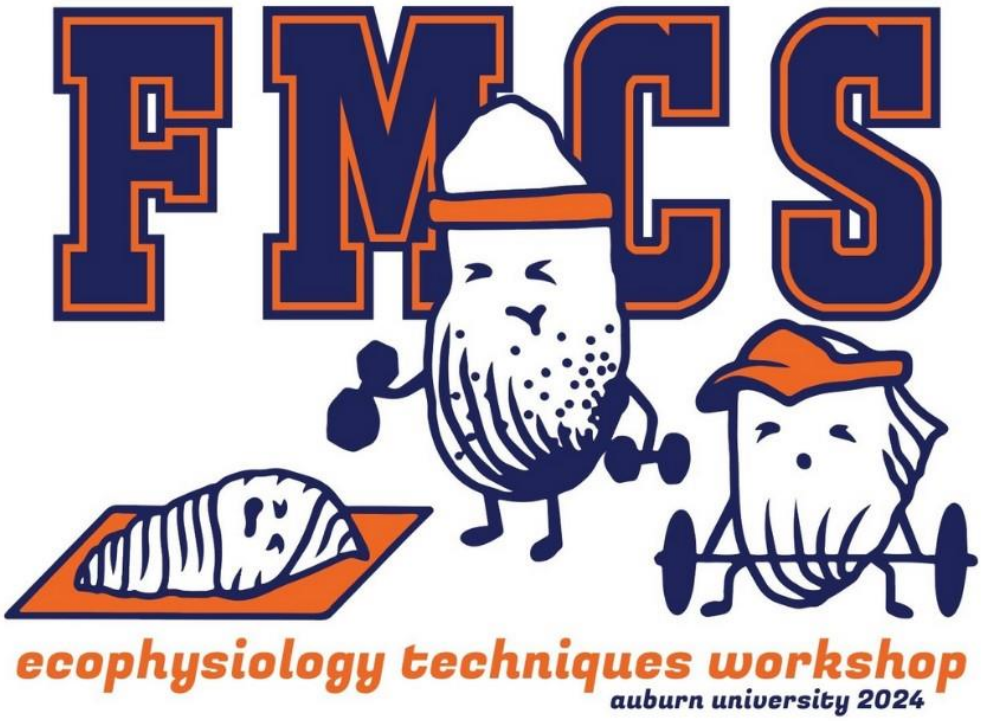
13th Biennial Workshop



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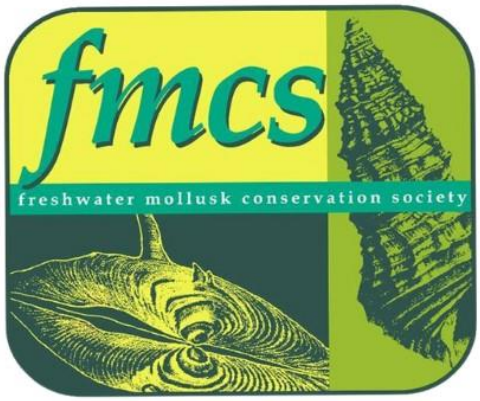

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Schedule Overview

Monday March 4th		
5:00 PM – 9:00 PM	Registration Social/ Mixer	540 at the Park

Tuesday March 5th		
8:00 AM – 9:00 AM	Registration & Coffee	540 at the Park
9:00 AM – 10:00 AM	Welcome & Keynote Speaker	
10:00 AM – 12:00 PM	Sessions: "Respirometry Techniques" & "Sublethal Indicators of Stress"	
12:00 PM – 1:00 PM	Lunch (Provided)	
1:00 PM – 2:25 PM	Session: "Indicators of Stress: Biomarkers"	
2:25 – 2:35 PM	Break	
2:35 – 3:45 PM	Session: "Energetic Models"	
3:45-3:55 PM	Break	
3:55 – 5:00 PM	Session: "Indicators of Stress: Growth and Survival"	
5:00 PM – 7:00 PM	Dinner (On your own)	
7:00 PM – 9:00 PM	Poster session* & mixer	The Corner Office

*3:30 – 5:30 PM – poster set-up at The Corner Office

Wednesday March 6th		
8:00 AM – 9:00 AM	Registration & Coffee	540 at the Park
9:00 AM – 10:15 AM	Session: "Lethal Stress Assays"	
10:15 AM – 10:25 AM	Break	
10:25 AM – 11:30 PM	Session: "Scaling up: Community Effect Modeling"	
11:30 AM – 12:30 PM	Lunch (Provided)	
12:30 PM – 2:30 PM	Session: "Management Needs: Species Recovery Planning"	
2:30 PM – 2:40 PM	Break	
2:40 PM – 3:40 PM	Session: "Management Needs: Habitat Suitability modeling"	
3:40 PM – 3:50 PM	Break	
3:50 PM – 5:00 PM	Session: "Management Needs: Contaminants and Die-offs"	
6:00 PM – 9:00 PM	Dinner (Fish Fry Mixer)	North Auburn Pavilion

Thursday March 7th		
9:00 AM – 12:00 PM	Hands on, Concurrent Workshops	E.W. Shell Fisheries Center
12:00 PM – 1:30 PM	Lunch (Provided)	
1:30 PM – 4:30 PM	Hands on, Concurrent Workshops	

Table of Contents

Keynote Speaker

Paul Johnson has been the Program Supervisor of the Alabama Aquatic Biodiversity Center for 19 years. Prior to that appointment was the Chief Research Scientist and Director of the Tennessee Aquarium Research Institute. He has been involved with mussel and gastropod research and conservation efforts across the Southeastern United States for nearly 30 years.

USING ENVIRONMENTAL DATA TO PROMOTE FRESHWATER MOLLUSK CONSERVATION AND HABITAT RECOVERY EFFORTS

Paul D. Johnson

Alabama Department of Conservation and Natural Resources, Alabama Aquatic Biodiversity Center, Marion, AL. paul.johnson@dcnr.alabama.gov

Under the Clean Water and Endangered Species acts, federal and state natural resource agencies are obligated to review environmental threats to at-risk species. Typically, these challenges have been toxicological in nature, with specific point source concentrations for many compounds determined through established testing protocols (aquatic life ambient water quality criteria – WQC). While WQC have been established for several compounds hazardous to aquatic life aquatic life (e.g., metals, salts), WQC are not always protective for freshwater mollusks. Specifically, toxicological and physical water quality limits for freshwater gastropods are sorely lacking. Additionally, some physical water quality parameters (e.g., discharge, temperature, dissolved oxygen) have recently been utilized to initiate operational changes to several large dams. Operational changes have been made to dams in the Coosa, Duck, Elk, and Tennessee rivers, where listed mollusks are present in dam tailwaters. Some of these adjustments have initiated spectacular improvements in populations of target species, but other projects have not. Laboratory research to determine toxicological or physical limits of at-risk mollusks can have profound positive impacts on riverine habitats and populations of at-risk species. The data these studies generate, especially when focused on regional fauna and hydrologic parameters, can optimize future habitat restoration projects, and increase their likelihood of success.

Workshop Location(s)

540 at the Park & the Corner Office

- 570 Devall Drive, Auburn, AL 36832
- Registration, talks, provided meals, mixers, and the poster sessions on Monday 3/4-Wednesday 3/6 will take place at 540 at The Park (570 Devall Drive Auburn, AL 36832). Free parking is located in the parking lot adjacent to the venue.

North Auburn Pavilion

- 950 Auburn Lakes Road Auburn, Alabama 36830 (32.68266N 85.50668W)
- For dinner on Wednesday 3/6 there will be a Fish Fry mixer at North Auburn Pavilion.
- Turn off of Auburn lakes road into the pavilion area (road denoted in dark blue). Parking is in grassy area around the pavilion (denoted in light blue). Carpool if possible.

E.W. Shell Fisheries

- 2101 N. College Street Auburn, AL 36830
- The hands-on techniques workshop on Thursday 3/7 will take place at the E.W. Shell Fisheries Center.
- Free parking is provided in the facilities parking lot and the grassy area around the parking lot.

Detailed Topic Schedule and Abstracts

Tuesday March 5th		
540 at the Park		
Time	**Speaker**	**Topic/Title**
9:00 AM	Jim Stoeckel & Kaelyn Fogelman	Welcome: 2024 Workshop on Ecophysiology & Environmental Tolerance Techniques
9:10 AM	Paul Johnson	Keynote: Using Environmental Data to Promote Freshwater Mollusk Conservation and Habitat Recovery Efforts
Respirometry Techniques Moderator: Jim Stoeckel		
10:00 AM	Jim Stoeckel	Respirometry Techniques and Relevant Endpoints
10:25 AM	Jonathan Lopez	Self-contained chambers for field and ecosystem respirometry with freshwater mollusks
10:45 AM	Discussion: Respirometry Techniques	
Sublethal Behavioral Indicators of Stress Moderator: Jim Stoeckel		
11:00 AM	Peter Hazelton	Oh, behave! Freshwater mollusk behavioral responses to environmental stimuli
11:25 AM	Jessica Kozarek	Valveometry
11:45 AM	Discussion: Sublethal Behavioral Indicators of Stress	
12:00 PM	Lunch (Provided)	
Indicators of Stress: Biomarkers Moderator: Peter Hazelton		
1:00 PM	Teresa Newton	Biomarkers of energy in native freshwater mussels
1:25 PM	Diane Waller	Freshwater mussel metabolomic studies: knowledge gained and knowledge gaps
1:50 PM	Matthew Jenny	Incorporation of Transcriptomics into a Multi-Biomarker Approach with Applications Toward Ecophysiology and Conservation
2:10 PM	Discussion: Indicators of Stress: Biomarkers	
2:25 PM	Break	
Energetic Models Moderator: Peter Hazelton		
2:35 PM	Andrea Darracq	Use of the cellular energy allocation model for evaluating mollusk health
3:05 PM	Jessica Radich	General techniques and applications of the scope for growth model
3:30 PM	Discussion: Energetic Models	
3:45 PM	Break	
Indicators of Stress: Growth and Survival Moderator: Peter Hazelton		
3:55 PM	Wendell Haag	Assessing stream health and mussel fitness with in situ exposures and shell thin sections

