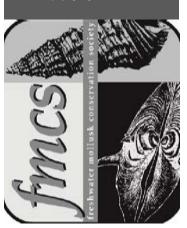


Mollusk Health & Disease Workshop March 13-15, 2018

11<sup>th</sup> Biennial Workshop



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U.S. Geological Survey

Upper Midwest Environmental Sciences Center

Megan Bradley & Nathan Eckert U.S. Fish and Wildlife Service Genoa National Fish Hatchery

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Shelly Bartsch-Poster section

Heidi Dunn- Mollusk Die-off Section Coordination

Corey Puzach- Laboratory Section Coordination and Local Arrangements

Emily Grossman- Management of Exchequer

Louise LaVictoire- Program layout and coordination

Greg Cope-Presenter selection

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### Awards

W. Gregory Cope - North Carolina State University greg cope@ncsu.edu

Teresa Newton - Upper Midwest Environ. Science Center

tnewton@usgs.gov

Emy Monroe - Midwest Fisheries Center emy\_monroe@fws.gov

# **Environmental Quality & Affairs**

Steve McMurray - Missouri Dept. of Conservation

stephen.mcmurray@mdc.mo.gov

Braven Beaty - The Nature Conservancy bbeaty@tnc.org

# **Gastropod Status and Distribution**

Nathan Whelan - Auburn University <a href="mailto:nwhelan@auburn.edu">nwhelan@auburn.edu</a>

### Genetics

David J. Berg - Miami University bergdj@miamioh.edu

Dave Zanatta- Central Michigan University zanat1d@cmich.edu

### **Guidelines and Techniques**

Ryan Schwegman - EnviroScience, Inc.

RSchwegman@EnviroScienceInc.com

Lisie Kitchel- Wisconsin Dept. Nat. Resources lisie.kitchel@wisconsin.gov

# **Information Exchange Newsletter**

John Jenkinson - Clinton, Tennessee jjjenkinson@hotmail.com

### **Information Exchange Journal**

W. Gregory Cope - North Carolina State University greg cope@ncsu.edu

Wendell R. Haag - U.S. Forest Service whaag@fs.fed.us

David J. Berg - Miami University bergdj@miamioh.edu

### **Mussel Status and Distribution**

Arthur E. Bogan – North Carolina State Museum of Natural Sciences

arthur.bogan@ncdenr.gov

John L. Harris - Arkansas State University omibob1@gmail.com

### Nominations

Leroy Koch - U.S. Fish and Wildlife Service leroy koch@fws.gov

### Outreach

Jennifer Archambault – N.C. State University <u>imarcham@ncsu.edu</u>

# Propagation, Restoration, & Introduction

Rachael Hoch – N.C. Wildlife Resources Comm.

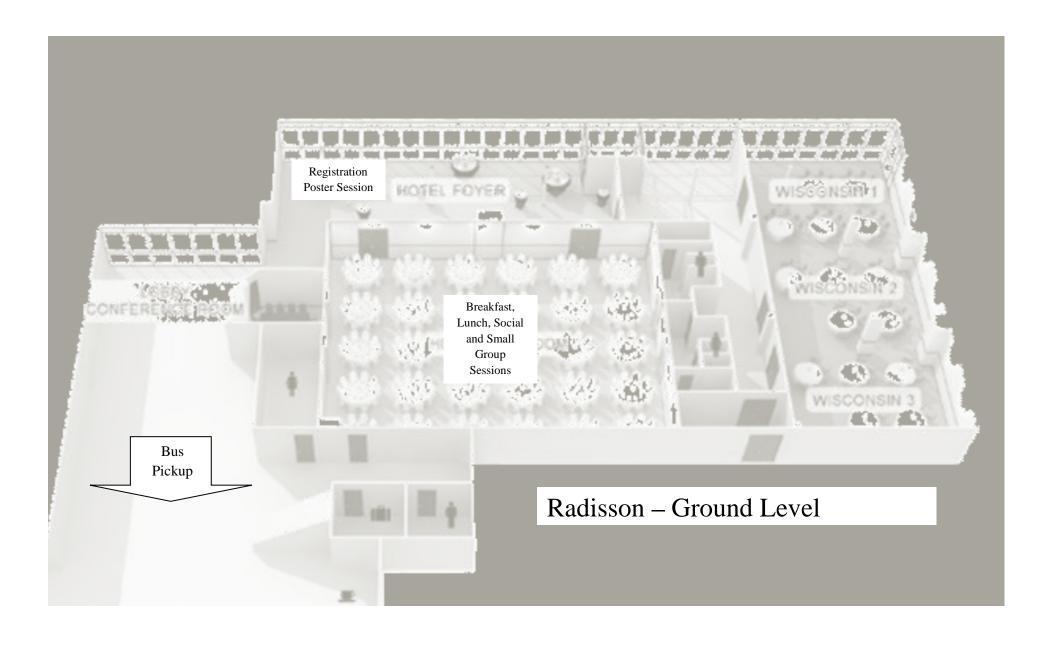
rachael.hoch@ncwildlife.org

Tim Lane- Virginia Dept.of Game & Inland Fisheries

tim.lane@dgif.virginia.gov

Symposium Jeremy Tiemann - Illinois Natural History Survey

itiemann@illinois.edu



The Freshwater Mollusk Conservation Society (FMCS) is dedicated to the conservation and advocacy of freshwater mollusks for public education and the conservation science of freshwater mollusks, North America's most imperiled fauna.

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# Schedule

Monday 3/12	Tuesday 3/13	Wednesday 3/14	Thursday 3/15
	Breakfast 7:30-8:00 am	Breakfast 7:30-8:00 am	Breakfast 7:30-8:00 am
	Welcome		
	8:00-8:10		
	The Uncertain State of Molluscan Health		Small Group Sessions
	Dr. Greg Cope and Diane Waller 8:10-8:40	Small Group Sessions 8:00-11:30	8:00-11:30
	Back to the Drawing Board: Assessing Causes of Freshwater Mussel Declines		1.1
	Wendell Haag 8:45-9:30	Lab session: group 1 (pink heelsplitters- red dot on	Lab session: group 3 (yellow sandshell- yellow
	Viruses of freshwater mussels (and other species): expanding the invertebrate "virosphere" in the pursuit of mussel health and conservation	nametag) 8:00am bus leaves Risk characterization	leaves  Risk characterization session: group 1 (pink heelsplitters- red dot on nametag)- Radisson ballroom n:
		session: group 2 (green	
	Break 10:00-10:15	floaters-green dot on nametag)- Radisson	
	An Overview of the Bacteriological Examination of Freshwater Mussels ballroom	ballroom	
	Eric Leis 10:15-10:45	rasites and Diseases Contributing to the Decline of Freshwater Mussels (Bivalvia, Unionida)?  group 3 (yellow sandshell-yellow dot on nametag)- Radisson ballroom green do	
	Are Parasites and Diseases Contributing to the Decline of Freshwater Mussels (Bivalvia, Unionida)?		group 2 (green floaters- green dot on nametag)-
	Andrew McElwain 10:45-11:15		Radisson ballroom
	Perspective from the Marine Realm: Contemporary Challenges and Approaches to Bivalve Mollusc Health Management		
	Ryan Carnegie 11:15-12:00		
	Plated Lunch 12:00-1:30	Groups 1 & 2 lunch provided, Group 3 lunch on own	Group 3 lunch provided, Groups 1 & 2 lunch on own

Monday 3/12	Tuesday 3/13	Wednesday 3/14	Thursday 3/15
	Overview of Health Assessment Tools in Native Freshwater Mollusks		
	Dr. Greg Cope and Dr. Teresa Newton 1:30-1:45		
	Omics	Small Group	
	Ieva Roznere 1:50-2:05	1:00-4:30	
	Use of Condition Indexes for Mussel Health Assessment	Lab session: group 2	
	Serena Ciparis 2:10-2:25	(green floaters-green	
	Hemolymph chemistry profiles and fatty acid analysis as tools for evaluating freshwater mussel health	dot on nametag) 12:00pm bus leaves	
	Andrea Fritts 2:30-2:45	Risk characterization	
	Influence of water and sediment on the digestive gland microbiome in the Alabama rainbow (Villosa nebulosa)	ballroom National Fish Die-offs and kills Hatchery,	
	Cova Arias 2:50-3:10		Tour of Genoa
	Break 3:10-3:25		National Fish
	Disease Risk Analysis – Applications for the Management of Fresh Water Mussels		Bus Leaves 1pm
	Tiffany Wolf 3:25-3:45		1:00-5:00
	Do the Infectious Diseases of Dreissenids Represent a Threat to North American Unionid Populations?	ballroom	
	Daniel Molloy 3:50-4:05		
	Invasive snails and their potential to serve as hosts for parasites in the Midwest		
	Greg Sandland 4:10-4:25		
	Factors Related to Growth Inhibition in Juvenile Mussels Exposed to Ambient Stream Conditions		
	Wendell Haag 4:30-4:45		
Registration Open	1999 Ohio River Mollusk Kill Assessment: The Gastropod Study	FMCS Board Meeting 5:00-7:00	
4:00-9:00	Janet Clayton 4:50-5:05		
	Freshwater mussel die-offs: insights from a compilation of known cases.		
	Jordan Richards 5:10-5:30		
	Poster Session and Social 7:00-9:00	Social and Jam Session 7:00-9:00	

# Speaker Biographies:

**Ryan Carnegie** is an Associate Research Scientist at Virginia Institute of Marine Science, Department of Environmental & Aquatic Animal Health. He received a BA in Biology from Rutgers University, MA in Marine Science from VIMS, and PhD in Marine Biology from the University of Maine. His research interests include parasitology and invertebrate pathology; shellfish genetics and molluscan population biology and host-parasite interactions.

**Dr. W. Gregory Cope** is a William Neal Reynolds Distinguished Professor in the Department of Applied Ecology at NC State University. He also serves as Department Extension Leader and Coordinator of the NC Agromedicine Institute. Dr. Cope's research interests are in aquatic toxicology, ecology, and physiology, and in the transport, fate, and effects of aquatic pollutants and other human-mediated stressors on freshwater mollusks and fish. His research utilizes sentinel aquatic organisms, biomarkers of exposure, effect, or susceptibility, or alternative toxicological models from which linkages to environmental and human health are evaluated. Major areas of research focus include the assessment of pesticides, persistent organochlorine contaminants, and metals in surface waters, the effects of waterborne and sedimentassociated contaminants on fish and native mollusks in inland waterways, the efficacy of constructed wetlands and other Best Management Practices for reducing non-point source pollution from urban (e.g., polycyclic aromatic hydrocarbons) and agricultural (e.g., nutrients) watersheds, and the effects of contaminant availability and cycling on imperiled species. Dr. Cope is a Charter Member of FMCS and has held numerous officer and committee positions in the Society, including President and Chair of the Awards Committee, and currently serves as Co-Editor of the Society's Journal, Freshwater Mollusk Biology and Conservation.

**Andrea Fritts** is a biologist with the USGS Upper Midwest Environmental Sciences Center. Her past research has included aspects of early life history, sclerochronology, and physiology of freshwater mussels, while her current research uses stable isotopes and fatty acids to study food web interactions. Andrea received her PhD from the University of Georgia.

