatant, and perform viral titers using the TCID50 method. Additionally, humans are at risk of dye inhalation when exposed to cotton, wool, and other synthetic fibers that are complexed with azo dyes. When these fibers break down, they can shed microfibers or nanofibers, which are potentially small enough to be inhaled deep into the lung. Azo dyes bound to these microfibers can make their way into the body and desorb from the fibers, leading to potentially negative health outcomes. We hypothesize that the combined effect of azo dyes complexed to microfibers will lead to decreased lung cell viability and increased susceptibility to IAV infection. Initial steps to test this hypothesis will involve determining the viability of SAECs exposed to microfibers alone, and the viability of SAECs exposed to azo dyes bound to microfibers. Sorption assays would be performed to determine the adsorption capacity of azo dyes to microfibers in the lung environment. These efforts work to better understand an understudied area of research, which is the impact of inhalation exposure to azo dyes and microfibers.

Elucidating Molecular Mechanisms of Harmful Algal Blooms on Respiratory Effects

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The overgrowth of certain types of harmful algae in the ocean (harmful algal blooms, HAB) occurs worldwide in coastal regions, including Florida. Commonly, these HABs are known as "red tide" because they often cause the discoloration of ocean water to a rusty red and are notorious for degrading marine ecosystems. Neurotoxins produced by the algae are known to kill aquatic species (i.e. fish, shellfish) in significant numbers. Additionally, humans also report respiratory symptoms such as coughing, sneezing, and the interruption of breathing during active HABs, particularly in individuals with chronic respiratory conditions. Yet the mechanism by which HABs cause these respiratory effects is unknown. This research project will employ human lung epithelial and immune cells to determine how aerosolized components of HABs (algae and toxins) alter immune responses. The hypothesis is that components of algae induce the secretion of exosomes from lung cells that traffic signaling lipids to immune cells, causing upregulation of immune responses. The experimental design includes exposing cells to aerosol extract of toxins, algae and a combination of the two at various doses. Exosomes are then isolated and lipid analysis performed by mass spectrometry. Immune analysis will be performed using PCR and ELISA techniques. To date, results indicate that aerosol extracts of algae upregulate exosome production and alter lipid profiles. Next experiments will determine whether these lipid molecules alter immune markers in other cell types. Overall, these data are the first to shed light on mechanisms involved in respiratory effects associated with HAB exposures.

Exploring in vitro Phase I biotransformation of brevetoxin (PbTX-2) in northern Gulf of Mexico fish microsomes

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Brevetoxins (PbTX) are potent marine neurotoxins produced by *Karenia* spp., associated with recurrent blooms of "red tide" along the Gulf of Mexico coast. Most toxicological research on PbTX has focused on shellfish and marine mammals, even though fish also serve as vectors for PbTX. Numerous studies have shown that PbTXs are rapidly accumulated and metabolized in shellfish and mammals, including rodents and humans, but there is only limited research on the metabolism of PbTXs in fish. In this study, we aimed to fill the knowledge gap surrounding PbTX metabolism in fish by exploring *in vitro* biotransformation of PbTX-2, a major constituent of *Karenia* spp. toxin profiles. We used hepatic microsomes prepared from several species of northern Gulf of Mexico fish, which represented several taxonomic and trophic groups. The metabolism assays focused on Phase I reactions mediated by cytochrome P450 monooxygenase (CYP) enzymes, which were confirmed active in the prepared microsomes prior to their use in PbTX-2 biotransformation experiments. Samples were analyzed by ultrahigh-performance liquid chromatography high-resolution tandem mass spectrometry to monitor PbTX-2 depletion and to aid in the identification of PbTX metabolites. Our results show that fish microsomes rapidly deplete PbTX-2, resulting in 70–98% reduction in PbTX-2 peak areas. We observed production of several oxidation, reduction, and hydrolysis metabolites forming throughout these experiments, including the previously identified congeners PbTX-3, PbTX-9, and BTX-B5. However, comparison of metabolite formation across fish suggests that interspecific variation in PbTX-2 metabolic pathways may drive divergent PbTX profiles in fish. These results confirm that fish are capable of similar PbTX biotransformations as reported for shellfish and mammals, and provides evidence to support variation in PbTX-2 metabolism across fish species. Collectively, these data have important implications for the ecotoxicological fate of PbTXs in marine food webs.