Tuesday March 5th CONTINUED	
540 at the Park	
4:50 PM	Discussion: Indicators of Stress: Growth and Survival
5:00 PM	Dinner (on your own)
7:00 PM	Poster Session & Mixer

*3:30 – 5:30 PM – poster set-up at The Corner Office

Wednesday March 6th		
540 at the Park		
Time	**Speaker**	**Topic/Title**
Lethal Stress Assays Moderator: Kaelyn Fogelman		
9:00 AM	Charles Randklev	Using thermal tolerance information to assess risks associated with natural and human-mediated changes in water temperature
9:35 AM	Kaelyn Fogelman	Interpretation and use of physiological thermal performance curves
10:00 AM	Discussion: Lethal Stress Assays	
10:15 AM	Break	
Scaling Up: Community Effect Modeling Moderator: Kaelyn Fogelman		
10:25 AM	Irene Sánchez González	Methods for In-Situ Collection of Freshwater Mussel Excretion and Egestion Samples
10:50 AM	Carla Atkinson	Scaling the impact of mollusks on ecosystem function across environmental gradients
11:15 AM	Discussion: Scaling-up: Community Effect Modeling	
11:30 AM	Lunch (Provided)	
Management Needs: Species Recovery Planning Moderator: Valerie Kearny		
12:30 PM	Nicole Rankin *(Virtual)*	Conserving Species: Listing and Review in the Southeast
12:55 PM	Maureen Walsh *(Virtual)*	SSA: General Framework + 3 Rs
1:25 PM	Carrie Straight	Recovery planning for species listed under the endangered species act
1:55 PM	Megan Bradley	Strides in propagation and culture of native freshwater mussels to restore declining populations & further best science practices
2:15 PM	Discussion: Management Needs – Species Recovery Planning	
2:30 PM	Break	

Wednesday March 6th CONTINUED		
540 at the Park		
Management Needs: Habitat Suitability Modeling Moderator: Valerie Kearny		
2:40 PM	Adam Kaeser *(Virtual)*	Advancing freshwater mussel conservation via side scan sonar imaging and habitat mapping automation technology
3:05 PM	Andy Hartzog	A watershed-wide approach to Species' Recovery, Habitat Assessment and Stream Restoration
3:25 PM	Discussion: Management Needs: Habitat Suitability	
3:40 PM	Break	
Management Needs: Contaminants and Die-offs Moderator: Valeria Kearny		
3:50 PM	Jordan Richard	Assessment of freshwater mussel die-offs using metagenomic markers
4:15 PM	Greg Cope	Understanding contaminants of emerging concern and their adverse effects on freshwater mussels
4:35 PM	Rob Tawes *(Virtual)*	The Georgia pesticide pilot program: an emerging conservation partnership
4:50 PM	Discussion: Management Needs: Contaminants and Die-offs	
6:00 PM	Fish Fry Mixer	

Thursday March 7th		
E.W. Shell Fisheries Center		
Session	**Leader**	**Location**
Mussel Silo Construction	Amy Maynard	Patio
Mussel Silo Placement	Wendell Haag	Chewacla Creek (32.535979, -85.496697)
Laboratory Respirometry	Jim Stoeckel	Counting Shed
Field Respiration	Jonathan Lopez	Ponds & Multipurpose Room
Microrespirometry	Kaelyn Fogelman	Teaching Classroom 115
Hemolymph + Swabs	Madi Polera	Teaching Classroom 115
Field Excretion	Matt Lodato	Ponds
Valveometry	Jim Stoeckel	Counting Shed

Speaker Abstracts – Tuesday March 5th

SESSION: RESPIROMETRY TECHNIQUES

10:00 AM – 11:00 AM

RESPIROMETRY TECHNIQUES AND RELEVANT ENDPOINTS

Jim Stoeckel

School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn AL 36849

Respirometry is increasingly being used as a tool to investigate the response of aquatic ectotherms to environmental stressors and effects of stressors on energetic health. Recent advances in respirometry systems have led to greatly improved techniques and accuracy of resultant data. Standardized methodology and endpoints associated with respirometry have largely been developed for fishes. While these can also be applied to other taxa, the ability of some mollusks such as mussels and snails to periodically close and switch to anaerobic respiration provides some challenges to standard approaches and interpretation of data. In this talk I will discuss the different types of respirometry, relevant endpoints of each, and ways that these endpoints can be interpreted with regard to environmental stressors and energetic health of organisms. For example, closed respirometry can be used to determine the dissolved oxygen threshold below which an organism can no longer obtain enough oxygen to meet its' energetic needs and must switch to less efficient anaerobic respiration. Intermittent respirometry can be used to assess energetic costs and provide insight into food requirements and energy budgets of organisms under a range of conditions. The bewildering array of endpoints associated with respirometry such as DOcrit, regulation index, standard metabolic rate, resting metabolic rate, routine metabolic rate, and maximum metabolic rate will be defined and explained. The goal of this talk will be to provide a basic understanding of respirometry, how to interpret results, and the types of practical management and conservation questions to which it can be applied. Subsequent talks regarding scope-for-growth, aerobic scope, and thermal performance curves will build from this general respirometry talk and show how respirometry can be incorporated into various frameworks regarding physiological responses to environmental stressors.

SELF-CONTAINED CHAMBERS FOR FIELD AND ECOSYSTEM RESPIROMETRY WITH FRESHWATER MOLLUSKS

Jonathan W. Lopez, Matthew B. Lodato, Carla L. Atkinson

Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA

Mollusks can provide important ecosystem services through their ecophysiological processes. In systems where their abundance and biomass are high, freshwater mollusks can support the flow of energy and resources through the ecosystem through metabolic functions like excretion and respiration. However, the most common method to measure metabolic rates in mollusks is to conduct laboratory trials. While laboratory trials allow collection of precise time-integrated metabolic data, the method has pitfalls, namely handling and captivity stress. Here, we outline two methodologies to collect respiration rate data for freshwater mollusks in the field. First, we outline the use of small portable chambers that can be used to calculate a two-point respiration rate by incubating mollusks in an isolated, air-free environment. This method allows for the direct estimation of mollusks' organismal respiration rates. Then we discuss a novel self-contained chamber design that can be installed directly into the benthic zone to evaluate mollusks' impacts on freshwater ecosystems in situ, across natural environmental gradients. When combined with measurements of organismal respiration, this method allows for the disentanglement of mollusks' direct and indirect impacts on ecosystem respiration via experimental manipulation. The field methods we describe can be integrated with complementary laboratory studies to generate a more holistic understanding of how and when mollusks alter energy flow in ecosystems. Quantifying mollusk-generated ecosystem services and the environmental conditions that constrain them provides scientific grounds for their conservation and helps predict the consequences that ecosystems stand to endure with the global defaunation of freshwater mollusk communities.

Speaker Abstracts – Tuesday March 5th

SESSION: SUBLETHAL BEHAVIORAL INDICATORS OF STRESS

11:00 AM – 12:00 PM

OH, BEHAVE! FRESHWATER MOLLUSK BEHAVIORAL RESPONSES TO ENVIRONMENTAL STIMULI

Peter D. Hazelton[1], Robert B. Bringolf[1], Teresa J. Newton[2] & W. Gregory Cope[3]

[1]University of Georgia, Athens, GA; [2]U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI; [3]North Carolina State University, Department of Applied Ecology, Raleigh, NC

Behavioral changes are among the most rapid responses animals display to environmental changes, making them potentially sensitive biomarkers of stress. Behaviors can be observed non-lethally and are often linked to underlying physiochemical pathways. Some behavioral responses are adaptive and may be associated with increased fitness, but alterations of reproductive, foraging, and movement behaviors can result in consequences at the organismal, population, and ecosystem levels of organization. Relative to other charismatic megafauna, freshwater bivalves and gastropods may not be known for elaborate displays of behavior (host attraction in mussels notwithstanding). Nevertheless, responses to external stimuli can help us understand linkages between biochemical responses and consequences for the individual. In this presentation, we explore stress responses in mollusk behavior including movement and burrowing, host attraction, and larval parturition. Specifically, we aim to review examples from the literature and our work on mollusk behavioral responses to environmental stimuli, including pollutants, thermal stress, and water level changes. We will summarize behavioral endpoints used in laboratory and field environments. We will emphasize linkages between behavioral and biochemical responses and discuss hypothetical consequences of behavioral shifts. Ultimately, we advocate for an integrated approach using behavioral and physiological endpoints to provide a comprehensive understanding of stress responses in freshwater mollusks.