**Tony Goldberg** is a professor of epidemiology at the University of Wisconsin-Madison, Department of Pathobiological Sciences School of Veterinary Medicine. He received a BA from Amherst College, a PhD from Harvard University, and DVM from University of Illinois. His research focuses on the ecology, epidemiology and evolution of infectious disease, combining field and laboratory studies to understand how pathogens in dynamic ecosystems are transmitted among hosts, across complex landscapes, and over time.

Wendell Haag is a Research Fisheries Biologist with the U.S. Forest Service, Southern Research Station. His research on freshwater mussels spans 30 years and has explored a variety of topics, including life history, fish-host relationships, age and growth, population and assemblage dynamics, biogeography, and conservation issues. He has authored over 50 peer-reviewed papers, book chapters, and books, including *North American Freshwater Mussels: Natural History, Ecology, and Conservation* (2012). His current research focuses on identifying causes and mechanisms of mussel declines. He has served as co-editor of *Freshwater Mollusk Biology and Conservation* since 2012 and was Associate Editor of *Freshwater Science* from 2009 to 2015.

**Susan Knowles** is a diagnostic pathologist at the USGS National Wildlife Health Center, Madison, WI. She received a BA from Bucknell University, DVM from Virginia-Maryland Regional College of Veterinary Medicine, and PhD from the University of Georgia. Her research interests include wildlife and aquatic animal pathology, including bivalves and novel virus discovery.

**Eric Leis** is a fish biologist at the UFWS La Crosse Fish Health Center. He studies parasite infections in fish and the morphological and genetic identification of these parasites. He also develops cell lines from fish and amphibians applied in the fields of virology and toxicology.

**Ieva Roznere** is a postdoctoral researcher at The Ohio State University. She is a physiological ecologist researching how environmental stress affects freshwater mussel metabolism using metabolomic and transcriptomic techniques.

Heidi Dunn is a malacologist, with EcoAnalyst (formerly Ecological Specialists). She holds a BS in Wildlife Science from Purdue University and a MS in Biology from Southern Illinois University at Edwardsville, IL. She was introduced to freshwater mussels in 1978 while working as a summer technician with USFWS, and started sampling and working with other malacologists in the Mississippi River basin in the 1980s. She has been working with these animals ever since and never ceases to be amazed at their diversity, beauty, and behavior. She has conducted over 600 mussel study designs, surveys, relocations, Biological Assessments, and workshops since founding Ecological Specialists in 1990. She has sampled throughout the interior basin, on the Atlantic coast and down to Texas. She is also currently an instructor for USFWS Conservation of Freshwater Mussel Biology at the National Conservation Training Center in Shepherdstown, WV and President of the Freshwater Mollusk Conservation Society.

**Dr. Teresa J. Newton** is a fishery biologist with the U.S. Geological Survey's Upper Midwest Environmental Sciences Center in La Crosse, WI. She received a B.S. in Biology in 1985 from Central Michigan University, a M.S. in Biology in 1987 from Tennessee Technological University, and a Ph.D. in Fisheries Biology and Toxicology in 1990 from Iowa State University. She has been employed at the USGS lab since 1990 where her research interests focus on the conservation and ecology of freshwater mussels, a group of benthic animals in which 70% of the North American species are considered threatened. Mussels are keystone species in many rivers and their catastrophic decline may lead to the decline of other faunal groups and the alteration of ecosystem processes. She uses a combination of comparative and experimental approaches to understand factors affecting the distribution and abundance of freshwater mussels, identify biochemical and physiological indicators of stress, and determine the roles that mussels play in large river food webs. Collectively, these studies provide information to help resource managers design effective restoration and conservation strategies for native freshwater mussels.

Andrew McElwain is a visiting assistant professor at The State University of New York, Oswego. He received a BS from Westfield State College, MS from Middle Tennessee State University, and PhD from Auburn University. He uses light and scanning electron microscopy to study tissue structure of fishes and freshwater mussels to better understand how cells, tissues and organs functions. He is especially interested in histopathology of parasitic infections.

**Dan Molloy** is a freshwater invertebrate pathologist conducting fundamental research on the diseases of North American and Eurasian populations of *Dreissena* spp. With a passion for environmental protection, his research also includes developing target-specific, pathogen-based methods for managing these mussels. He is, for example, the patent inventor of using the bacterium *Pseudomonas fluorescens* strain CL0145A as a highly-specific control agent against these mussels – the approach used in the commercial product Zequanox<sup>®</sup>. A Research Scientist and Adjunct Professor at the State University of New York Great Lakes Center at Buffalo, he also maintains affiliations with the State University of New York at Albany and the University of Illinois Natural History Survey at Urbana-Champaign. In addition, he directs Molloy & Associates, LLC – a firm specializing in developing credible prevention, detection, rapid response, and eradication/control programs for *Dreissena* spp.

**Dr. Nick Phelps** is the Director of the Minnesota Aquatic Invasive Species Research Center and Assistant Professor in the Fisheries, Wildlife and Conservation Biology Department at the University of Minnesota. His research focuses on emerging threats to the health and sustainability of aquatic ecosystems at the intersection of humans, animals and the environment. He aims to identify threats, mitigate risks and develop long-term science-based management solutions to meet the needs of all stakeholders involved.

Tiffany Wolf is an assistant professor in the Veterinary Population Medicine Department of the University of Minnesota (UMN) College of Veterinary Medicine; she also holds an appointment as an Associate Fellow at the UMN Institute on the Environment. With a background in wildlife epidemiology and ecosystem health, she maintains a broad interest in understanding diseases of wildlife populations at the interface of humans, animals, and the environment. She is particularly interested in the dynamics of infectious diseases in multi-host pathogen systems and optimizing epidemiological, ecological, and molecular approaches to assess changes in health and mitigate disease impacts on populations. Her work entails working across scientific disciplines to find solutions to complex health problems. Current projects include understanding the role of health in a declining moose populations, *Parelaphostrongylus tenuis* and *Mycobacterium tuberculosis* complex transmission in multihost systems, human-primate disease transmission, wildlife disease surveillance, and contaminants of emerging concern in aquatic ecosystems.

TUESDAY MARCH 13, 2018		
7:00 – 8:00 a.m.	CONTINENTAL BREAKFAST	
8:00 – 8:10 a.m.	WELCOME AND ANNOUNCEMENTS	
8:10 – 8:40 a.m.	THE UNCERTAIN STATE OF MOLLUSCAN HEALTH Diane L. Waller & Dr. W. Gregory Cope	

Assessing the health of native freshwater mussels and snails, whether at the population or organismal level, is difficult because of the lack of established benchmarks by which to judge what is considered suitable or normal. Moreover, the lack of development of techniques, assays, and diagnostic tools that are critical in assessing the relative health and condition of mollusks has been hampered by concerted research efforts and limited financial resources. In this presentation, we outline the goals and objectives of the 2018 FMCS Freshwater Mollusk Health Workshop and summarize the history of progress in this topic area since the first workshop on freshwater mussel health and die-offs was held in 1986. Additionally, we will provide an overview of some of the existing and emerging health assessment tools that will be discussed in more detail throughout the workshop. We assert that a world-wide effort should be made in the areas of freshwater molluscan health and disease diagnosis and treatment, similar to those that currently exist for fish health and disease. As a greater number of state and federal fish hatcheries are undertaking mollusk propagation and culture programs for restoration and other efforts, it will be critical to understand the health of mussels held in captivity as well as those in the wild.

	8:45 – 9:30 a.m.	BACK TO THE DRAWING BOARD: ASSESSING CAUSES OF FRESHWATER MUSSEL DECLINES
l		Wendell Haag

Freshwater mussel declines are on par with declines in pollinators, amphibians, bats, and other organisms but have received much less attention. Many mussel declines share the following characteristics: 1) commenced about 1970–1990; 2) affect entire assemblages; 3) curtailment of recruitment, followed by gradual faunal loss; 4) occurrence in otherwise intact streams; 5) upstream progression in some cases. Conventional explanations have three main characteristics: 1) focus on long-term, cumulative factors, such as sedimentation; 2) include multiple, unrelated factors, invoked to varying degrees; 3) causal factors often vague (e.g., "poor land use practices") and mostly untested. These explanations do not correspond well to characteristics of recent declines. Rather, the similarity in characteristics and timing of declines suggests a single, severe factor specific to mussels acting rapidly across a large area. Vague and untested assumptions about causes provide little guidance for conservation and may have directed resources unproductively. I propose that mussel conservation be refocused along the following three points. 1) Largely discard conventional wisdom about causes of declines until it is evaluated critically. 2) Build a body of case studies that provide rigorous evaluation of the success of conservation strategies. Evaluation should emphasize short-term response variables such as individual condition or performance and deemphasize assemblage- and population-level responses, which occur slowly. 3) Increased emphasis on research and adaptive management that can identify specific causes of mussel declines. Previously ignored, but potentially widespread factors such as disease and the effects of invasive *Corbicula fluminea* should be revisited.

9:30 – 10:00 a.m.

# VIRUSES OF FRESHWATER MUSSELS (AND OTHER SPECIES): EXPANDING THE INVERTEBRATE "VIROSPHERE" IN THE PURSUIT OF MUSSEL HEALTH AND CONSERVATION

Tony Goldberg, Chris Dunn, Jordan Richard, Diane Waller, Eric Leis & Joel Putnam

Recent advances in metagenomic technology have revealed a remarkable diversity of heretofore unknown viruses in invertebrates and their environments. As this "virosphere" continues to expand, it will become increasingly important to understand what the presence or a virus (or multiple viruses) means for population health and conservation. This talk presents results of several "virus hunting" efforts that have used these emerging technologies to identify the causes of population declines across multiple species. Data show that many species have a "normal" or "typical" virome consisting of a tractable number of endemic and apparently non-pathogenic viruses. As a consequence, defining the "baseline" virome of a species is critical for documenting changes, such as introduction of a new virus or enhanced replication of an endemic virus. Challenges to these approaches include the management of "big data," choosing appropriate bioinformatic methods, distinguishing "real" viruses from viruses that have integrated into host genomes, and interpreting the clinical significance of results. In the case of freshwater mussels, these problems are amplified by the extreme divergence of the novel viruses we have found from their known relatives, as well as limited comparative data from other species. Nevertheless, this overall approach has great potential to elucidate the role of viruses in freshwater mussel health and conservation. A recent die-off event in the Clinch River, Tennessee, will be used to illustrate these principles.

Resources:

Redefining the Invertebrate Virosphere available at https://molluskconservation.org/Library/pdf/MARCH2018/nature20167.pdf with password FMCS\_workshop\_2018

# Morning Break 10:00 - 10:15 a.m.

10:15 - 10:45 a.m.