Investigating Impacts of Irbesartan on PPARγ in Aquatic Species via Non-Target Analysis and In Vitro Bioassay Testing

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Lack of knowledge about different chemicals in diverse water systems gives researchers limited knowledge of which contaminants are of public health concern. To address this, we are employing 'non-targeted' high-resolution mass spectrometry (MS) to generate chemical fingerprints in collected water samples to determine whether toxicity data are (or not) readily available for these chemicals in open-source federal databases (i.e. ToxCast). The goal is to identify understudied chemicals in environmental samples. To accomplish this, water samples were collected from surface waters and wells in Haiti (n=13), North Carolina (n=13), and Sri Lanka (n=11), and chemical fingerprints were generated by MS. In total, 169 chemicals were identified in Haiti (111 chemicals unique to Haiti), 144 chemicals in Sri Lanka (96 chemicals unique to Sri Lanka), and 323 chemicals in North Carolina (256 chemicals unique to North Carolina) with 11 chemicals shared by all regions. The majority of the contaminants identified had no representation in ToxCast with percent representation of identified chemicals values per sites ranging from 35-45% across all three sampling sites. One chemical class of interest that was shared by all sites with limited tox data Angiotensin Type 1 Receptor Antagonists (ARB) that are used to control blood pressure. These compounds can activate PPARγ, but limited information is available for aquatic species. To begin to understand the toxicity of ARBs, we performed bioactivity assays for irbesartan and rosiglitazone (positive agonist) by using transactivation reporter assays for human and zebrafish PPARγ. For irbesartan, results showed AC50 values of 11 µM and 13 µM for human and zebrafish, respectively. For rosiglitazone, results showed AC50 values of 0.2 µM and 2.3 µM for human and zebrafish, respectively. The next steps would be to test the chemicals in an in vivo zebrafish embryo model measuring endpoints such as adipogenesis, energy metabolism, heart rate, and morphology. These efforts should lead to a better understanding of exposure and health effects of ARBs to fish in aquatic environments.

Evaluation of air mass back trajectory utility for determining ONG emission influence

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The Bakken Shale Formation is a substantial contributor to US oil and natural gas (ONG) production and subsequent volatile organic compound (VOC) emissions. These VOCs impact air quality and greenhouse gas (GHG) emissions on a local, regional, and global scale. Previous efforts to use air mass back trajectories to verify the influence of ONG sources on discrete air samples have yielded inconsistent results. Back trajectories for the time period covering the Bakken Air Quality Study were compared with source apportionment model results and air mass photochemical age estimates to evaluate the reliability of back trajectory analysis. NOAA's Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model was used to generate and cluster 48-hour back trajectories. The air mass age for each air sample was estimated using the alkyl nitrate photochemical clock. Source apportionment was performed using a positive matrix factorization (PMF) model. Five back trajectory clusters were identified but did not have statistically different photochemical ages. The five identified PMF groups had more of a statistical difference in air mass age than the trajectory clusters; in particular, samples dominated by the ONG source were significantly younger than samples dominated by combustion sources or background air. Approximately 56% of samples that had short distance, localized back trajectories were also dominated by the ONG PMF factor. Back trajectories have not proven to be a reliable indicator of ONG influence as an individual model, but it can provide useful information when combined with other models such as PMF source apportionment and photochemical age estimates. Future studies will focus on variations of the HYSPLIT model including different meteorological datasets and resolutions and different back trajectory timing options as well as evaluating other photochemical air mass age models.

Automated Morphology Assessments for Improved Larval Zebrafish Screening

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The U.S. Environmental Protection Agency utilizes larval zebrafish (Danio rerio) morphological changes as a tool to screen chemicals for potential developmental disruptions. Previous larval assessments of zebrafish in developmental toxicity assays have been confined to the visual examination under a microscope by a trained human observer. The human observer will record and classify any larval abnormalities, but this visual examination is rarely quantified. While these approaches increase our understanding of morphological abnormalities, oftentimes there can be discrepancies among laboratories because definitions of abnormalities are not standardized and may vary by laboratory. Our laboratory is transitioning to more quantifiable, objective methods of larval zebrafish assessment by integrating computer automation and visual assessments. These newer methods produce both quantitative and qualitative data, as well as photos of each larva that may be referenced later. We use the Vertebrate Automated Screening Technology (VAST) system, a robotic imaging system that captures each larva in lateral, ventral, and dorsal positions within a relatively short amount of time (https://www.unionbio.com/vast). Using FishInspector, a program developed by the Helmholtz Center for Environmental Research (UFZ, Leipzig, Germany), we are able to quantitatively measure the major organs of the larva using these images. Through machine-learning, Fishinspector detects and measures each feature of the larva, including, but not limited to, body length, spinal curvature, swim bladder size, jaw, otolith presence, and head-trunk angles, requiring minimal human correction. Using KNIME data analytics software, we are able to summarize the morphological data through an automated workflow (Teixidó et al. 2019). All of this considered, we are able to not only optimize the efficiency of our assessments but also increase the standardization and capacity of our analysis. (This abstract may not necessarily reflect official Agency policy.)