Speaker Abstracts – Tuesday March 5th

SESSION: INDICATORS OF STRESS: BIOMARKERS

1:00 PM – 2:25 PM

BIOMARKERS OF ENERGY IN NATIVE FRESHWATER MUSSELS

Teresa J. Newton[1], W. Gregory Cope[2] and Madison E. Polera[2]

[1]U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI; [2]North Carolina State University, Department of Applied Ecology, Raleigh, NC

Research on native freshwater mussels has advanced considerably over the past 25 years, especially in the areas of ecology, toxicology, and propagation. Comparatively less is known about their physiology, immunology, and biochemistry, and this has hampered the development of tests, assays, and diagnostic tools that are critical in assessing the relative health and condition of mussels. The development of biomarkers—a change in a biological response at the molecular, cellular, biochemical, physiological, or behavioral level—could advance knowledge of the exposure, effects, and susceptibility of mussels to environmental pollutants and other stressors. This presentation will (1) provide an overview of biomarkers, (2) discuss the history and application of biomarkers of energy (i.e., glycogen) in mussels, (3) review the applicability of recent advances in biomarker research in other aquatic organisms for their use in mussels, (4) provide examples how biomarkers have been used to inform management decisions, and (5) offer guidance for the next steps in biomarker research with native freshwater mussels. Improved tools and techniques are needed to help resolve long-standing questions about the relative health of mussels, unexplained die-offs, and their sensitivity to environmental pollutants. Implementation of a suite of biomarkers, across multiple biomarker classes, could help advance efforts to conserve and restore mussel assemblages.

FRESHWATER MUSSEL METABOLOMIC STUDIES: KNOWLEDGE GAINED AND KNOWLEDGE GAPS

Diane Waller and Nolan Steiner, USGS Upper Midwest Environmental Sciences Center, La Crosse, WI, US

Louise Lavictoire, Freshwater Biological Association, The Hedley Building, Lakeside, Newby Bridge, LA12 8BD.

Metabolites are the substrates, intermediate products, and end-products of cellular metabolism. Metabolomics, the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind" is an emerging tool used to evaluate biologically relevant molecules in organismal tissues and fluids. This approach can identify metabolites linked to biochemical processes and provide insight into pathways disrupted by environmental stressors such as contaminants, starvation, and pathogens. Metabolomic studies of freshwater mussels have shown its use for detecting responses to contaminants, laboratory holding, thermal stress, and a mortality event. The metabolome is a snapshot in time of an organism's metabolic state and interpretation of metabolomic data is complex and challenging given the thousands of chemicals that may be detected and the factors that can contribute to variability across time and space and among individuals. External factors such as season and site and organismal factors such as species, sex, and age, and natural variability influence metabolism and may confound interpretation of data. Metabolite concentrations also vary among tissue; therefore the tissue selected for study will depend on the metabolic pathways of interest, as well as the analytical platform. We review approaches that have been used for applying metabolomics as a mussel health tool and what we have learned to date. Considerations for including metabolomics in mussel monitoring and health assessment studies and future knowledge needs will be discussed.

INCORPORATION OF TRANSCRIPTOMICS INTO A MULTI-BIOMARKER APPROACH WITH APPLICATIONS TOWARD ECOPHYSIOLOGY AND CONSERVATION

Matthew J. Jenny

University of Alabama, Tuscaloosa, AL 35487

The transcriptome represents the sum of all of an organism's RNA transcripts including protein-coding mRNAs and non-coding RNA genes, such as microRNAs and long non-coding RNAs. Over the last couple of decades, changes in technology have revolutionized our ability to sequence and assess global changes in gene expression from cells and tissues, progressing from microarrays, which can evaluate expression changes in a defined set of genes, to more advanced RNA sequencing (RNA-seq) technologies that allow for high-throughput sequencing of all expressed transcripts. The ability to assess global changes in gene expression in different tissues and under different experimental conditions allows for the identification of gene expression signatures that may be useful in identifying unique sets of biomarkers in response to specific ecological stressors. Furthermore, such assessments can provide novel information on gene regulation and new insight into associated physiological changes, particularly if the gene expression signatures can be phenotypically anchored to other biological responses from the cellular to organismal level that change in response to environmental factors. Thus, transcriptomics has the potential to serve as a powerful diagnostic tool that can contribute to the development of ecological forecasting models that can aid in predicting how specific populations of organisms may respond to current and changing ecological systems. Such models have the potential to improve restoration and conservation efforts on threatened species, including freshwater mussels. In this presentation, we will review available transcriptomic technologies and highlight the current state of genomic and transcriptomic resources available to freshwater mussel researchers. In addition, we will also discuss both the advantages and limitations associated with the application of transcriptomic sequencing through the lens of published and current research efforts.

Speaker Abstracts – Tuesday March 5th

SESSION: ENERGETIC MODELS

2:35 PM – 3:45 PM

USE OF THE CELLULAR ENERGY ALLOCATION MODEL FOR EVALUATING MOLLUSK HEALTH

Andrea K. Darracq[1], Traci Dubose[2], Steven Price[2], Kaelyn Fogelman[3], Jim Stoeckel[3], and Wendell Haag[4]

[1]Department of Biological Sciences, Murray State University, Murray, KY, USA, [2]Department of Forestry and Natural Resources, University of Kentucky, Lexington, KY, USA, [3]School of Fisheries, Aquaculture & Aquatic Sciences, University of Auburn, Auburn, AL, USA, [4]Southern Research Station, Center for Bottomland Hardwoods Research, U.S. Forest Service, Frankfort, KY, USA

Implementation of conservation measures for freshwater mollusks requires simple but effective monitoring tools that provide information about organismal health. The cellular energy allocation (CEA) model assesses the effect of stress on the energetic health of an organism. Available energy (E_a) is assessed by quantifying energy reserves (i.e., lipids, glycogen, proteins) while energetic costs (E_C) are estimated via an enzymatic assay (ETS: electron transport system assay). The CEA value is then calculated as Ea – Ec or Ea/Ec. The assumption is that a reduction in the CEA value indicates a negative effect on energetic health. This approach has been effective for assessing effects of environmental stressors (e.g., metal and microplastic pollution, temperature, and habitat characteristics) on aquatic mollusks. However, recent laboratory studies suggest that under some circumstances, the individual components of the CEA model provide a more accurate assessment of stress than the CEA estimate itself. Specifically, in a starvation experiment we found that the CEA did not differ between fed and starved juvenile mussels. However, total carbohydrates and protein levels and ETS activity were all greater in fed mussels. Thus, the utility of the CEA model is likely context dependent and may be influenced by valve closure in response to a stressor, organism-specific responses, and temperature. Challenges when using the CEA approach include problems with low tissue mass when using juvenile mussels or small snails, the amount of replication required, and limitations associated with the use of the ETS assay as an estimate of energetic cost. The CEA can be a valuable tool for assessing effects of stress on mollusks in the field and laboratory, but effective use requires an understanding of the strengths and limitations of this approach.

GENERAL TECHNIQUES AND APPLICATIONS OF THE SCOPE FOR GROWTH MODEL

Jessica Radich[1,2], Jim Stoeckel[2], Evelyn Pieper[2]

[1] U.S. Fish and Wildlife Service, Warm Springs, GA; [2] Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn, AL

Various environmental factors can affect the physiological responses of mollusks, resulting in an increase or decrease in energy available for a range of functions. Scope for growth (SFG) is a modeling approach that estimates net energy available to an organism for growth and reproduction. A mollusk's energy gains (e.g. food consumption) and costs (e.g. respiration) can be directly measured and converted into energetic units. A positive SFG value occurs when energy inputs are greater than the energetic losses incurred by basic maintenance, food acquisition, digestion, and absorption. This indicates an energy surplus that can be invested in growth and reproduction. A negative SFG occurs when energetic costs are greater than energy gains and indicates that an organism must use previously acquired energy stores just to meet the basic costs of maintenance and survival. Scope for growth provides a valuable, non-lethal approach for measuring sublethal stress in an organism exposed to pollutants, low oxygen, varying temperature, and other environmental stressors. While SFG has rarely been applied to freshwater mollusk taxa, it has been more widely used in marine systems. We therefore discuss research on fresh and saltwater mollusks and the modifications needed to accommodate a range of taxa and applications. We aim to provide a general understanding of standard SFG methodology and stimulate thoughts and discussion on potential applications of SFG to management and conservation of freshwater mollusks.

Speaker Abstracts – Tuesday March 5th

SESSION: INDICATORS OF STRESS: GROWTH AND SURVIVAL

3:55 PM – 5:00 PM

ASSESSING STREAM HEALTH AND MUSSEL FITNESS WITH IN SITU EXPOSURES AND SHELL THIN SECTIONS

Wendell R. Haag

US Forest Service, Southern Research Station, Frankfort, KY

Evaluating mussel responses to ambient stream conditions can provide essential information for understanding causes of mussel declines and evaluating candidate streams for restoration. I will discuss application and usefulness of methods for evaluating mussel responses. An increasingly common method is evaluating in situ responses of juvenile mussels exposed to stream conditions in silos or cages. Silos are durable, easy to deploy, and have minimal cage effects, but they may provide water-only exposures. Cages provide more exposure to sediments, but they are difficult to deploy and have a higher likelihood of cage effects. Survival during in situ exposures typically is high and uninformative. Growth is highly variable and potentially informative, but it is strongly affected by temperature, productivity, and other intrinsic factors, making comparisons among streams challenging. In situ exposures also are useful for evaluating physiological responses, pathogen burdens, and other questions. Growth histories and other features from shell thin sections can provide information about responses of adult mussels, but few studies have examined associations with specific factors. Similar to juvenile growth, adult growth is strongly influenced by physiochemical factors, and it is essential to account for age-related variation. Studies have examined chemical composition of shells over time, but the effect of shell composition on fitness is unknown, and composition can be influenced by age, species, and perhaps other factors. Using growth as an indicator of stream health or mussel fitness is challenging because of its high dependence on the physiochemical environment and a lack of clarity about interpretation. For example, the common assumption that higher growth is unequivocally good does not consider hypotheses about expected mussel responses to climate change and eutrophication/nutrient enrichment, as well as tradeoffs between growth rate, life span, and other life history traits.