BACTERIOLOGY

Eric Leis

The diagnosis of bacterial disease in freshwater mussels is greatly hindered by a lack of information regarding the microbial communities associated with these animals. This makes identification of the causative agent(s) during mussel mortality events difficult, if not nearly impossible. A necessary initial step is the documentation of the normal bacterial flora. This basic information is essential to provide much needed insight into these assemblages; potentially resulting in the identification of pathogens. While there have been a few studies focused on the collection of this data, seasonally examining Unionid populations throughout geographically widespread locations will be necessary to properly evaluate bacterial species associated with mussels. The identification of these communities will also require careful examination as there are many aspects of sampling freshwater mussels which can make the interpretation of results challenging. Bacteria are a typical dietary component for many mussels and their presence may simply represent a food source, not necessarily indicating either a symbiotic or pathogenic relationship. Additionally, the close contact of the internal organs to the aquatic environment, which increases the likelihood for sample contamination, can further complicate interpretation. In an effort to examine the bacterial communities associated with Unionids in the Upper Mississippi River (UMR) basin, we cultured bacteria from the hemolymph of several mussel populations throughout the 2017 field season. Molecular methods targeting the 16S rRNA gene were used to identify the isolates. Hemolymph was selected due to its relative disconnect with the aquatic environment and role in the circulatory system. The most commonly cultured bacteria from mussels in the UMR were identified as belonging to the genus *Bacillus*. In October 2017, additional bacterial isolates were identified from hemolymph samples acquired during a mortality event of pheasantshells (Actinonaias pectorosa) on the Clinch River near Kyles Ford, Tennessee. These isolates were then compared to the bacteria obtained from mussels in the UMR which revealed that both mussel populations shared many common bacteria as well as some differences. Future bacteriology investigations will improve our understanding of the microbial communities associated with freshwater mussels, allowing us to identify those bacterial species capable of causing disease, and ultimately lead to improved Unionid restoration efforts.

10:45 - 11:15 a.m.

# ARE PARASITES AND DISEASES CONTRIBUTING TO THE DECLINE OF FRESHWATER MUSSELS (BIVALVIA, UNIONIDA)? Andrew McElwain

Freshwater mussels or pearly mussels (Mollusca: Bivalvia: Unionida) comprise approximately 843 nominal species in six families worldwide, but nearly half of these species are imperiled. Mussels have a unique life history starting with a parasitic larva that attaches to skin or gill of a fish for several days before transforming into a sedentary suspension feeder in the benthos. Gravid females brood their larvae in their gills for several weeks or months before releasing them, and many species have intriguing host attraction strategies to ensure the attachment of their larvae. This life history renders mussels vulnerable to toxic contaminants and the loss of benthic sediments, which are significant causes of mussel declines. Etiological agents have the potential to be contributing factors, but parasites and diseases of freshwater mussels remain understudied relative to marine bivalves. Mussels may host a wide variety of eukaryotic organisms including ciliates (Ciliophora), trematodes (Platyhelminthes: Aspidogastrea, and Digenea), roundworms (Nematoda), "moss animals" or bryozoans (Ectoprocta, Entoprocta), oligocheates (Annelida: Oligocheata), leeches (Annelida: Hirudinida), mites (Arthropoda: Acari), copepods (Arthropoda: Copepoda), insects (Arthropoda: Insecta), and fish eggs (Chordata: Actinopterygii). Some of these groups contain species that are considered parasitic because they appear to injure their host through attachment, feeding or otherwise invade tissue (e.g., digeneans). The literature also contains many observations of small organisms living in the mantle cavity or attached to the shell in which the relationship between the two parties is unclear (e.g., oligochaetes). Regarding diseases, there are a few published accounts of mass mortality events known as "die-offs" and "mussel kills", and only one of these documents gross and histopathological changes to tissues. Additionally, there are some observations of tumors or neoplasms, and several accounts of shell anomalies such as parasite-induced pearl formation, and deformities such as infoldings and protuberances of the periostracum, erosion to the nacre and or periostracum, and misshapened shells. The cause of diseases and shell deformities has rarely been established and more often subject to speculation. With the above in mind, we are facing a significant biodiversity crisis and it is the intention of this review to highlight the need to understand the health of wild and captive mussels.

11:15 - 12:00 a.m.

# PERSPECTIVE FROM THE MARINE REALM: CONTEMPORARY CHALLENGES AND APPROACHES TO BIVALVE MOLLUSC HEALTH MANAGEMENT

Ryan B. Carnegie

Understanding and managing health and disease in freshwater mussels can be informed by long experience in shellfish pathology in marine systems. Marine bivalve molluscs are infected by myriad microbial pathogens, the most notable of which have usually been protozoan but with bacteria also contributing and viral pathogens causing acute contemporary concern. Several of these pathogens, representing major protozoan lineages, are notifiable to the World Organisation for Animal Health. For many of these pathogens, devastating initial effects on shellfish populations and economies dependent on them were followed by success in control, for example through natural selection or selective breeding for disease resistance. Enlightened management includes strong elements of conservation, such as the use of sanctuaries from harvest to promote natural selection for resistance evolution in oyster populations. Yet many challenges remain. Disease surveillance in many areas for many mollusc species is inadequate, detection of emerging bacterial and viral pathogens in particular is problematic, basic life history knowledge for many pathogens is lacking. Difficulty in meeting these challenges is particularly troublesome given the rapid growth of shellfish aquaculture, which may promote disease emergence by creating new interactions between pathogens and host populations that have no adaptation to them.

As aquaculture production expands around the world, managing the health of aquacultured populations, including preventing undesired disease interactions with wild animal populations, will increasingly be a challenge. Despite best efforts to maintain biosecurity, widening commerce alters distributions of pathogens and hosts, producing outbreaks of established diseases and the emergence of new ones as noted above. Anthropogenic impacts on the marine environment including ocean warming contribute to shifting host and pathogen distributions and introduce other possible influences on disease processes, from harmful algal blooms and ocean acidification to organic pollutants and microplastic contamination—all of which can affect freshwater environments as well. In this evolving environmental landscape, the path to more effective management of animal health in aquaculture is not fully clear. Application of contemporary genetic methods for pathogen detection and discovery such as next-generation sequencing has garnered recent attention as part of the solution. Regardless of available diagnostics, however, disease occurrence remains a product of host and environmental factors in addition to those associated with the pathogen. Thus meeting the challenges of protecting aquaculture systems in a changing world will require incorporation of broader expertise into aquatic animal health, including chemistry and toxicology, physiology, genetics, oceanography, and ecology in addition to traditional areas of parasitology, microbiology, and virology to better understand more complex and multifactorial etiologies. Capacity to explore disease-associated factors in controlled experiments will remain essential. Fundamental to maintaining capacity for understanding disease in aquatic ecosystems will be societal commitment to broad-based science programs in support of the goal of aquaculture to support a growing human population in the 21st century, but also to maintain the ecological integrity of aquatic ecosystems worldwide.

Plated Lunch 12:00 – 1:30 p.m.

1:30 – 1:45 p.m.

OVERVIEW OF HEALTH ASSESSMENT TOOLS IN NATIVE FRESHWATER MOLLUSKS

Dr. W. Gregory Cope & Dr. Teresa J. Newton

The study of freshwater mollusks has advanced substantially in many topical areas (e.g., propagation, toxicology, ecology) over the past 25 years. Although these advances have propelled the science of mollusk conservation forward, areas such as physiology, immunology, and basic biochemistry have received much less attention, largely due to limited financial resources and few investigators conducting research in these areas. The deficit of advances in these particular areas has hampered the development of tests, assays, and diagnostic tools that are critical in assessing the relative health and condition of mollusks. To address the continued questions about mollusk health, as well as to assess the new issues of disease, unexplained die-offs, and emerging contaminant exposures, improved tools and techniques are needed. Here we provide an overview of the major categories of existing tools (i.e., biomarkers) used to assess mollusk health and make inference to those requiring additional research and development. For our purposes, a biomarker is a change in a biological response at the molecular, cellular, biochemical, physiological, or behavioral level that can be related to exposure, effects, or susceptibility. Although highly useful and informative in assessing relative condition of mollusks and other organisms, connections between biomarker responses and tangible outcomes for characterizing "good health" will be imperative to future mollusk conservation and protection efforts.

Resources:

Newton and Cope, Chapter 10 Biomarker Responses of Unionid Mussels to Environmental Contaminants pdf available at https://molluskconservation.org/Library/pdf/MARCH2018/Newton\_Cope-Ch10.pdf with password FMCS\_workshop\_2018

1:50 – 2:05 p.m.

**OMICS** Ieva Roznere

What is omics? How has it been used in research on mollusks? Can these techniques be applied to improve conservation and management efforts? This presentation will address these questions with a focus on transcriptomics and metabolomics. Omics refers to fields of study that aim to detect and characterize a group of biological molecules in an organism, such as genes (as in the field of genomics), transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics). Transcriptomics is the study of transcripts, the subset of genes that are being expressed at a certain time period. Metabolomics is the study of metabolites, the intermediates and products of metabolism that are produced by enzymatic chemical reactions. Because gene expression and metabolite production are closely associated with environmental conditions, studying changes in these biological molecules is especially helpful in understanding how organisms react to different environmental stressors. For example, transcriptomics and metabolomics have been used to identify stress-responses of freshwater mussels to heat, drought, pollutants, and translocation. The different fields of omics can be used as health assessment tools to provide snapshots of molluscan physiology at various levels of biological organization. Although still in its infancy, the application of omics techniques in the management of species of concern holds great potential for advancing the field of conservation biology.

2:10 – 2:25 p.m.

# USE OF CONDITION INDEX FOR MUSSEL HEALTH ASSESSMENT

Serena Ciparis, Ty Stephenson, Garrett Rhyne & Susan Lingenfelser

Energy storage and allocation are critical metrics of freshwater mussel health due to direct relationships to survival, growth, and reproductive capability. Condition indices (CIs) assess energy storage, or 'fatness', by normalizing weight to size. Crosby and Gale (1990) recommended a standard method for determining bivalve CI, where standard CI = dry soft tissue weight (g) \*1000/ internal shell cavity capacity (g). However, this method is rarely applied in studies of freshwater mussels. We compared eight potential methods for determining CI of adult *Lampsilis fasciola* exposed to clean (pond) water, clean (pond) sediment, elevated major ion concentrations (simulated Powell River water) and coal-contaminated sediment (Powell River sediment) in a 2x2 factorial design. Calculated CIs included two methods for live mussels (ratios of weight to length and weight to volume) and six methods for dissected mussels (ratios of tissue wet weight and tissue dry weight to shell length, cavity volume, and cavity weight). Glycogen content of mantle tissue and activities of enzymes associated with the glutathione antioxidant system in digestive gland tissue were also determined. There were no statistically significant differences between treatments for CIs calculated using live mussels or using tissue wet weight of dissected mussels. However, all CIs calculated using tissue dry weight indicated statistically significant differences between water treatments, with the standard CI yielding the most precise measurements. Glycogen content of mantle tissue was also significantly different between water treatments for male mussels only. We hypothesize that exposure to major ions caused mussels to utilize energy stores to compensate for osmotic stress, with males utilizing glycogen and females utilizing lipids from oocyte resorption. Differences between treatments would not have been detected without dissection, emphasizing the utility of the standard CI and the need for correlation with non-lethal endpoints. Results of other studies

A Review and Evaluation of Bivalve Condition Index Methodologies with a Suggested Standard Method is available at https://molluskconservation.org/Library/pdf/MARCH2018/Crosby\_Gale\_1990.pdf with password FMCS\_workshop\_2018

2:30 - 2:45 p.m.