Ontogenetic differences of major ion toxicity in the mayfly, *Neocloeon triangulifer*

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Freshwater salinization has become an emerging ecological concern due to the alarming decline of sensitive aquatic organisms in affected areas. Our physiological understanding of how changing major ion concentrations impact aquatic insects, such as mayflies, is limited. Importantly, many organisms exhibit greater sensitivity to salinity and other toxicants at early-stage development compared to more mature stages. Thus, we hypothesize that the lab-reared, Baetid mayfly, *Neocloeon triangulifer*, may have ontogenetic differences of major ion toxicity across their ~24 day larval life span. Because previous work in our lab has demonstrated the importance of studying ion-specific effects, rather than total salinity, we are performing a series of 96-hour toxicity bioassays across multiple larval life stages for three different major ions: sodium, calcium, and sulfate. We observed that mature larvae were more tolerant of sodium (17-21 days old, LC_{50}

= 546 mg L^{-1} Na) than earlier life stages (2-6 days old, LC_{50} = 385 mg L^{-1} Na), but these differences were not statistically significant (p = 0.07). Next, we observed that similar concentrations of calcium were less toxic than sodium and thus, we were unable to calculate LC_{50} values. However, at the highest concentration (980 mg L^{-1} Ca) there were not statistically significant differences between survival rates of the four larval life stages (ranging 20-46%, p = 0.18). Studies with sulfate are ongoing. To date, our results indicate that ontogenetic differences for major ion toxicity are not profound for *N. triangulifer*, but may be more important in other taxa (e.g., fish or crustaceans). We also are relieved to find that using larger, late-stage *N. triangulifer* larvae is not significantly underestimating the physiological effects and toxicity of salinity.

The Impact of Select PFAS-free Aqueous Film-Forming Foams (AFFF) on the Eastern Oyster, *Crassostrea virginica*

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Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals that are environmentally persistent, are known to bioaccumulate, and have been linked to changes in physiology and metabolism. These chemicals have historically been used in a number of industrial processes, but one source of PFAS chemicals in the environment is from the use of traditional firefighting foam formulations. Regulations, such as the Clean Water Act, have acted to curb the presence of particularly alarming fluorinated compounds in drinking water. Since these PFAS substances are not yet regulated in the US by any specific environmental mandates or laws, the development of PFAS-free foams would help proactively reduce additional PFAS entering the environment. The Strategic Environmental Research and Development Program (SERDP) has been tasked with helping to assess PFAS-free aqueous film-forming foams (AFFF) to help identify potential AFFF replacements, but there is a lack of basic toxicological information available for these PFAS-Free formulations. The objectives of this work focus on determining the potential toxicological impact of these novel AFFFs on the Eastern Oyster, Crassostrea virginica. To support these objectives, an initial toxicity ranking of these formulations relative to a currently used PFAS-containing AFFF will be determined using recognized standard toxicological protocols that quantify effects on survival and shell growth. It is expected that the outcome of these studies will help to inform decisions surrounding the replacement of currently used fluorinated AFFFs.

Prospective Research in Enantioselectivity of Reductive Dehalogenase Exposed to Metals

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The microbial reductive dehalogenation of polychlorinated biphenyls (PCBs) can be an important step to remediate regions contaminated with the persistent organic pollutant. Several researchers in recent years have identified reductive dehalogenases (RDase), one enzymatic pathway responsible for dechlorination of PCBs. However, the mechanisms underlying the enantioselective transformation of chiral PCBs are unexplored. Enantiomers are one of two stereoisomers that are non-superimposable mirror images of each other. Field samples from around the world indicate non-racemic enantiomeric fractions of PCBs, demonstrating that one enantiomer is in higher concentration than the other. Commercial PCBs were released into the environment at racemic levels, so the observations propose the question as to how chiral PCBs degraded enantioselectively. There are several components, such as cofactors, that can promote enzymatic functions that could affect enantioselectivity. Recently, hazardous metals have been shown to inhibit RDase function, implying that metals play a role in the enzymes' pathway. Metals have also been shown to serve as electron donors or acceptors and can form ligands that interact with enzymes. We propose to explore how naturally occurring, essential metals such as zinc, magnesium, iron, and cobalt affect the function of RDase and enantioselective dehalogenation. This research will be conducted using lab microcosms from sediment cores from contaminated areas of Lake Hartwell, SC, USA. This Superfund site is ideal for this prospective research because the only contamination is from PCBs, and previous research by our group has confirmed enantioselective degradation is occurring in the sediments. From when the plant was opened in 1955 to its closure in 1977, it was estimated that over 220 tons of PCBs were released into Town Creek that feeds into Lake Hartwell. We will present a brief literature review, an experimental design for our study to discover the relationships among essential metals and enantioselective dehalogenation.