Speaker Abstracts – Wednesday March 6th

SESSION: LETHAL STRESS ASSAYS

9:00 AM – 10:15 AM

USING THERMAL TOLERANCE INFORMATION TO ASSESS RISKS ASSOCIATED WITH NATURAL AND HUMAN-MEDIATED CHANGES IN WATER TEMPERATURE

Charles R. Randklev[1], Dorothea Mildenberger[1], & Xenia L. Rangaswami[2]

[1]Texas A&M Natural Resources Institute and Department of Rangeland, Wildlife, & Fisheries Management
[2]The Bay Institute, San Francisco, California

Water temperature is an important factor shaping the distribution and abundance of aquatic species. Because of this, estimating thermal tolerances of aquatic species is of considerable value to resource managers. For example, thermal tolerance information can be used to evaluate the risk of thermal stress and guide development of water quality and quantity criteria to protect aquatic species and their habitats. Unionid mussels are sensitive to thermal stress due to their unique life history, which has likely played a role in their global decline. Unfortunately, thermal tolerance data is lacking for a vast majority of mussel species and so there remains an urgent need for scientists and practitioners to collect this information. In this presentation, we share several case-studies on mussel thermal tolerance to highlight the utility of this information for conservation and restoration and to provide a roadmap for further research. Our specific goals are to: 1) show how mussel thermal tolerance data can be estimated using laboratory methods; 2) discuss how to use thermal tolerance data to quantify thermal exceedances using in situ water temperature; and 3) show how to translate exceedance data into information that can guide policy and regulations. The case studies presented are from Texas, located in the southwestern United States, which harbors a number of rivers already experiencing thermal stress and our focal species range from those presently considered common to species of high conservation need.

INTERPRETATION AND USE OF PHYSIOLOGICAL THERMAL PERFORMANCE CURVES

Kaelyn Fogelman

Department of Biological and Environmental Sciences, Troy University, Troy, AL 36082

Conservation of aquatic taxa is impeded by a lack of understanding of the linkages between physiological, behavioral, and thermal tolerance endpoints. Thermal performance curves (TPCs) allow us to trace thermal stress, physiological, and behavioral responses across multiple levels of biological organization (cellular to population). Combining TPCs with empirical measurements of energetic, behavioral, and lethal endpoints allow researchers and managers to interpret what a TPC means for the organism (e.g. does a peak in respiration rate or enzymatic activity indicate the optimal temperature for an organism, or a stressful temperature threshold beyond which death is imminent?). Previous attempts to link TPCs and thermal endpoints have yielded promising information by which to better understand unionid thermal ecology. Thermal performance curves can also be combined to develop scope for growth, cellular energy allocation, and aerobic scope models to evaluate optimal and stressful thermal thresholds. With further understanding, TPCs have the potential to reduce the number of assays and number of individuals required or sacrificed for thermal ecology studies. For example, TPCs associated with scope for growth and electron transport system assays can be constructed using sublethal approaches and may, by themselves, provide useful information regarding optimal, stressful, and lethal temperatures once linkages with endpoints from a range of other assays have been verified. This talk aims to discuss TPCs generated from a variety of techniques, and how they may be interpreted to aid in conservation of unionids and other aquatic ectotherms.

Speaker Abstracts – Wednesday March 6th

SESSION: SCALING UP: COMMUNITY EFFECT MODELING

10:25 AM – 11:30 AM

METHODS FOR IN-SITU COLLECTION OF FRESHWATER MOLLUSK EXCRETION AND EGESTION SAMPLES

Irene Sánchez González[1,2], Carla L. Atkinson[2], Garrett W. Hopper[3]

[1]University of Georgia, Athens, GA. [2]University of Alabama, Tuscaloosa, AL. [3]Louisiana State University and Agricultural Center, Baton Rouge, LA

Freshwater mollusks can elicit strong local effects on biogeochemical cycling. As mollusks filter-feed or graze, they convert energy and associated nutrients in their food into soft tissue, shell, and biodeposits (feces and pseudofeces), and release bioavailable dissolved nutrients that support primary producers and detritus-based food webs. Quantifying mollusk excretion and egestion offers information on the supporting ecosystem services they provide. We present comprehensive methods encompassing detailed information on equipment, materials, and protocols, for the measurement of excretion and egestion of freshwater mollusks directly at the sampling site. By conducting sample collection and processing on-site, we eliminate the need for transporting animals and site water, reducing the stress on the organisms and enhancing the reliability of the obtained data. Our methods apply to different freshwater habitats and we aim to provide fellow researchers with a standardized methodology applicable to diverse mollusk species.

SCALING THE IMPACT OF MOLLUSKS ON ECOSYSTEM FUNCTION ACROSS ENVIRONMENTAL GRADIENTS

Carla L. Atkinson[1], Garrett Hopper[2], Jonathan W. Lopez[1], Irene Sánchez González[3]

[1]University of Alabama, Tuscaloosa, AL. [2]Louisiana State University and Agricultural Center, Baton Rouge, LA. [3]University of Georgia, Athens, GA.

Freshwater mollusks can exert strong controls on nutrient cycling and organic matter dynamics, provide habitat for invertebrates and fish, and alter channel geomorphology. Given their complex effects on ecosystems, there are challenges to quantify their impact on ecosystem structure and function. The relative importance of animals in regulating ecosystem structure and function is variable across species and ecosystems and can be influenced by several external drivers such as temperature, ambient nutrient concentrations, water movement (i.e. hydrodynamics), and ecosystem size (e.g. stream discharge). Here we will discuss approaches for scaling individual-level mollusk physiological rates and patch-scale measurements of biomass to the assemblage and ecosystem-levels, respectively. Given the multitude of mollusk effects on energy and nutrient dynamics, we will focus on approaches for scaling biofiltration, excretion, and nutrient storage and production to the ecosystem. We will present a case study from the Sipsey River, Alabama, showing how both mussel assemblage composition and biomass alter the fluxes of energy and nutrients, highlighting the critical role mussels play at the ecosystem-scale. These approaches allow researchers to place mollusks into a broader framework regarding their importance to ecosystem services as well as allowing comparisons among other aquatic taxa and ecosystems. This is critical for management as research quantifying the functional importance of mollusks can improve science-based consideration of the social, ecological, and economic value of mollusk communities to healthy aquatic ecosystems.

Speaker Abstracts – Wednesday March 6th

SESSION: MANAGEMENT NEEDS: SPECIES RECOVERY PLANNING

12:30 PM – 2:30 PM

CONSERVING SPECIES: LISTING AND REVIEW IN THE SOUTHEAST (VIRTUAL)

Nicole Rankin and Rebecca Harrison

U.S. Fish and Wildlife Service

The U.S. Fish and Wildlife Service's (Service) priority is to implement and administer the Endangered Species Act (Act) effectively and efficiently. At-risk species conservation is one Service initiative promoting proactive conservation of fish, wildlife, and plants with partners before the species warrant protections under the Act. Our Listing and Classification program uses the best available science to determine whether to add a species, petitioned or discretionary, to the federal list of endangered and threatened wildlife and plants. This presentation will briefly present how our at-risk species efforts connect with our listing and classification work in the Southeast Region. It will focus on species' listing prioritization, National Listing Workplan, upcoming species for status review, and roles of partners in at-risk species proactive efforts and species status assessments to inform listing decisions.

RECOVERY PLANNING FOR SPECIES LISTED UNDER THE ENDANGERED SPECIES ACT

Carrie A. Straight

U.S. Fish and Wildlife Service, Atlanta, GA 30345

Once species are listed under the Endangered Species Act (Act), the U.S. Fish and Wildlife Service (Service) has statutory requirements to write a recovery plan for the listed species and to assess the status of the species once every five years. This presentation will focus on presenting a brief overview of species listing and delisting and summary of the Service's statutory requirements for recovery planning and 5-year status reviews. Along with the information about statutory requirements, I will discuss species information needs (research), roles of partners in recovery planning and implementation, and places to look for potential funding for implementing recovery actions.

STRIDES IN PROPAGATION AND CULTURE OF NATIVE FRESHWATER MUSSELS TO RESTORE DECLINING POPULATIONS & FURTHER BEST SCIENCE PRACTICE

Megan Bradley

U.S. Fish and Wildlife Service, Genoa, WI

Over the last 30 years efforts to improve their conservation have resulted in improvements in propagation and culture techniques. In 2004 there were 7 established freshwater mussel propagation facilities in the United States and Canada and today there are at least 30. This surge was driven by the listing of species at the state and federal level, publication of the National Strategy for the Conservation of Freshwater Mussels in 1998 (updated in 2016) and the implementation of a policy for controlled propagation by the USFWS and NMFS in 2000. Three freshwater mussel propagation and culture workshops have taken place since 2002, the USFWS hosts a yearly class and a manual of protocols for propagation of native mussels will be published this year. Refinement of techniques for holding host fish, producing juveniles without hosts, wild and laboratory grow out and novel marking methods have resulted in hundreds of thousands of cultured mussels being used for restoration of populations in rivers across the United States, Canada and Europe.