# HEMOLYMPH CHEMISTRY PROFILES AND FATTY ACID ANALYSIS AS TOOLS FOR EVALUATING FRESHWATER MUSSEL HEALTH

Andrea Fritts, Robert Bringolf, Brent Knights, Lynn Bartsch & Michelle Bartsch

Since 1999 the Canadian waters of the St Clair delta have been known to possess a rich and diverse freshwater mussel fauna including many species which have since been listed under the Canadian *Species at Risk Act*. As these waters are found in the relatively undisturbed wetlands of Walpole Island First Nation it was hoped that they would act as refuge for many species from the impacts of dreissenids and other pollutants. In 2003, Environment Canada established a network of index monitoring sites within this area and these sites were resampled by Fisheries and Oceans Canada in 2011 and again in 2016 to investigate the status of this refuge. Sites were sampled by snorkelling along predefined transects until a mussel was detected. Once detected, the location was marked and a circular plot (65 m²) was searched with the location of the animal at the centre of the plot. All animals were collected, identified, measured and all dreissenid mussels were enumerated and removed. Each transect was searched until 10 plots were assessed or until the transect reached the shore. Dreissenid burden (# of dreissend mussels/unionid) has declined steadily over the study period at all sites while unionid density (#/m²) within the circular plots initially declined between 2003 and 2011 but has rebounded in 2016 to equal or exceed 2003 levels at most sites. However, unionids have become more patchy with the average number of plots/transect declining from 26 in 2003 to only 14 in 2016 resulting in an overall reduction in the number of unionids found within the study area from 814 in 2003 to 674 in 2016 (18% decline). Although overall species richness has remained relatively stable between 2003 (19 species) and 2016 (18 species) there has been a shift in dominance with *Lampsilis siliquoidea* representing 66% of all individuals in 2016 compared with only 33% in 2003. Four of the seven species at risk found in 2003 were not detected in 2016 calling into question the potential for the delta to act as an ongoing refuge for many species.

2:50 – 3:10 p.m.

# INFLUENCE OF WATER AND SEDIMENT ON THE DIGESTIVE GLAND MICROBIOME IN THE ALABAMA RAINBOW (VILLOSA NEBULOSA)

Alison K. Aceves, Cova R. Arias, Paul Johnson, and Stephen A. Bullard

Freshwater mussels (Bivalvia: Unionidae) are the most imperiled faunal group in North America. Alabama harbors the highest biodiversity of freshwater mussels in the world and currently leads restoration efforts across the Southeast. In collaboration with the Alabama Department of Conservation and Natural Resources, we are investigating the role of bacterial communities, using 16S rRNA gene sequencing, found in cultured and in-stream mussels and how factors such as water and sediment influence these communities. We collected a total of 22 Alabama rainbows (Villosa nebulosa) from the Alabama Aquatic Biodiversity Center reared from two different wild stocks (Shoal Creek = 5 and Flannigan Creek = 5) and collected in-stream (Shoal Creek = 10, Flannigan Creek = 2). Overall, the microbial communities between hatchery-reared and wild mussels were significantly different. Rearing environment exerted a stronger effect than population although differences were noted between mussels collected from Shoal Creek and Flannigan Creek. The most dominant phylum from cultured V. nebulosa was Tenericutes (>43.3%), followed by Proteobacteria (25.3%), Firmicutes (12.6%), Cyanobacteria (8.3%), and Bacteroidetes (3.1%). The top five phyla from in-stream Shoal Creek V. nebulosa were Tenericutes (36.4%), Chlamydiae (18%), Proteobacteria (17.3%), Bacteroidetes (9.2%), and Firmicutes (7.1%); however the top five phyla from in-stream Flannigan Creek V. nebulosa were Proteobacteria (44.5%), Bacteroidetes (17.3%), Firmicutes (10.2%), Acidobacteria (6.5%), and Actinobacteria (6.1%). Analysis of similarity percentages showed that the most abundant genus in cultured Shoal Creek mussels was *Ureaplasma* sp. (41.4%), whereas, in cultured Flannigan Creek mussels Spiroplasma sp. (23.5%) was the most abundant genus, both belonging to the phylum Tenericutes. Results from multidimensional scaling and analysis of similarity showed that water and sediment have no influence on the diversity of gut bacterial communities found in mussels. These results collectively show that novel microbial communities exist between cultured and in-stream freshwater mussels and that their gut bacterial communities may be independent from the influence of environmental factors.

# Afternoon Break 3:10 – 3:25 p.m.

3:25 – 3:45 p.m.

Disease Risk Analysis – Applications for the Management of Fresh Water Mussels

Tiffany Wolf, Phil Miller, Alex Primus & Dominic Travis

Disease risk analysis (DRA) is a structured, evidence-based process that helps decision making in the face of uncertainty by characterizing the potential impact of infectious and non-infectious diseases on ecosystems, wildlife, domestic animals and people. Since 1992 the Conservation Planning Specialist Group (CPSG, previously the Conservation Breeding Specialist Group) of the IUCN Species Survival Commission (IUCN-SSC) has been facilitating collaboration between experts in zoo and wildlife veterinary medicine, disease ecology and population management to develop a set of tools for analysis of wildlife disease risks. In 2010, CPSG, in partnership with three other IUCN-SSC specialist groups (Wildlife Health, Reintroduction and Invasive Species), undertook a global needs analysis survey in this area. The results demonstrated that wildlife disease concerns are global, broad in scope and involve a wide diversity of people from multiple disciplines. The resulting Manual of Procedures for Wildlife Disease Risk Analysis built upon a large body of work on disease risk analysis, in particular that of the World Organisation for Animal Health (OIE), and extends this to biodiversity conservation. The University of Minnesota College of Veterinary Medicine's Risk Analysis Unit (https://www.cahfs.umn.edu/risk-analysis-service) brings together expertise and resources across the University of Minnesota to develop science and risk-based tools to meet new challenges in the areas of animal health, food safety and wildlife. In partnership, CPSG and UMN have developed a mini workshop series aimed at helping conservationists, animal resource managers and industry professionals integrate science and policy in order to frame, characterize and manage health risks using these international standards. Participation in this workshop will help managers and policy makers: transition health policy problems into a scientific riskbased framework; organize and communicate with diverse stakeholder working groups; design a structured approach to identifying and prioritizing disease hazards; develop a first iteration conceptual risk assessment model.

3:50 – 4:05 p.m.

DO THE INIFECTIOUS DISEASES OF DREISSENIDS REPRESENT A THREAT TO NORTH AMERICAN UNIONID POPULATIONS? Daniel P. Molloy

The spread of dreissenids (zebra and quagga mussels) across North America has had a significant negative impact on unionid populations. High densities of dreissenids settling on their shells often lead to the death of unionids, resulting in severe population declines. But is there any evidence that these invasive mussels might also represent an additional threat to unionids due the parasites they carry? This presentation will examine what parasites dreissenids have been documented to carry in North America and in their native Eurasia and what evidence there is that these parasites are crossing over to infect unionids on these continents.

4:10 – 4:25 p.m.

INVASIVE SNAILS AND THEIR POTENTIAL TO SERVE AS HOSTS FOR PARASITES IN THE MIDWEST Gregory J Sandland

Abstract: Invasive species have important impacts on the ecology and economics of regions across the world. Snails are an important group of aquatic invaders because they have the potential to transmit parasites that are problematic for both wildlife and people. As part of this presentation I will 1) highlight some of the key invasive snails found in freshwater systems throughout the Midwest and 2) outline their potential to serve as hosts for flatworm parasites in the region.

4:30 – 4:45 p.m.

FACTORS RELATED TO GROWTH INHIBITION IN JUVENILE MUSSELS EXPOSED TO AMBIENT STREAM CONDITIONS. Wendell R. Haag, Jacob Culp, Monte McGregor, Steven Price & Lesley Sneed

In a previous study, caged juvenile mussels survived but did not grow in streams that have previously lost their mussel fauna; growth was as much as two orders of magnitude higher in streams that continue to support mussels. Growth inhibition was associated with agricultural contaminants (pesticides, nitrates) in some streams but not in others, and no potential causal factor was common to all streams. We measured growth of hatchery-reared juvenile mussels exposed to ambient conditions in 10 streams, five that previously showed growth inhibition and five that did not. We examined other potential causal factors and other measures of performance associated with growth inhibition. Diatom assemblages showed a geographical pattern but no relationship to growth. Microbe assemblages showed no pattern related to geography or growth; these results do not support differences in food resources as explanations for low growth. C and N stable isotope signatures showed that mussels with growth inhibition assimilated available food resources to a lesser extent than mussels with normal growth. Mussels that showed growth inhibition had a distinctive metabolomic profile in which citric acid cycle activity and amino acid metabolism were greatly reduced, but fatty acid oxidation was elevated; these traits distinguished those individuals reliably from individuals with normal growth. This profile likely reflects a starvation response in which lipids are catabolized to provide energy in the absence of energy normally produced via the citric acid cycle. The factors responsible for this metabolomic response and failure to assimilate available food resources are unknown at this time.

4:50 – 5:05 p.m.

# 1999 OHIO RIVER MOLLUSK KILL ASSESSMENT: THE GASTROPOD STORY.

Janet L. Clayton & Patricia A. Morrison

An extensive mollusk kill occurred on the Ohio River in 1999 resulting from a toxic release of hexavalent chromium and the treatment thereof with a molluscicide compound. The kill originated in the Belleville Pool below Marietta, OH. The kill assessment was conducted by Ecological Specialists and consisted of semi-quantitative transects and excavated quadrats at five known mussel beds below the alleged discharge. Impacts were observed nearly 30 miles downstream. While the primary objective was to assess the unionid kill, the quadrat excavations provided an opportunity to also assess the impact to the snails. The dominant snail observed was *Pleurocera canaliculata* with three additional species observed. It was believed that 100% mortality of unionids occurred at the most upstream assessed bed, and total mortality at the five beds was estimated at 870,000 individuals; approximately 11 million gastropods died at those sites alone. Losses in between and beyond those sites are immeasurable. Both unionid and gastropod recovery has been monitored over the past 18 years. Snails were much slower to rebound and whereas the population was primarily made up of *P. canaliculata* prior to the kill it is now composed of *P. canaliculata* and *Lithasia verrucosa*. An explosion of *Birgella subglobosus* occurred in 2007 but has only barely persisted since. Episodic settlement and growth of zebra mussels continue to threaten the full recovery of both the unionids and gastropods.

5:10 – 5:30 p.m.