Speaker Abstracts – Wednesday March 6th

SESSION: MANAGEMENT NEEDS: HABITAT SUITABILITY MODELING

2:40 PM – 3:40 PM

ADVANCING FRESHWATER MUSSEL CONSERVATION VIA SIDE SCAN SONAR IMAGING AND HABITAT MAPPING AUTOMATION TECHNOLOGY

Adam Kaeser[1] and Cameron Bodine[2]

[1]U.S. Fish and Wildlife Service, Panama City, Florida, [2]Northern Arizona University, Flagstaff, Arizona

Freshwater mussels are often distributed throughout deep, turbid, and non-wadeable streams of the Southeastern Coastal Plain. These conditions pose significant challenges to the identification and characterization of mussel habitats, and the development of standardized mussel sampling approaches across landscapes. Nearly twenty years ago, a type of sonar called side scan sonar (SSS) first appeared on the recreational market, opening the door to underwater investigation via remote sensing to anglers and biologists alike. Side scan sonar produces a picture-like image of the underwater environment across wide swaths (up to ~90 meters). Data can be collected relatively quickly (8 km/hr), processed into sonar image mosaics, and then interpreted to produce classified habitat maps. Until recently map production was a labor intensive step, requiring significant expertise, but newly emerging (and free) software tools based on machine learning make it possible to both process raw data into mosaics and automatically produce classified substrate maps in a fraction of the time. The utility of these maps for designing sampling approaches, and developing models of the distribution and abundance of mussels via habitat associations is under exploration, and access to these no-cost tools offers the promise of greater rates of adoption and implementation in the future. Now is the time to harness the power of imaging technology and automation to advance mussel conservation at the landscape scale.

A WATERSHED-WIDE APPROACH TO SPECIES' RECOVERY, HABITAT ASSESSMENT AND STREAM RESTORATION

Andy Hartzog, Chris Metcalf

U.S. Fish and Wildlife Service, Panama City Florida, Fish and Wildlife Conservation Office

Using a watershed-wide approach to species recovery allows us to affect conservation within critical habitat that often occurs on private lands. Accelerated stream sedimentation impairs water quality, decreases biological productivity, and alters channel morphology, and aquatic habitats. Federal and state governmental agencies actively seek to conserve and restore both critical habitat and populations of threatened, endangered, and species of special concern. Recovery plans for listed species often call for restoration within critical habitat. What does that restoration look like?

Case Study, The Chipola River. The Chipola River Threats Assessment has identified over 1,200 potential threats to the basin including small streams, tributaries, and head water stream segments. The 1,200 sites were assessed using a Habitat Evaluation Form that collects fluvial geomorphological data using a bank Erosion Hazard Index (BEHI) dataset. In addition, 141 un-paved road crossings in the Chipola watershed were evaluated using the Sedimentation Risk Index (SRI). Threats were identified using GIS software and field verified. Threats include non-point source pollution from cows, livestock, agricultural run-off, lack of riparian buffer for any reason, development, erosion potential, stream bank erosion, agricultural threats, silvicultural, un-paved road crossings, upstream fish passage blockage issues (culverts, ponds, lakes, and dams), and poor land use practices. In conjunction with multiple partners, restoration actions have implemented conservation across 13,777 acres of agricultural lands in the Chipola River Watershed that protected over 74 acres of riparian habitat and, to date, have implemented 20 projects across five private properties. Project objectives include planting 4,000 trees; installing seven solar-powered livestock watering stations and 16 miles of cattle exclusion fencing; and restoring 10 miles of stream buffers and 15,000 feet of riverbank.

Speaker Abstracts – Wednesday March 6th

SESSION: MAAGEMENT NEEDS: CONTAMINANTS AND DIE-OFFS

3:50 PM – 5:00 PM

UNDERSTANDING CONTAMINANTS OF EMERGING CONCERN AND THEIR ADVERSE EFFECTS ON FRESHWATER MUSSELS

W. Gregory Cope[1], Robert B. Bringolf[2], Peter D. Hazelton[2], and Teresa J. Newton[3]. [1]North Carolina State University, Department of Applied Ecology, Raleigh, NC; [2]University of Georgia, Warnell School of Forestry and Natural Resources, Athens, GA; [3]U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI

Contaminants of emerging concern, collectively known as CECs, include many classes of compounds such as industrial chemicals, pharmaceutical and personal care products, pesticides, and nanomaterials. As defined by the U.S. Environmental Protection Agency, CECs are chemicals and other substances that have no regulatory standard, have been "recently discovered" in natural streams (often because of improved analytical chemistry detection methods), and potentially cause adverse effects on aquatic life at environmentally relevant concentrations. CECs include compounds that have long been present in the environment, but whose presence and significance are only now being evaluated (e.g., Per- and polyfluoroalkyl substances) or new chemicals and substances that are increasingly being detected at low levels in surface water (e.g., nanomaterials). Research on CECs and native freshwater mussels has advanced relatively slowly over the past 25 years compared to fish and other wildlife. This slow pace is primarily due to the lack of understanding on the basic biology, physiology, immunology, and biochemistry of mussels, and because CEC effects are often not acutely lethal, but rather involve reproductive, neuroendocrine, or energetic pathways. This presentation will (1) provide an overview of CECs, (2) discuss the history and evolution of CEC research in mussels, (3) describe case studies with mollusks in which CECs have been linked to adverse effects, (4) discuss the challenges of using non-lethal endpoints to inform management decisions, and (5) provide an overview of CECs on the horizon with suggestions for future funding and research opportunities.

THE GEORGIA PESTICIDE PILOT PROGRAM: AN EMERGING CONSERVATION PARTNERSHIP

Robert Tawes[1], Stanley Culpepper[2], and Taylor Randell-Singleton[2]

[1] U.S Fish and Wildlife Service, Southeast Regional Office, 1875 Century Boulevard, Atlanta, GA 30345. [2] University of Georgia, Agricultural Extension Program, Coastal Plain Experiment Station, 2360 Rainwater Road, Tifton, GA 31793

The Georgia Pesticide Pilot Program, which involves the U.S. Fish and Wildlife Service, Georgia Department of Agriculture, University of Georgia, Georgia farmers, and the Environmental Protection Agency, began in 2022. Initially formed to provide improved mapping and science to inform the national Endangered Species Act consultations on pesticide registrations, it has also created new relationships that we believe will foster additional conservation for Federal trust resources while reducing regulatory burden on Georgia's agricultural producers. During this presentation we will briefly outline the national pesticide consultations, discuss efforts to eliminate pesticide exposure to listed species, outline species mapping efforts, and look towards possible future innovative conservation collaborations with Georgia's vast agricultural sector, including those that could benefit freshwater mollusks.

Poster Session Abstracts

1. Alex B. Dunahoo (Student)

PREDICTING HOTSPOTS OF MUSSEL BIODIVERSITY FOR ANALYSIS OF CONSERVATION PRIORITIES

Alex B. Dunahoo[1], Robbyn J.F. Abbitt[2], David J. Berg[3].

[1] Department of Biology, Miami University, Oxford, OH, USA; [2] Department of Geography, Miami University, Oxford, OH, USA; [3] Department of Biology, Miami University, Hamilton, OH, USA

Traditional approaches to developing and managing conservation landscapes often fail to consider freshwater biodiversity, and those that do may not adequately focus attention on freshwater invertebrates. The state of Missouri has a diverse network of protected areas and regions of high conservation priority, however, it is unknown whether the state's diverse freshwater mussel assemblage is adequately protected. We identified hotspots of mussel diversity and compared them to existing conservation boundaries utilizing a geographic approach to planning (GAP) analysis. Maxent was used to predict the distribution of 53 mussel species using occurrence records obtained from the Missouri Department of Conservation (MDC) Mollusk Database in combination with mussel-relevant environmental variables. Distribution modelling was completed in replicate within the known range of each species and then filtered based on average model performance (AUC > 0.8). The 45 species with models that passed through the filters were projected to the entirety of Missouri. State-wide projections were then converted to a presence/absence format and stacked to estimate species richness and rarity-weighted richness (RWR) within each 1 square kilometer. Those cells with high predicted diversity metrics were identified as "hotspots" and compared to various boundaries representing conservation priority areas and protected areas. Based on an initial analysis, the vast majority of mussel diversity hotspots in Missouri are not being protected, with less than 11% of species richness and RWR hotspots occurring within land managed for biodiversity (GAP Status 1 and 2). Additionally, less than 2% of hotspots are contained within MDC's natural areas or Priority Geographies, which have high priority for conservation efforts. Our results identify areas of high mussel diversity while also demonstrating that such hotspots need to be further incorporated into future freshwater conservation efforts.

2. Susan Fuller (Student)

A REVIEW OF SALINITY TOLERANCES AMONG NORTH AMERICAN FRESHWATER MUSSELS (BIVALAVIA: UNIONIDA)

Susan Fuller[1], Shannon Brewer[1], Maureen Walsh[3], Jennifer Archambault[4], Jim Stoeckel[1], Kaelyn Fogelman[1].

[1] Troy University, Troy, AL; [2] Auburn University, Auburn, AL; [3] U.S. Fish and Wildlife Service, Panama City Beach, FL, [4] U.S. Fish and Wildlife Service, Raliegh, NC.

North America is a biodiversity hotspot for freshwater unionid mussels – of which nearly 1/3 are considered imperiled. One threat that freshwater species are facing is the increased salinization of freshwater systems through an increase in extreme weather events such as droughts and hurricanes or human activities such as irrigation and use of road de-icing salts. To facilitate conservation and management efforts associated with mussel conservation it is important to understand stressful and lethal thresholds of tolerance to environmental stressors such as increased salinity. To support this need we conducted a systematic literature review to summarize existing salinity tolerance data for North American unionids by life stage and taxonomy. We identified published studies on salinity tolerances for 22 North American species within the families Margaritiferidae and Unionidae, including the tribes Amblemini, Anodontini, Lampsilini, Pleurobemini, Popenaidini, and Quadrilini. Lethal endpoints identified included the salt concentration at which 50% of individuals tested experienced a desired endpoints (generally mortality; EC50), at which 50% of individuals tested experienced mortality (LC50) and the time until death for 50% of individuals tested (TUD50). The primary salt used in exposure studies was sodium chloride (NaCl), but tolerance assays have also been conducted using exposures to beetjuice salt brines, commercial salts (Liquidow brine, Morton salt, and Cargill road salt), and sodium cyanides. Most exposures have been conducted on glochidia and juvenile life stages, although three species of adult unionids have had upper lethal tolerance limits evaluated. The next steps of this review include summarizing and synthesizing lethal thresholds for known species across life stages and taxonomy to identify lethal salinity thresholds for aquatic systems that unionid mussels reside in.

3. Michael M. Gangloff (Professional)

FIFTY YEARS OF CHANGES TO MUSSEL ASSEMBLAGES IN FOUR EAST-CENTRAL ALABAMA WATERSHEDS

Michael M. Gangloff[1] and Andrew M. Gascho Landis[2]

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[2]Department of Fisheries, Wildlife and Environmental Science, SUNY Cobleskill, Cobleskill, NY 12043

East-Central Alabama has one of the fastest growing human populations in the southeastern US. Prior to 2000 landuse in this region was largely mixed agricultural and timber production; however, industrial expansion along the Interstate 85 corridor has led to increasing demands on the region's natural resources. Recent droughts, combined with an increased need for water, have increasingly stressed aquatic resources in the region, including numerous populations of federally-listed mussels. Four streams (Halawakee, Saugahatchee, Uchee and Uphapee creeks), divided between the Chattahoochee and Tallapoosa drainages, historically supported populations of at least 36 mussel species, including 7 taxa currently designated as threatened or endangered by the USFWS. Surveys conducted by J. C. Hurd and J. J. Jenkinson between 1970-1973 found 35 species suggesting that very little had changed in the > 50 y since H. H. Smith and R. L. Howard with the Geological Survey of Alabama initially conducted surveys. In contrast, surveys during the period extending from 1999-2002 revealed that although diverse mussel assemblages were still present in several streams including Chewacla and Uchee creeks, populations of three federally-listed species were extirpated from these systems. More recent (2010-2020) surveys indicate that the extent of diverse mussel assemblages has been further reduced in these systems and only one federally-threatened mussel, the Finelined pocketbook, is still found at >50% of historically-occupied sites in the Uphapee Creek watershed. These results are alarming and reflect an urgent need for watershed-level protections including more serious efforts to reduce the degree of streamflow loss to a massive quarry in the heart of the Chewacla-Uphapee Creek watershed, as well as better addressing impacts of increased stormflow runoff and wastewater discharge to increasingly stressed waterbodies.

4. Gabriel T. Inoshita (Student)

ZERO-INFLATED MODELING OF TRANSECT SAMPLING TO OPTIMIZE POPULATION MONITORING OF FRESHWATER MUSSELS

Gabriel T Inoshita[1], Alfredo A Ascanio[1], Daniel A Trujilio[2], Nathan Thompson[2], Kentaro Inoue[3], Steve R Hein[1], and David J Berg[4].

[1]Department of Biology, Miami University, Oxford, Ohio; [2]New Mexico Department of Game and Fish, Santa Fe, NM, [3]Daniel P. Haerther Center for Conservation and Research, John G. Shedd Aquarium, Chicago, IL; [4]Department of Biology, Miami University, Hamilton, Ohio.

Threats to freshwater mussel survival makes monitoring of population abundances essential. Most studies estimate mussel density, but abundance is the key value for making conservation decisions. A >20 year monitoring program for the federally endangered Texas Hornshell (*Popenaias popeii*) in the Black River of New Mexico has provided such estimates of abundance. However, the transect censusing method used has limited statistical power and is very costly, in both time and personnel. To meet the goal of reducing sampling costs in the future while keeping good population estimates, we studied data from 2011-2012 and 2017-2018 censuses, assessing previous sampling and statistical methods used for monitoring. Our aim is to improve these methods. When exploring the data, we found that between 40% and 49% of the transects were zeros, which greatly inflated the variances of our population estimates. To account for this excess of zeros in our censuses, we propose the use of Poisson or zero-inflated Poisson models, that further consider the sampling year, habitat, site, transect, and channel position as covariates for estimating mussel counts. Using a model calibration approach, with 2023-2024 census data for testing, we will provide the best predictive model for total mussel abundance. Potential sampling design avenues to decrease logistic costs will be created by removing data from the models of best fit until they no longer have predictive power. This will tell us the minimum amount of sampling effort required to still have good predictive models. Through similar analysis of censusing methods in hard-to-detect ecosystems, agencies can produce optimized censusing procedures that reduce costs while allowing them to monitor populations in an effective and efficient way.

5. Molly Martin (Student)

EVALUATION OF SEDIMENT AND WATER QUALITY TO SUPPORT FRESHWATER MUSSELS IN THE CONASAUGA RIVER, GEORGIA

Molly Martin[1], Kelly Robinson[2], Brian Irwin[2], Robert Bringolf[3], and Peter Hazelton[3].

[1] Georgia Cooperative Fish and Wildlife Research Unit, Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602, [2] U.S. Geological Survey, Georgia Cooperative Fish and Wildlife Research Unit, Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602, [3] Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602.

Freshwater mussels are some of the most imperiled taxa in the world. Historically, threats to mussel populations have been attributed to habitat degradation or loss from dams, pollution, invasive species, and siltation. These disturbances are often correlatively associated with declines, yet research has often lacked rigorous methods of testing for causative factors of enigmatic loss. We plan to implement a combination of field and laboratory exposure trials to assess the effects of multiple contaminant stressors on freshwater mussel survival and growth in the Conasauga River, located in northwest Georgia and southern Tennessee. We will conduct substrate-exposure studies in the laboratory using sediment collected throughout the watershed following established sediment toxicity test conditions. Sediments will be collected in the Conasauga at multiple sites representing a gradient of municipal and agricultural contamination and varying land use practices. Additionally, we will deploy juvenile mussels in silos at the same sites utilized for the toxicity trials to assess responses to waterborne contaminants *in situ*. Mussel silos are concrete structures that allow for containment and retrieval of mussels. We expect to find differences in survival and growth across the contamination gradient. This study will inform reintroduction efforts for freshwater mussels in the Conasauga River as well as focus stream conservation efforts in the basin. Additionally, data collected will further our understanding of the role sediment and waterborne contaminants play in the decline of freshwater mussels.

6. Amy Maynard (Professional)

FRESHWATER MUSSEL PROPAGATION AT NEOSHO NATIONAL FISH HATCHERY

Amy Maynard[1] and Nathan Eckert[1].

[1]Neosho National Fish Hatchery, United States Fish and Wildlife Service, Neosho, MO.

Neosho National Fish Hatchery (Neosho NFH) propagated approximately 390,000 juvenile mussels of six species of freshwater mussel for research and conservation in 2023 including: Fragile Papershell, White Heelsplitter, Giant Floater, Plain Pocketbook, Neosho Mucket, and Wabash Pigtoe. Our current projects include an *in-situ* water quality study of juvenile mussel growth and survival within Shoal Creek (Missouri), propagation of Federal Endangered Neosho Mucket in two distinct management units in the Illinois River (Arkansas/Oklahoma), freshwater mussel restoration within tributaries to the Verdigris River (Oklahoma), and propagation of Wabash Pigtoe for U.S. Geological Survey (USGS) salinity studies at multiple life stages. After conducting preliminary surveys of the Neosho River with the Kansas Department of Wildlife and Parks (KDWP), we released 1,374 Neosho Mucket that were collaboratively cultured by Neosho NFH and the Kansas City Zoo. Additionally, we distributed 3,786 Wabash Pigtoe to USGS, and 15 Bleufer to the National Conservation Training Center for education. To increase growth, survival, and culture capacity, we constructed two pulsed-flow-through units. We have also moved our propagation and culture equipment to a building that offers more space, insulation, electrical capacity, and access to pond water. We are currently holding 12,359 juveniles propagated in 2023 and an additional 13,667 juveniles and sub-adults propagated in 2021-2022 for stocking and grow-out next year.

7. Dorothea Mildenberger (Student)

AN EVALUATION OF THE THERMAL TOLERANCES OF COMMON AND THREATENED MUSSEL SPECIES IN EAST TEXAS

Dorothea Mildenberger[1], Timothy Bonner[2], Terry Corbett[3], Clinton Robersten[4], Roel Lopez[1], & Charles R. Randklev[1]

[1]Texas A&M Natural Resources Institute, Texas A&M AgriLife Research Center at Dallas, Dallas, TX; [2]Department of Biology/Aquatic Station, Texas State University-San Marcos, San Marcos, TX; [3]Lower Neches Valley Authority, Beaumont, TX; [4]Texas Parks and Wildlife Department, Inland Fisheries Division, Management and Conservation Branch, San Marcos, TX.