# FRESHWATER MUSSEL DIE-OFFS: INSIGHTS FROM A COMPILATION OF KNOWN CASES

Jordan Richard

In 1986 a symposium was conducted to address the emerging threat of unexplained mass die-offs of freshwater mussels in the United States. Although the symposium brought significant attention to the topic at the time, publications and records of die-offs in subsequent years are rare. As part of an effort to focus the investigation of the ongoing die-off of freshwater mussels in the Clinch River, TN, we attempted to review data and conclusions from all documented die-off events. We issued calls for information through both the unio listserv and the FMCS mailing list. Including the data from the 1986 workshop, our compilation presently contains 48 cases of varying magnitude from seven countries. The cases include a number of events with known causes, but also include many mass die-offs that went entirely unexplained. Based on significant spatial and temporal biases in the cases reported, we believe the list is largely incomplete, and are actively seeking additional responses. Here, we discuss some of the most significant and well-documented cases, and share evidence of insights derived from commonalities in the case reports. Among these, we find evidence that: 1) Die-offs driven by various mechanisms leave unique "signatures" that can guide investigations based on simple, inexpensive analysis of an in-progress die-off; 2) Die-offs often persist over multiple years; and 3) Many of the populations affected by die-offs have never recovered.

Poster Session & Social 7:00-9:00

WEDNESDAY MARCH 14, 2018			
7:30 – 9:30 a.m.	CONTINENTAL BREAKFAST		
8:00 – 11:30 a.m. 12:00 – 4:30 p.m.	LABORATORY SESSION: pp.19 8:00AM BUS LEAVES GROUP 1(PINK HEELSPLITTERS- red dot on nametag) 12:00PM BUS LEAVES AT GROUP 2(GREEN FLOATERS-green dot on nametag)	Expert Leads: Eric Leis, Corey Puzach, Katie Bockrath, Susan Knowles, Andrew McElwain	
8:00 – 11:30 a.m. 1:00 – 4:30 p.m.	RISK CHARACTERIZATION SESSION: pp. 21 8:00AM- GROUP 2 (GREEN FLOATERS-green dot on nametag) 1:00PM- GROUP 3 (YELLOW SANDSHELL- yellow dot on nametag)	Expert Leads: Tiffany Wolf, Alex Primus and Phil Miller Facilitator: Megan Bradley	
8:00 – 11:30 a.m. 1:00 – 4:30 p.m.	DIE-OFFS AND KILLS SESSION: pp. 20 8:00AM - GROUP 3 (YELLOW SANDSHELL- yellow dot on nametag) 1:00PM- GROUP 1(PINK HEELSPLITTERS- RED DOT ON NAMETAG)	Expert Leads: Heidi Dunn, Dr. Nick Phelps & Jordan Richards Facilitator: Diane Waller	
Social & Jam Session 7:00-9:00			

THURSDAY MARCH 15, 2018			
7:30 – 9:30 a.m.	CONTINENTAL BREAKFAST		
8:00 – 11:30 a.m.	LABORATORY SESSION: pp.19 8:00AM BUS LEAVES GROUP 3 (YELLOW SANDSHELL- yellow dot on nametag)	Expert Leads: Eric Leis, Corey Puzach, Katie Bockrath, Susan Knowles, Andrew McElwain	
8:00 – 11:30 a.m.	RISK CHARACTERIZATION SESSION: pp. 21 8:00AM- GROUP 1(PINK HEELSPLITTERS- red dot on nametag)	Expert Leads: Tiffany Wolf, Alex Primus and Phil Miller Facilitator: Megan Bradley	
8:00 – 11:30 a.m.	<b>DIE-OFFS AND KILLS SESSION:</b> pp. 20 8:00AM- GROUP 2 (GREEN FLOATERS-green dot on nametag)	Expert Leads: Heidi Dunn, Dr. Nick Phelps & Jordan Richards Facilitator: Diane Waller	
1:00 – 5:00 p.m.	FIELD TRIP TO GENOA NATIONAL FISH HATCHERY		

# **Laboratory Session Outline**

# Eric Leis, Corey Puzach, Katie Bockrath, Susan Knowles, Andrew McElwain

- I. Introduction-Microscope use
- II. Mussel Anatomy
  - A) Overview of mussel organs and tissues
    - 1. Mantle
    - 2. Gills and glochidia
    - 3. Labial palps
    - 4. Integument of the visceral mass
    - 5. Foot
    - 6. Digestive gland
    - 7. Stomach
    - 8. Crystalline style sac
    - 9. Intestine
    - 10. Gonads
    - 11. Nephridium
    - 12. Heart
    - 13. Hemocytes and hemolymph
    - 14. Ganglia and nerves
    - 15. Shell damage and shell deformities
- III. Histological samples
  - A) Proper fixation and storage of mussels for histology
    - 1. Prop shell open with wooden dowels
    - 2. Cut adductor muscles
    - 3. Remove adductor and mantle from one side of the shell
    - 4. Long term storage in ethanol
    - 5. Comparing fixation/preservation methods (formalin, ethanol, freezing)

- 6. Proper fixation of snails and small clams
- B) Sampling specific tissues for histology
  - 1. Sampling from live animals
  - 2. Sampling from fixed animals
  - 3. Sampling from infected/lesioned tissue
  - 4. Grossing/trimming tissue samples
- C) Optimizing images
  - 1. White balance
  - 2. Brightness
  - 3. Scale bar
  - 4. Optimizing photographs using Adobe Photoshop
  - 5. Making figures or plates with Adobe Illustrator
- IV. Bacteriology
- V. Virology
- VI. Parasitology
  - A) How to perform a necropsy
    - 1. Open shell
  - B) Cursory inspection of mantle cavity
    - 1. Remove and inspect specific tissues
      - i. Mantle
    - 2. Gill
    - 3. Integument of visceral mass
    - 4. Foot
    - 5. Anterior visceral mass
    - 6. Posterior visceral mass
    - 7. Dorsal visceral mass

# **Mollusk Die-off Session**

Dr. Ryan Carnegie (VIMS) – Mortality events across a continuum: lessons from marine bivalve disease outbreaks
Dr. Nick Phelps (UMN) – Standardizing and simplifying fish kill reporting
Heidi Dunn (Ecoanalysts) – Investigation and monetary values of fish and freshwater mollusk kills Effective sampling design and techniques for assessment of mussel populations
Jordan Richard (USFWS – Virginia) – Review of a case study: Clinch River mussel die-off

# Disease Risk Analysis – Applications for the Management of Freshwater Mussels

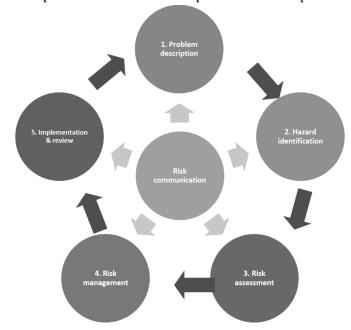
Tiffany Wolf, Phil Miller, Alex Primus, & Dominic Travis

# Background and Resources

The following brief overview and resources are included as a user-friendly guide to outline the process of Disease Risk Analysis (DRA) and define common terminology. For a more thorough introduction to the DRA, participants are referred to the IUCN's *Manual of Procedures for Wildlife Disease Risk Analysis*.

# Overview of the Process

The DRA consists of five general steps, with risk communication with stakeholders being a key component throughout (Figure 1). Risk analysis refers to the overall process; distinct from Risk Assessment, a stage of the process. DRA starts with problem description (the process of describing and justifying the problem or question) and follows with Hazard Identification, Risk Assessment, Risk Management, and Implementation and Review. Again, Risk Communication should occur frequently throughout the process and engage technical experts, key stakeholders, and policy makers to maximize the quality of the analysis and enhance the likelihood of implementation of resulting recommendations. Problem Description is the first step of the process and critical to the success of the DRA. In this step, participants should clearly define the context of the problem, the specific question to be addressed, the goal and scope of the DRA, assumptions and limitations, and the acceptable level of risk. Hazard Identification involves the identification of all possible hazards, both infectious and non-infectious. Criteria should be developed related to the defined problem to aid in prioritizing the list of hazards, consequences, both direct and indirect, of each of the hazards should be



considered to identify which should be further evaluated. The Risk Assessment stage of the DRA is a more formal approach to assessing 1) the likelihood of release (introduction) of a hazard into a system, 2) the likelihood of the species of interest being exposed to the hazard following release and 3) the consequences of exposure. This may be accomplished through qualitative, semi-quantitative, or quantitative methods. Risk Management entails a review of potential risk reduction or management strategies for mitigation that may be employed along with their potential outcomes. This forms the basis for DRA recommendations. Finally, the DRA process concludes with development of a plan for the process and timeline for monitoring, evaluation and review of the proposed risk management actions.

Figure 1: Overview of the Disease Risk Analsysis process taken from the IUCN Manual of Procedures for Wildlife Disease Risk Analsysis

Glossary of Terms- Select terminology and definitions taken from IUCN Manual of Procedures for Wildlife Disease Risk Analysis.

acceptable risk	A level of risk that is so small in terms of likelihood of occurrence or consequences that, in comparison with the expected benefits, stakeholders are willing to accept it
consequence assessment	The process of describing the relationship between specified exposures to a hazard and the consequences of those exposures. A causal process must exist by which exposures produce adverse health or environmental consequences, which may in turn lead to socioeconomic consequences and consequences for conservation. The consequence assessment describes the consequences of a given exposure and estimates the probability of them occurring
disease	Any impairment of the normal structural or physiological state of a living organism resulting from its physiological response to a <i>hazard</i>
disease risk analysis	The application of <i>risk analysis</i> to identify diseases that may enter a specified animal population to identify the likelihood of such introductions, assess their consequences and identify measures that may be applied to mitigate either the likelihood of introduction or the magnitude of consequences
endemic	A disease or parasite the prevalence of which does not exhibit wide fluctuations through time in a defined location. The term 'enzootic' is sometimes applied when referring to non-human populations
epidemic	A sudden, rapid spread or increase in the prevalence or intensity of a parasite or disease. An epidemic is often the result of a change in circumstances that favor parasite transmission such as a rapid increase in host population density or the introduction of a new parasite. Having an established baseline is essential for detecting epidemics. The term 'epizootic' is sometimes applied when referring to non-human populations

exposure assessment	The process of describing the biological pathway(s) necessary for exposure of animals and humans in a particular environment to the hazards (in this case the pathogenic agents) released from a given risk source, and estimating the probability of the exposure(s) occurring, either
	qualitatively or quantitatively
hazard	A biological, chemical or physical agent in, or a condition of, an animal or animal product with the potential to cause an adverse health effect. See also disease
hazard identification	The process of identifying the pathogenic or hazardous agents that could potentially be introduced into a specified animal population or environment by the activity being considered
host	Any animal that is capable of harboring a parasite, regardless of whether it plays a role in the further transmission of the parasite
incidence	The number of new health events (infection, disease, etc.) experienced by a given population over a specific period of time. (cf. prevalence, the total number, new and old, in a given population in a specified time period)
incubation period	The time that elapses between infection with a parasite and the onset of disease
infection	The entry and development or multiplication of a parasite in the body of a host, where it may or may not cause disease
infectious period	Period during which the infected individual is able to transmit the infection
infestation	Subsistence of a macroparasite on the external surface of a host regardless of whether the infestation results in disease
latent period	The period when an individual is infected but not yet capable of transmitting the infection