River water temperature is a key factor that determines habitat suitability for aquatic organisms. Climate change and human-driven impacts on freshwater ecosystems can increase water temperatures above species-specific ranges, posing a significant threat to freshwater ecosystem composition, function, and the services they provide to people. Unionid mussels are globally imperiled and many species are living near or at their upper thermal limit, which means future increases in water temperature will likely have negative impacts on their long-term persistence. Despite this threat, there remains a significant knowledge gap on sublethal and lethal temperature thresholds for the vast majority of mussel species. To begin addressing this issue, we tested the upper thermal limits (LT05 and 50) of three species (*Cyclonaias pustulosa*, *Fusconaia askewi*, and *Pleurobema riddellii*) for which thermal tolerance information is lacking. Mussels were collected from east Texas, located in the southwestern United States. The region has a subtropical-humid climate that has not yet been studied for thermal exceedances. Mussels were acclimated to 27°C then tested at four experimental temperatures (30, 33, 36, 39°C) and a non-acclimated control (20°C) for 10 days (240-hr). Lethal tolerance estimates resulting in 5% mortality ranged from 31.69°C – 37.22°C and lethal tolerances resulting in 50% mortality ranged from 34.29°C – 38.43°C. Relating field water temperature to our laboratory-derived thresholds revealed a small number of exceedances, which increased under drought-like conditions. Climate projections for East Texas show that droughts will become more commonplace in the future, which means that while thermal stress may not be an issue now it may become a threat in the future. Overlapping thermal tolerance estimates with *in situ* water temperature and discharge data provides a useful approach for evaluating thermal risk to mussels and for guiding efforts focused on protecting and restoring instream flows.

8. Joshua D. Millwood (Student)

THE MOLECULAR AND PHYSIOLOGICAL RESPONSES TO THERMAL STRESS OF FOUR UNIONID SPECIES

Joshua D. Millwood[1], Paul D. Johnson[2], and Matthew J. Jenny[1].

[1]University of Alabama, Tuscaloosa, AL; [2]Alabama Aquatic Biodiversity Center, Alabama Department of Conservation & Natural Resources, Marion, AL.

As global temperatures continue to rise most groups of organisms, terrestrial and aquatic, are being affected. Impacts on these organisms include drought, flooding, thermal stress, etc. Unionid mussels, being sessile organisms, are certainly not immune from these environmental changes and how they may respond is not well known. To better understand how various species of mussel may react to elevated temperatures we performed a mesocosm study utilizing adult mussels. We investigated species with various habitat ranges and various brood strategies to gain a better understanding of how distribution and life history may influence thermal stress response. Individuals of *Fusconaia cerina, F. escambia, Cambarunio nebulosus,* and *Leaunio lienosus* were placed into one of three temperature treatments (low: 22°C, mid: 25°C, and high: 28°C) for approximately 6 weeks. This experimental design was utilized to produce a sub-lethal response to collect molecular (differential gene expression) and physiological (respiratory, metabolic, and enzymatic) response data. Data from this experiment is still being analyzed, but preliminary results show a potential correlation between extent of thermal stress response and brood strategy. Gaining a better understanding of the physiological response, and molecular underpinning of those responses, to thermal stress could be instrumental in protecting this important and highly impacted group of organisms.

9. Lauren Morris (Student)

ELEMENTAL COMPOSITION CHANGES OF FRESHWATER MUSSEL SHELLS (UNIONIDAE) DURING DECOMPOSITION IN AN ALABAMA RIVER.

Lauren M. Morris[1], Garrett W. Hopper[2], Jonathan W. Lopez[1], Carla L. Atkinson[1].

1.University of Alabama, Tuscaloosa, AL; 2. Louisiana State University, Baton Rouge, LA.

Freshwater mussels (Family: Unionidae) often occur in dense aggregations in rivers providing critical ecosystem services, such as storing carbon (C), nitrogen (N), and phosphorus (P) in their soft-tissues and shells. After mussels die, C, N, and P are slowly leached from the shell over the course of months, years, or even decades. However, there is not much information regarding how shell thickness impacts decomposition of freshwater mussel shells and whether there is preferential nutrient leaching from shell material over time. Previous findings in evaluating shell stoichiometry suggest life history strategies could be related to nutrient composition of shells, which ultimately determine species growth rates. Depending on the life history strategy and associated growth rate, freshwater mussel shell nutrient composition can vary. We hypothesize that thinner-shelled species will have faster decomposition rates than thicker-shelled species due to their shell nutrient composition differences. Additionally, we hypothesize that mussel shells will preferentially leach N initially over C and P due to decay of the periostracum firstly and that thicker-shelled mussel species will be composed of more P and less C and N than thinner-shelled species. To test these hypotheses, we collected shells of seven mussel species with varying shell thicknesses, and deployed them in mesh bags along three sites in the Sipsey River, Greene County, AL. Each bag was anchored to the bottom of the river and then were retrieved across four different sampling dates over the course of four years and measured for change in total mass. Our next steps will include cutting lateral cross sections from each shell and analyzing each sample for C, N, and P composition. Linear regressions will be performed to determine if species identity and associated shell thickness, and location in the river have an impact on decomposition rates and overall nutrient retention. By evaluating and comparing decomposition rates across freshwater mussel species, we can better understand whether these organisms act as short-term nutrient capacitors or longer-term nutrient sinks in freshwater systems.

10. Ieva Roznere (Professional)

ASSESSING THE QUANTITY AND QUALITY OF RNA EXTRACTED FROM FRESHWATER MUSSELS.

Rachel Obler[1], Kailee Kretzinger[1], Ieva Roznere[1].

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Freshwater mussels are an increasingly imperiled group of animals, but there is limited knowledge of their health and appropriate health assessment techniques. Investigation of gene expression changes in response to environmental stressors is one potential health assessment tool. The objectives of this study were to determine the quantity and quality of RNA extracted from various tissues, and the impact that tissue collection may have on mussel mortality. *Eurynia dilatata* (n=87) were collected from the Kokosing River in Ohio and transported to the Watters Aquatic Conservation Center. Tissue from the foot, gill, and mantle were biopsied from 10 mussels each and frozen in liquid nitrogen. RNA was extracted using a RNeasy Mini Kit. Hemolymph samples were collected from another 20 mussels and stabilized in either RNAprotect Animal Blood Tubes or EDTA. RNA was extracted from 10 samples using a RNeasy Protect Animal Blood Kit for the Hemo(A) group, and from 10 samples using a QIAamp RNA Blood Mini Kit for the Hemo(B) group. RNA concentration and integrity were assessed using a Qubit Fluorometer and 4200 TapeStation, respectively. The rest of the mussels (n=37) were used as a control group to assess any biopsy-induced impacts on mortality. A Kurskal-Wallis test was used to determine any significant differences in RNA concentration and integrity between the treatment groups. A Chi-Square test was used to determine impacts on mortality. Gill tissue yielded the highest RNA concentrations and integrity values. In general, tissue samples yielded higher concentrations than hemolymph samples. Gill and Hemo(B) samples had RNA integrity values above 7, while Mantle, Foot, and Hemo(A) samples had average values lower than 7. Sample collection had no significant impact on mortality. We conclude that, although gill tissue yields the best results, either gill, foot, mantle, or hemolymph samples can be used for non-lethal sampling and RNA extraction.

11. Ashton J. Schardt (Student)

ASSESSING DIETARY ADDITIVES FOR ENHANCING THE NUTRITION AND GROWTH OF JUVENILE FRESHWATER PLAIN POCKETBOOK MUSSELS *LAMPSILIS CARDIUM*.

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Freshwater mussels are invaluable for aquatic ecology and consist of indicator species in these environments. However, nutrition research is in the infancy of discovering how certain commercial feeds and additives may support growth and survival of hatchery raised freshwater mussels by providing essential nutrients and energy. This 14-week study evaluated various combinations of commercial algae and additives as dietary treatments. In a completely randomized arrangement, nine treatments were designed including a negative control (NC; no feed), four treatments of paste algae and four treatments of freeze-dried algae with or without additives (probiotic, worm casing, and a commercial algae replacement). Groups of 20 mussels (0.02075g ± 0.04786) were stocked into 36 9L tanks (n=4) in a static system with partial water exchanges (33%) occurring every 8 hours using an automated setup. The mussels were fed once a day and water quality parameters were tested weekly. Every two weeks the mussels in each tank were sampled to access length (in millimeters using Image-Pro Plus software), weight gain, and survival. Daily rations were adjusted after each sampling event according to tank mussel biomass. The results of the study revealed significant ($P \leq 0.05$) increases in length and weight when all additives were fed in combination with algae, whereas the mussels in the NC lost weight. Whole-body composition analysis including dry matter, protein, and energy are underway. This study provides new insights on the nutrition of freshwater mussels raised in captivity and may help in optimizing conservation/aquaculture endeavors.

12. Allison Sieja (Professional)

LINKING FRESHWATER MUSSEL HABITAT CONDITIONS TO RESTORATION OUTCOMES AND ECOSYSTEM SERVICES

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Freshwater mussels are among the most imperiled taxa globally. Once prevalent in the landscape, anthropogenic stressors such as habitat alteration, contaminants, and climate change have led to steep population declines. Such declines have altered habitat functionality through the loss of ecosystem services they provide, such as water quality improvements, sequestration/processing of nutrients, stream stability, and diverse food web structure. This project aims to restore mussel assemblages and their ecosystem services, as well as provide systematic habitat assessments for locations of variable land usage. We studied the Clinton River, located within an urban watershed near Detroit, Michigan, USA, with varying water quality issues, strong urban influence, and wastewater input. In the initial phase of the study, we conducted a field study that integrates water quality, habitat conditions, and in situ mussel exposures to assess the potential role of contaminants and habitat stressors on the suitability for mussel restoration and potential uplift. The field study incorporated measures of sediment and water contaminants, water quality analysis, passive samplers, water quality sensors housed within a silo, and monitoring native mussels placed in situ. Fatmucket, *Lampsilis siliquoidea*, (13 mm) were placed in silos in the river for 78 days at five different sites with existing mussel beds to assess their survival and growth. Additionally, every two weeks, we visited the silos to collect water samples for water quality and particle count analyses. Mussel survival was high, greater than 93%, across all sites, and mussel growth averaged 0.6-2.5 mm, depending on the site. These data will be used to inform the placement of mussels in the river in 2024 and to predict the likelihood of restoration success. Ultimately, we seek to re-establish diverse representative mussel assemblages and evaluate the resulting ecosystem services provided by freshwater mussels including the impacts on water quality and increased macroinvertebrate diversity.