macroparasites	Parasites that in general do not multiply within their hosts but instead produce transmission stages (eggs and larvae) that pass into the external environment (e.g. the parasitic helminths (worms) and arthropods).
model	In the context of DRA, a graphical or computational representation of an actual system used to predict disease dynamics and impacts, and the effect of management interventions on those dynamics and impacts
monitoring	The intermittent performance and analysis of routine measurements and observations, aimed at detecting changes in the environment or health status of a population
parasite	An agent that lives on or within a host and that survives at the expense of the host regardless of whether a disease state follows. This definition includes both microparasites (e.g. bacteria, viruses) and macroparasites (e.g. helminths, arthropods)
pathogen	Any disease-causing parasite
prevalence	The proportion of the host population with infection, disease or antibody presence, often expressed as a percentage. A measure of how widespread an infection, disease or exposure to an infectious agent is at a point in time
qualitative risk assessment	An assessment in which the outputs on the likelihood of the outcome or the magnitude of the consequences are expressed in qualitative terms such as high, medium, low or negligible
Quantitative risk assessment	An assessment in which the outputs of the risk assessment are expressed numerically
release assessment	The process of describing the biological pathway(s) necessary for a particular activity to 'release' (that is, introduce) hazards into a particular environment or ecosystem, and estimating the probability, either qualitatively or quantitatively, of that complete process occurring

rocorvoir	
reservoir	Any animate (humans, animals, insects, etc.) or inanimate object (plant, soil, feces, etc.) or any
	combination of these serving as a habitat of a parasite
	that reproduces itself in such a way as to be transmitted
	to a susceptible host
risk	The likelihood of the occurrence and the likely
	magnitude of the consequences (biological, economic,
	etc. as defined by a specific risk analysis question) of
	an adverse event or effect to animal or human health
risk analysis	The process composed of problem description, hazard
	identification, risk assessment, risk management and
	risk communication
risk assessment	The evaluation of the likelihood and the consequences
	of entry, establishment or spread of a pathogenic agent
	within a specified animal population or environment
risk	The interactive exchange of information and opinions
communication	throughout the <i>risk analysis</i> process concerning risk,
	risk-related factors and risk perceptions among risk
	assessors, risk managers, risk communicators, the
	general public and other interested parties
risk management	The process of identifying, selecting and implementing
	measures that can be applied to reduce the level of risk
surveillance	The systematic ongoing collection, collation and
	analysis of information related to animal health and the
	to know so that action can be taken
uncertainty	The lack of precise knowledge of the input values that
•	is due to measurement error or to lack of knowledge of
	the steps required, and the pathways from hazard to
	risk, when building the scenario being assessed
vector	An insect or any living carrier that transports an
	infectious agent from an infected individual to a
	susceptible individual or its food or immediate
	surroundings. The organism may or may not pass
	through a development cycle within the vector
	1 · · · · · · · · · · · · · · · · · · ·
zoonosis	A disease naturally transmitted between humans and
·	timely dissemination of information to those who need to know so that action can be taken  The lack of precise knowledge of the input values that is due to measurement error or to lack of knowledge of the steps required, and the pathways from hazard to risk, when building the scenario being assessed  An insect or any living carrier that transports an infectious agent from an infected individual to a susceptible individual or its food or immediate surroundings. The organism may or may not pass

# POSTER SESSION ABSTRACTS, TUESDAY MARCH 13, 2018-7:00PM

### BIG DARBY CREEK, OHIO, 2016 MUSSEL DIE-OFF

A.M. Sasson<sup>1</sup>, G.T. Watters<sup>2</sup>, A. Boose<sup>3</sup>, D. Symonds<sup>2</sup>, J. Gordon<sup>4</sup>, C. Byrne<sup>2</sup>. <sup>1</sup>The Nature Conservancy, Dublin, OH 43017; <sup>2</sup>The Ohio State University, Columbus, OH 43212; <sup>3</sup>Columbus and Franklin County Metro Parks, Westerville, OH 43081; <sup>4</sup>Lawhon & Associates, Columbus, OH 43212.

# Die-off Section Poster 1

In late 2016 and early 2017, a large die-off of mussels across all species was observed in Ohio's Big Darby Creek, a tributary in the Scioto River basin. Big Darby Creek is a State and National Scenic River due to its outstanding fish and mussel diversity, whose 144,000 ha (555 mi²) watershed is dominated by agricultural land use. The die-off was first observed in October 2016, with lingering effects continuing into early 2017. An unusually high number of distressed and fresh dead mussels were observed throughout ~93 km (~58 mi) of Big Darby Creek. Based on about 200 person-hours of survey and collection efforts, the number of fresh dead shells observed and collected at many sites was estimated at often one to two orders of magnitude greater than has been known to be observed by well-practiced observers familiar with mussels in this watershed. Of the 43 recorded species and 35+ known to be presently extant in the watershed, it appeared that all species of freshwater mussels were affected in the Big Darby Creek die-off, from the most common (e.g., spike, kidneyshell, fatmucket, plain pocketbook) to federally endangered (northern riffleshell, clubshell). Mussel deaths appeared to occur over a period of weeks to months, ending by spring 2017. There were no known observations of fish mortality, and no cause was determined through state and federal agency water and tissue sampling. Six sites were surveyed on Little Darby Creek although a die-off was not observed in this tributary.

# THE DIE-OFFS OF FRESHWATER PEARL MUSSEL (MARGARITIFERA MARGARITIFERA) IN SWEDEN; AN OVERVIEW OF THE CURRENT SITUATION AND POSSIBLE CAUSES.

Niklas Wengström<sup>1,2</sup>, Håkan Söderberg<sup>3</sup>, Johan Höjesjö<sup>1, 1</sup>University of Gothenburg, Department of Biological and Environmental Sciences, Box 463, SE-405 30 Gothenburg, Sweden; <sup>2</sup>Swedish Anglers Association, Sjölyckan 6, 416 55 Gothenburg, Sweden; <sup>3</sup>The county administrative board of Västernorrland, Pumpbacksgatan 19 871 86 Härnösand.

# Die-off Section Poster 2

The freshwater pearl mussel *Margaritifera margaritifera* is an endangered species in Sweden with more than 600 known populations distributed in 16 counties. Approximately one third of these populations can be considered viable and healthy as they show sign of recent recruitment of juveniles (<50 mm). The problem with recruitment failure in Sweden is associated with turbidity and sedimentation of fine substrates and other alterations in their habitat. Almost every running water has been used historically for lodging and/or mills and since the beginning of the 20<sup>th</sup> century hydropower plants has been a problem. In the late 1960's acidification of streams and lakes became one of Sweden's biggest environmental problem and in 1977 a liming program started to mitigate the effects of acidification in surface waters. Many of these problems have been solved or are about to get solved. Since the 1980's Sweden have measured freshwater pearl mussel populations with quantitative methods and we use juvenile recruitment as one factor indicating a healthy environment. Recently, there have been reports of freshwater pearl mussel die-offs in five different counties resulting in population decreases between 22 – 100 %. The underlying reason for this sudden death in many systems is not clear but we believe that it is due to both biotic and abiotic factors. We have therefore started a small-scale monitoring project aiming to detect possible factors and we will in this presentation provide a map visualizing the current knowledge of where freshwater pearl mussel die-offs have been found in Sweden.

Die-off Section Poster 3	EU LIFE IP PROJECT FRESHABIT: CONSERVATION OF THE TWO MAIN REMAINING MARGARATIFERA MARGARATIFERA POPULATIONS OF SOUTHERN FINLAND  Jouni Taskinen¹, Jukka Pakkala², Eero Mäenpää², Anu Suonpää³, Juha-Pekka Vähä³, Esko Vuorinen⁴ and Panu Oulasvirta⁵. ¹Department of Biological and Environmental Science, University of Jyväskylä, Box 35, FI-40014 JYU, Finland; ²Centre for Economic Development, Transport and the Environment of South Ostrobothnia, Finland; ³Association for Water and Environment of Western Uusimaa, Finland; ⁴Silvestris Nature Surveys Ltd., Finland. ⁵Alleco Ltd., Finland.  The freshwater pearl mussel (eastern pearl shell, Margaritifera margaritifera, FPM) is endangered throughout the range of its distribution in Europe. There are over 120 rivers inhabiting FPM in Finland, most of them being located in the north. Only two major FPM rivers are found from the southern Finland – River Ähtävänjoki/Essen and River Mustionjoki/Svartå, flowing to the Baltic Sea. The estimated FPM population size of River Ähtävänjoki has decreased form 50,000 to 500 from between 1987-2016. Reproduction of FPM was evident in River Ähtävänjoki from 1990 to 2006, but not in 2015-16. Steep decline of FPM was evident also in River Mustionjoki, from 3,000 to 1,300 between 2010-2016, accompanied with cessation of glochidia release. I will describe the planned/on-going actions of the EU Life IP project FRESHABIT (2016-2021) to prevent extinction of these last remaining southern Finnish FPM populations: transportation of adult mussels to tank facility to resume glochidia production by using artificial feeding, captive breeding, habitat restoration and fishway construction to restore the natural migration and reproduction of salmonid hosts.
Die-off Section Poster 4	EFFECTIVENESS OF TWO ANTIFUNGAL MEDICATIONS ON FUNGAL INFECTION IN SOUTHERN COMBSHELL, EPIOBLASMA PENITA (CONRAD, 1834)  Michael Buntin¹, Todd Fobian¹, Benjamin Beck², and Paul Johnson¹. ¹Alabama Department of Conservation, Alabama Aquatic Biodiversity Center, Marion, AL, 36756; ²USDA-Agricultural Research Service, Auburn, AL 36832.  Southern Combshell is a federally endangered unionid endemic to the Mobile River Basin in Alabama and Mississippi. Propagation of this species has been taking place at the Alabama Aquatic Biodiversity Center since 2010. In 2017, five female Southern Combshells taken from the Buttahatchee River, Lowndes County, MS became infested with fungal hyphae after two individuals had their glochidia extracted via water-filled syringe. The animals were initially grouped by species population in separate aerated 1.5 L covered containers stored in an 8°C communal incubator. Once the infection was observed, the Southern Combshells were quarantined in a separate incubator. Three individuals died before any treatment could be administered. The remaining two mussels were treated independently with Pimafix™ and amphotericin B. The amount of visible hyphae on the mantle or foot was reduced on both animals after multiple days of treatment but neither eliminated the infection entirely. Both treated Southern Combshells eventually died after the visible hyphae returned to pretreatment levels. No fungal hyphae were observed on any other mussel within the original brood stock incubator. The fungi were later identified by Dr. Cova Arias, using ITS-2 sequencing, as Didymella bellidis and Cladosporium sphaerospermum. While not a controlled experiment, the inability of these medications to eliminate the fungal infestation in these mussels emphasizes the need for sterile procedures and isolation of brood stock to prevent the spreading of potentially lethal infections among individuals and populations.
Die-off Section Poster 5	DISENTANGLING THE DIFFERENT ROLE OF PARASITES AND INVASIVE SPECIES IN THE DECLINE OF NATIVE MUSSELS  Nicoletta Riccardi¹, Maria Urbanska², Wojciech Andrzejewski², Angela Boggero¹, Caterina Fioroni¹, Jouni Taskinen³.¹ Institute of Ecosystem Study, National Research  Council, Italy; ² Poznan University of Life Sciences, Poland; ³ University of Jyväskylä, Finland.  Parasites have important negative consequences for freshwater mussels, but in the past they have been regarded as non-lethal and without any drastic impact upon mussel populations. Nonetheless, increased parasite densities impair mussel physiological conditions, which causes an increased mortality rate in mussels exposed to other stressors. A recently reported stressor is the spread of non-indigenous species (NIS), that can introduce new parasites and/or alter the dynamics of endemic parasites. To explore the interaction between biological invasions and growing parasites presence we profited of the sharp decline of the dominant native mussel (Unio elongatulus) in Lake Maggiore (Northern Italy), after the arrival of Corbicula fluminea and Sinanodonta woodiana. This study addressed whether: 1) the native species decline was determined by NIS co-introduction of own parasites; 2) introduced species triggered endemic parasite emergence; 3) habitat characteristics affect the interacting impacts of NIS and parasites. To answer these questions we evaluated:  1) the occurrence of parasites (alien/endemic) and the frequency of shared parasites in invasive and native bivalve species;  2) the prevalence and intensity of parasitic diseases in native species:  a. before and after NIS arrival (task 2);  b. in different habitat types and at different depths (task 3)

# MACROSCOPICAL AND MICROSCOPICAL FINDINGS FROM STUDIES OF ONGOING DECLINES IN SWEDISH FRESHWATER PEARL MUSSEL (MARGARITIFERA MARGARETIFERA) POPULATIONS

<u>ANDERS ALFJORDEN</u><sup>1</sup>, Håkan Söderberg<sup>2</sup>, Tomas Troschke<sup>3</sup>. <sup>1</sup>National Veterinary Institute, Sweden (SVA), S-751 89 Uppsala; <sup>2</sup>County administrative board of Västernorrland, S-871 86 Härnös; <sup>3</sup>County administrative board of, Gävleborg, S-801 70 Gävle.

# Poster 6

Several cases of increased mortality in freshwater pearl mussels (*Margaritifera margaritifera L.* 1758) have been detected during surveys of freshwater creeks and rivers during the last years (2011-2017) in Sweden. No etiological cause of these wild stock mortalities has been concluded yet. In some cases, samples from live animals have been collected for necropsy and histological investigations, during field surveys. During recent field surveys (2016-2017), freshwater mussels have been transported alive for further investigations to the National Veterinary Institute in Uppsala. These mussels have been compared with mussels collected the same day from healthy population where no signs of mortalities have been reported. Ocular screening of cross sections of these animals was investigated regarding pathological findings. Freshwater pearl mussels from affected population showed clear pathological changes compared to reference mussels both regarding macroscopical and microscopical appearances. The mussels from healthy population had firm and thick bodies, well developed gonadal organs and digestive glands filled with dark green digested feed content. In comparison the mussel from the affected population was in general much thinner with more relaxed bodies and had a lower body weight. Furthermore, the affected, lethargic mussels showed low or no presence of gonad cells (egg cells) and the digestive gland was pale with watery and transparent content. The microscopical investigation confirmed this pattern showing changes in the affected mussels compared to the reference population. The mussels from the declining populations had empty gonad follicles, enlarged swollen epithelial cells in digestive glands, where cells showed no presence of granular or vesicular content. Sometimes cellular infiltration was seen in the connective tissue surrounding these digestive glands. In the solid external body parts where connective and muscle fibers dominated, an increased cellular infiltration was observed in the emaciated mussels.

# NON-DESTRUCTIVE DIAGNOSIS OF TREMATODE PARASITISM IN FRESHWATER MUSSELS

# JOUNI TASKINEN, Jocelyn Mah Choo, Markus Tulolsela & Benjami Kumpalalnen

# Poster 7

Freshwater mussels (Unionoida) are declining throughout the world, possibly signaling poor health status of mussels. Digenean trematodes of the family Bucehpalidae and Gorgoderidae (e.g. *Bucephalus*, *Rhipidocotyle*, *Prosorhynchoides* and *Phyllodistomum* spp.) infect unionid clams, producing thousands of cecarial larvae and causing sterility of mussel host. Microscopical examination of mussels for trematodes requires destructive dissection of mussel tissues. Therefore, an alternative, non-destructive method would be useful as endangered mussels can only rarely be sacrificed for a parasitological survey. We studied if cercariae shedding could reliably reveal *Rhipidocotyle fennica* infection in the mussel *Anodonta anatina*. In the 1<sup>st</sup> experiment, individual mussels were kept in 2 L aquaria for 1 h, after which the water was checked for cercariae and the tissues examined microscopically. 26% of infections were found in ambient water temperature of 17 C, but 81% when temperature was increased to 21 C. Second experiment was conducted at field by incubating mussels in three time points during the cercariae shedding season, and by using water temperature 3 C higher than the ambient lake water. 63 % of infections were detected during the seasonal peak shedding period (mid-August) while on average 44% of infected individual were detected outside the optimal season (late July and late August). Thus, cercaria method could serve as a non-invasive way to investigate trematode infections in freshwater mussels.

# Poster 8

# COMMUNITY-BASED MONITORING OF FRESHWATER MUSSELS AS A RESEARCH AND CONSERVATION PRACTICE Celeste A. Searles Mazzacano. CASM Environmental, LLC, Portland, OR 97206.

Oregon is home to three genera of native freshwater mussels: *Margaritifera falcata* (Western Pearlshell), *Gonidea angulata* (Western Ridged Mussel), and several clades of *Anodonta* (Floater), including the Oregon/Western Floater clade (*A. oregonensis/kennerlyi*) and California/Winged Floater clade (*A. californiensis/nuttalliana*). Oregon's freshwater mussels are known to be declining, but they are also more poorly characterized than many other aquatic fauna, and detailed or long-term studies are lacking for most water bodies. With no federal protections in place for western mussels, funding mechanisms and legal obligations that help drive research and conservation are lacking. In addition, many practitioners do not know whether mussel populations are present in the waters they manage, and/or they lack the resources to characterize known or suspected populations. Community-based monitoring done by volunteers trained and overseen by content experts can be a powerful and cost-effective tool to improve freshwater mussel research, conservation, and education. This poster will describe best practices in community-based freshwater mussel monitoring and use case studies in Oregon to illustrate the benefits and challenges of these projects.

	TOXICITY EVALUATION OF AN EFFLUENT ENTERING THE DEEP FORK RIVER, OK, USA  JAMES KUNZ¹, Jeff Steevens¹, Ning Wang¹, Suzanne Dunn² and David Martinez². ¹US Geological Survey, Columbia, MO 65201: ²US Fish and Wildlife Service,  Tulsa, OK.
Poster 9	Effluents from a local manufacturing company were suspected as the cause of two mussel kills in 2005 and in 2011 in the Deep Fork River (DFR), OK. Previous studies reported elevated potassium in water contaminated by the effluent. The objectives of this study were to (1) assess the potential toxicity of the effluent to a unionid mussel ( <i>Lampsilis siliquoidea</i> ) and two commonly tested species (cladoceran <i>Ceriodaphnia dubia</i> ; fathead minnow <i>Pimephales promelas</i> ) in 7-day effluent tests; (2) evaluate the relative sensitivities of the three species to potassium in 7-day toxicity tests; and (3) determine the influence of water hardness on the acute toxicity of potassium in reconstituted waters. Preliminary results indicated that the effluent-contaminated water contained 50 times more potassium than upstream DFR water and was highly toxic to mussels and cladocerans. The mussel was more sensitive than the cladoceran and fathead minnow in all tests. The acute toxicity of potassium to the mussel and cladoceran did not substantially change in four reconstituted waters mimicking water quality characteristics in the DFR with a broad hardness range of 35 to 300 mg/L as CaCO <sub>3</sub> , and importantly, the 50% effect concentrations for mussels were below the potassium concentrations measured in the effluent in this and other studies.
	THE VALVE MOVEMENT RESPONSE OF UNIO TUMIDUS AND SINANODONTA WOODIANA EXPOSED TO NITRATE, SULFATE CHLORATE AND PHOSPHATE COMPOUNDS  Joanna Chmyst <sup>1</sup> , Maria Urbańska <sup>2</sup> . <sup>1</sup> Department of Ecology & Environmental Protection; <sup>2</sup> Institute of Zoolgy, Poznań University of Life Sciences, ul. Wojska Polskiego 28, 60-637 Poznań, Poland.
Poster 10	For last years bivalves have presented one of the most widely used organisms in monitoring water quality establishing the biology early warning system (BEWS). We compared the sensitivity of European native <i>Unio tumidus</i> and alien, <i>Sinanodonta woodiana</i> bivalves. <i>U tumidus</i> has been used as a water safety monitor for more than 8 million people in Poland. <i>S. woodiana</i> is one of the species that has recently been reported as being invasive worldwide. KNO <sub>3</sub> (50 mgl <sup>-1</sup> ), K <sub>2</sub> SO <sub>4</sub> (250 mgl <sup>-1</sup> ), NH <sub>4</sub> Cl (250 mgl <sup>-1</sup> ) and KH <sub>2</sub> PO <sub>4</sub> (0,75 mgl <sup>-1</sup> ) were tested in the continuous recirculation system. For each experiment 8 individuals of mussels were placed into the system. Both species react in similar way for each exposure to pollution taking into account both mean daily shell opening level and mean daily activity time. For the chloride and phosphate pollution there is no reaction in this measure for both species. The greatest reactions are for nitrate and sulfate exposure. While the mean daily shell opening level declines twice for <i>U. tumidus</i> , this leap is 12.5 times for <i>S. woodiana</i> . However, in absolute values this fall is smaller by 48% and 23%, respectively, so the detection may be easier in the former case. The mean daily activity time drops is by 57% and as much as 88% for <i>U. tumidus</i> and <i>S. woodiana</i> , respectively.
	IS IT POSSIBLE TO HIDE FROM DREISSENA POLYMORPHA? EXAMPLE OF NATIVE AND INVASIVE SPECIES OF UNIONIDAE FOULING BY ZEBRA MUSSELS  Maria Urbanska <sup>1</sup> , Wojciech Andrzejewski <sup>1</sup> and Henryk Gierszal <sup>2</sup> . <sup>1</sup> Institute of Zoology, Poznan University of Life Sciences, ul. Wojska Polskiego 28, 60-637 Poznań, Poland; <sup>2</sup> Department of Applied Computer Science, Adam Mickiewicz University, Umultowska 85, 61-614 Poznań, Poland.
Poster 11	Unionidae live partly buried in the sediments, usually with the posterior part of their shells exposed to the water column to enable filtration. This exposed part of the shell can therefore serve as a substratum for zebra mussels, which limit the host's ability to feed, move, and breed. We analyzed images of shells of 2 unionid species:  Anodonta anatina and alien Sinanodonta woodiana. 40 specimens of both species fouled or not by D. polymorpha were randomly collected. The recorded data comprised approximate area of the whole shell, the exposed part, and of the outer parts of D. polymorpha, protruding from the unionid shell. Our results show that these 2 species significantly differed in shell surface available to zebra mussels. A. anatina was consistently more heavily fouled than S. woodiana and had a greater surface area of the shell exposed in the water column. On average only 25.5% of the shell of S. woodiana was exposed above the sediments, compared to 45.4% of the shell of A. anatina.  Thus S. woodiana is strongly buried in the mud, which gives it better protection from the negative impact of zebra mussels and makes the Chinese species more competitive than the native A. anatina.