13. Allison Sieja (Professional)

INNOVATIONS IN THE MUSSEL SILO DESIGN WITH THE INTEGRATION OF WATER QUALITY SENSORS

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The mussel silo is a portable cage used to house juvenile mussels, which utilizes the Bernoulli effect to create an upwelling flow through the chamber that houses the mussels. In addition to being employed to evaluate in situ exposures to contaminants, silo usage has expanded to assist in the determination of potential reintroduction sites for restoration projects. We deployed silos for a restoration project in the Clinton watershed near Detroit, Michigan which historically had high levels of anthropogenic activities and varying water quality issues. We integrated dissolved oxygen, temperature, and conductivity sensors into the silo design so that the sensors would record measurements from within the chamber and provide continuous water quality data at 5-min intervals. A pressure sensor was also integrated to record continuous water level data, also at 5-min intervals, at each deployment location. Four silos with integrated sensors were deployed for 78 days, and sensor data was compared to water quality data taken from surface-level water obtained at two-week intervals. Generally, sensor data was similar to surface-level water samples, but conductivity values were lower in the sensor data than the surface-level measurements at some sites. We also used the water quality data from the sensors to parameterize a stream metabolism model that can directly link primary productivity with mussel growth. In addition to the water quality sensors, we explored how different water velocities affected the flow coming through the chamber. We placed a silo in a continuous-loop, racetrack-style flume housed at the Columbia Environmental Research Center and used particle imaging velocimetry to assess chamber flow at velocities ranging from 5 to 75 cm/s. As water velocity increased, chamber flow increased as well. We plan to add additional sensors to the silo and will deploy them in the summer of 2024 to investigate other potential restoration sites.

14. David J. Soucek (Professional)

COMPARING THE RESPONSE OF GLOCHIDIA AND JUVENILES OF COMMON AND FEDERALLY ENDANGERED FRESHWATER MUSSELS TO THREE CONTAMINANTS OF CONCERN

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Freshwater mussels are in sharp decline and while many factors are thought to contribute to this, contaminants and degraded water quality are often cited as significant factors. The state of Michigan is home to several populations of the federally endangered *Epioblasma triquetra* (Snuffbox), and long-term monitoring by numerous agencies indicates prevalence of mercury (Hg) and per- and polyfluoroalkyl substances (PFAS) both within watersheds inhabited by Snuffbox and more widely across the Great Lakes Basin. Little is known about how these contaminants may impact mussel health and recruitment. In addition, freshwater salinization, resulting from a variety of anthropogenic land use changes including resource extraction and road salting, has become a water quality issue in the region as it has globally. The most direct approach to assessing the risks of contaminants to federally threatened or endangered (TE) species like *E. triquetra* is to test them in the laboratory; however, there are often barriers to this approach like animal availability or permitting issues. Therefore, surrogate species such as *Lampsilis siliquoidea* (Fatmucket) which are more common and/or more amenable to culture in the laboratory are often tested in place of TE species. The extent to which the responses of common species of mussels like Fatmucket represent the responses of endangered species like the Snuffbox is poorly understood. Our objective was to compare the acute responses of Fatmucket and Snuffbox early life stages to Hg, the PFAS perfluorooctane sulfonate (PFOS), and NaCl (representing road salt), conducting 96- and 24-h bioassays with juveniles and glochidia, respectively. We observed similar sensitivities between the two species for juvenile responses, but Snuffbox glochidia were approximately 4- and 7-fold more sensitive than Fatmucket glochidia to PFOS and Hg, respectively. Conversely, Snuffbox glochidia were nearly 2-fold less sensitive to NaCl than Fatmucket; however, it should be noted that source population for snuffbox was from a site with background chloride concentration greater than 200 mg/L, which may have resulted in reduced Snuffbox sensitivity via adaptation. With similar juvenile responses but disparate effect levels for glochidia, it is evident more work is needed to ensure risks of environmental contaminants to early life stage endangered species are adequately assessed.

15. Kaitlin Ulin (Student)

DOES *IN VITRO* MEDIA COMPOSITION MATTER? A GENE EXPRESSION EXPERIMENT IN *LAMPSILIS SILIQUOIDEA*.

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Artificial propagation, called *in vitro*, has been commonly used to transform juvenile freshwater mussels without the need of a fish host, making it a useful tool for conservation biology. To improve our success of *in vitro* propagation, we must first expand our current limited knowledge of the larval development of mussels. The key to this is understanding what nutrients are needed for the successful development and propagation of healthy juvenile mussels. In this study, we compare media of various compositions to assess the growth of larval mussels. Growth is evaluated via analysis of the transcriptome, which can reveal internal processes happening within an organism. Glochidia were extracted from adult *Lampsilis siliquoidea* mussels, pooled, and separated into three media groups: Leibovitz's L-15 Medium (L-15), Medium 199 with Hank's balanced salts (M199), and M199 with Hank's balanced salts and 50 µL of lipids from concentrate. Glochidia developed in an incubator until signs of metamorphosis were present. Juveniles were collected from each dish, rinsed with sterile ultrapure water, and snap frozen in liquid nitrogen. RNA was extracted from the samples and sequenced on the Illumina NovaSeq 6000 sequencer with output as 100-base-pair paired-end reads. Assembly of the *de novo* transcriptome was performed and differentially expressed transcripts between the three groups were identified. This study combines the accessibility of *in vitro* propagation with gene expression analysis of larval growth between growth media of different compositions with the goal of improving *in vitro* juvenile mussel propagation. Exploring the differences in gene expression between the media types will allow us to better understand larval response to the culture media and provides foundational knowledge that is needed to improve the conservation of the highly imperiled and ecologically important freshwater mussels.

16. Matthew J. Ashton (Professional; Virtual)

SURFACE AND POREWATER AMMONIA CONCENTRATIONS IN RELATION TO DWARF WEDGEMUSSEL (*ALASMIDONTA HETERODON*) POPULATION STATUS IN MARYLAND, USA.

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Numerous abiotic stressors have been attributed to the decline of North American freshwater mussels. Degraded water quality, particularly excess ammonia in runoff, can be toxic to mussel species. We investigated whether concentrations of total and unionized ammonia (TAN and UIA-N) in surface and pore water may explain dwarf wedgemussel distribution and abundance within the four extant populations of Maryland's Coastal Plain. A prior study of two of these streams indicated it was the only water chemistry parameter that might explain recent distributional declines. Grab samples of filtered surface water and pore water samples using peepers were collected approximately monthly at each site from April through September, 2023. Preliminary results indicate that TAN and UIA-N a concentrations 1) correspond with their predominant watershed land use, 2) varied temporally and spatially, 3) often exceed the chronic concentration that is toxic to mussels in the agriculturally dominated streams, and 4) did not exceed thresholds in McIntosh Run and so do not appear to be related to the decline of Dwarf Wedgemussels. We cannot rule out the potential impact from acute ammonia concentrations as we sampled during base-flow over 28-days or synergistic effects, such as interactions with *Corbicula*, which is a recent invader to these streams, as both are known to affect mussel survival.

17. Katherine H. Philipp (Professional; Virtual)

CAN THE ADDITION OF NITRIFYING BACTERIA IMPROVE FRESHWATER MUSSEL CULTURE?

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Freshwater mussels are the most imperiled group of animals in North America and in many parts of Maryland their populations are reduced. The Maryland Department of Natural Resources (MDNR) has recently started a mussel propagation program to captively raise juvenile mussels and restock habitat that has recovered from prior degradation. Mussels are highly sensitive to ammonia, especially as juveniles. As a result, higher mortality in hatchery culture is often associated with elevated ammonia concentrations. We designed a semi-recirculating culture system and conducted a pilot study to test if the application of commercially available nitrifying bacteria can reduce ammonia levels by oxidizing ammonia to nitrite and therefore increase growth and survival rates of juvenile mussels. Two identical down-wellers held 500 juvenile Yellow Lampmussel (*Lampsilis cariosa*) in each system. One system was dosed with nitrifying bacteria a week prior to the experiment and was given a weekly dose for 30 days. Both systems were given identical feedings of commercial algae daily and cleaned three times a week. Growth and survival were measured weekly while ammonia, temperature and pH were measured twice a week. Survival and growth of juvenile mussels changed at similar rates between the treatment and control. The treatment's efficacy in the pilot experiment was unclear and we plan to run another trial in March. During the second trial we will culture mussels in sediment in addition to down-wellers. We predict that growth and survival rates will be higher when juvenile mussels are cultured in sediment and treated with nitrifying bacteria because the sediment may provide substrate for the bacteria to colonize. If our experiments show that commercially available nitrifying bacteria lowers ammonia concentrations and in turn increasing growth and survival, the treatment could greatly improve MDNR's mussel propagation effort by increasing the number of mussels they can stock.

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