# EFFECT OF TEMPERATURE ON SURVIVAL, GROWTH AND DEVELOPMENT OF MARGARITIFERA MARGARITIFERA LARVAE DURING EARLY PARASITISM IN SALMO SALAR GILLS.

Castrillo, P. A.<sup>1</sup>, Varela C.<sup>2</sup>, Ronza, P.<sup>1</sup>, Outeiro A.<sup>2</sup>, Bermúdez R.<sup>1</sup>, Quiroga M.I.<sup>1</sup>, Ondina P.<sup>2</sup>. Department of Anatomy, Animal Breeding and Veterinary Clinical Sciences; Department of Zoology, Genetics and Physical Anthropology. Universidade de Santiago de Compostela, Facultad de Veterinaria, 27002, Lugo, España.

Margaritifera margaritifera is a freshwater mussel categorized as Critically Endangered and protected by European conservation policies. Still, the propagation programs are constrained by its life cycle, characterized by a long-term and specific parasitic stage of the larvae (glochidia) on fish gills. It is known that temperature influences the metabolism and immune response of bivalves and fish, nevertheless, the effect of this factor on the interaction between fish and glochidia has not been investigated. The purpose of this study was to determine the effect of temperature on M. margaritifera larvae during the early parasitic stages on Salmo salar gills. Two fish groups were exposed to glochidia and maintained at 9 and 17 °C, respectively, for 30 days. Parr were euthanized and second left holobranches were assessed by stereomicroscopy. The number and the maximal diameter of glochidia between both groups were compared by Wilcoxon and ANOVA test, respectively. Moreover, remaining holobranches were processed for histological examination. In fish exposed at 17°C, larvae displayed higher number (P<0,05), bigger size (P<0,01) and more advanced organogenesis than those at 9°C. These results suggest that temperature influences survival, growth and development rates during early parasitic stages of M. margaritifera. Moreover, combination of stereomicroscopical and histological studies represent a good tool to study the host-parasite interaction, although further studies are needed to understand the subjacent mechanisms and the consequences in older stages of this freshwater pearl mussel. This work has been funded by the MarMaCul

# DEVELOPING KEY SKILLS IN FRESHWATER MUSSEL CONSERVATION AT THE NATIONAL CONSERVATION TRAINING CENTER (USFWS) Matthew Patterson. National Conservation Training Center, United States Fish and Wildlife Service, 698 Conservation Way, Shepherdstown, WV 25443

The plight of native freshwater mussels in North America and around the world has led to significant increases in the number of natural resource professionals spending all or a portion of their time on mussel conservation issues. Within the United States Fish and Wildlife Service alone, Ecological Services biologists are being asked to review project proposals and new listings, hatchery biologists are being asked to propagate mussels for recovery of threatened and endangered species, and national wildlife refuge biologists are being asked to survey mussel populations on their property. Unfortunately, many of these biologists have never received formal training in the biology, ecology, life history, or identification of freshwater mussels. A similar lack of formal training can be found at other federal, state, and non-governmental organizations. To help meet this training need, the National Conservation Training Center (NCTC) in Shepherdstown, West Virginia developed a freshwater mussel curriculum that currently includes three courses, Conservation Biology of Freshwater Mussels, Freshwater Mussel Propagation for Restoration, and Freshwater Mussel Identification. Since starting the curriculum in 2012, the NCTC has trained over 250 biologists in freshwater mussel biology, ecology, life-history, field sampling, propagation and culture, and identification in an effort to improve conservation efforts on the ground and stem the tide of population declines. This poster presentation will provide an overview of the mussel curriculum, a break-down of students trained by state and agency, as well as information on upcoming courses.

### THE MUSSELS OF LOLO CREEK

Doug Nemeth, Frank Mullins, Mike Murray, Chris Griffith. Idaho Fish and Wildlife Conservation Office, Orofino, Idaho.

project from the Fundación Biodiversidad and by a predoctoral contract of Xunta de Galicia.

Freshwater mussels are one of the most imperiled groups of animals in the world, so when a habitat restoration project in Lolo Creek, Clearwater River Basin, Idaho was going to result in dewatering a large number of western pearlshell mussels (*Margaritifera falcata*), agencies agreed to cooperatively translocate the mussels prior to dewatering. This provided an opportunity to investigate mussel quantification techniques and the demographic characteristics of Lolo Cr. mussels. Objectives were to estimate the numbers of mussels present using two different techniques and compare those estimates to the actual census (number of mussels translocated), determine the proportion of mussels on the surface to those buried and their lengths, and determine the recovery rate of PIT-tagged, translocated mussels. Floating downstream and counting all mussels within the prescribed area was more efficient than using quadrats and a systematic random sampling design. The number and proportion of mussels on the surface in Lolo Cr. was strongly influenced by water temperature. Mussels may have segregated by length through the substrate column. Disturbance and removal of mussels resulted in additional mussels recruiting to those areas. Recovery rate of PIT-tagged mussels was about 50% at 4°C, but may be higher at warmer temperatures when more mussels are on the surface.

# Poster 14

Poster 13

Poster 12

### PHYSIOLOGICAL PROFILE OF NATIVE UNIONID MUSSELS IN RESPONSE TO ZEBRA MUSSEL INVASION

Vince L. Butitta, Alyssa Ginther & Emily H. Stanley. Center for Limnology, University of Wisconsin-Madison, Madison, WI 53706.

### Poster 15

One of the fastest growing threats to North America's native freshwater mussel is the invasion of Eurasian Zebra mussel (*Dreissena polymorpha*). Since the introduction and rapid spreading of invasive Dreissenid mussels throughout North America, native Unionid species have experienced dramatic declines—with many reports of local extirpation, but some situations of possible coexistence. Despite well-documented declines in native mussel populations to Dreissenid invasion, the mechanisms through which Unionids are locally extirpated is not well understood. Zebra mussel populations began exploding in the Mendota Lake (Dane Co., WI) in 2016, they have since quickly colonized the three lakes downstream. We collected individuals from two common Unionid species (*Lampsilis siliquoidea* and *Pyganodon grandis*) across a Zebra mussel infestation gradient. Hemolymph samples were extracted for metabolomic analysis, soft tissues were dried, weighed, and assessed for glycogen content. Glycogen has long been used to quantify general poor body condition in mussels, but the powerful new tool of metabolomics has recently been shown to be extremely useful in understanding how mussels physiologically respond to stressful handling, hypoxia, food limitation, and certain toxicants. A metabolomic analysis of Unionids suffering from a zebra mussel invasion may provide a more detailed look into the mechanism through which Unionid populations collapse following zebra mussel invasions and could offer insight into possible coexistence.

### DEVELOPMENT OF WEB-BASED TAXONOMIC KEY TO IDENTIFY FRESHWATER GLOCHIDIA.

Heidi Dunn<sup>1</sup>, Kathy Koch<sup>2</sup>, Nate Jacobson<sup>2</sup>, Doug Bradley<sup>2</sup>, Chris Cieciek<sup>2</sup>, Jonathon Black<sup>3</sup>, Doug Dixon<sup>3</sup>. <sup>1</sup>EcoAnalysts, Inc, O'Fallon, MO 63366; <sup>2</sup>LimnoTech, Ann Arbor, MI 48108; <sup>3</sup>Electric Power Research Institute, New Salem, MA 01355.

# Poster 16

Taxonomists traditionally use a variety of hardcopy guides to identify freshwater unionid mollusk larvae (glochidia). These guides may not include contemporary identification characteristics and/or current nomenclature, may lack high resolution photographs or images, and may be out of print or difficult to obtain. Further, new morphological information observed at propagation facilities may not be published. With Electric Power Research Institute (EPRI) funding, a conceptual web-based lucid key has been designed to aid researchers and professionals with identification of freshwater unionid mollusk glochidia. The web-based platform is modeled on other taxonomic keys (freshwater and marine larval fish) developed by the EPRI and LimnoTech team and is intended to provide a single, open resource that supports the compilation and dissemination of taxonomic information, allows for the incorporation of new and high-quality information and imagery, and facilitates improved identification. The project is presently adding species-level information and images to the conceptual key framework to improve the database and support the accurate identification of freshwater glochidia. Current activities also include collaboration with malacology experts to identify additional data and image resources to further enhance the database, and broaden the exposure and access of glochidia identification among scientists.

### PARASITISM IN UNIONID MUSSELS: A THREAT FOR SPECIES AT RISK

Joseph P. Carney & Andrea Strawson. Department of Biology, Lakehead University, Thunder Bay, ON, P7B 5E1, Canada.

# Poster 17

Freshwater mussels can host a variety of helminth parasites, including digenean sporocysts. Host sterility, can result from sporocyst infection. The purpose of this study was to assess the helminth parasite fauna of freshwater mussels from the Lake Winnipeg drainage. A total of 1021 freshwater mussels (Bivalvia: Unionidae) representing 12 species collected from 35 rivers in Manitoba, Saskatchewan and North Dakota was examined for helminth parasites. Five helminth species infected a total of 10 mussel species. Adult *Aspidogaster conchicola* infected 5 mussel species, and adult *Cotylogaster occidentalis* infected 9 mussel species with no apparent effect to the hosts. Metacercariae, a larval digenean, parasitized 6 mussel species, with no apparent effect on the host. Five mussel species were infected with 2 types of digenean sporocyst. A sporocyst releasing rhopalocercous cercariae infected 3 mussel species with no apparent effect on mussel reproduction. A second sporocyst, releasing furcocercous cercariae, infected 5 mussel species and was responsible for either reducing mussel reproductive capacity, or completely sterilized the mussel. Subsequent histological study of *Pyganodon grandis* demonstrated the absence of gonad tissue associated with heavy infection by this second type of sporocyst. Decreasing reproduction, either partially or completely, both lowers individual fitness, and decreases the effective breeding size of the population. These parasite induced pathologies need to be considered when conservation efforts are directed toward mussel species at risk.

# Poster 18 Poster 18 Poster 18 At 1,1 La spe pro RI VI Sa Ma Th sin ha KI Poster 19 Co de

# PROPAGATION AT VIRGINIA FISHERIES AND AQUATIC WILDFLIFE CENTER

BEN DAVIS¹, Amy Maynard², Rachel Mair¹, Brian Watson³, Michael Odom¹, Bryce Maynard¹. ¹U.S. Fish and Wildlife Service, Charles City, VA 23030; ²Conservation Management Institute, Virginia Polytechnic & State University, Charles City, VA 23030; ³Virginia Department of Game and Inland Fisheries, Forest, VA 24551.

Virginia Fisheries and Aquatic Wildlife Center (VFAWC) is a cooperative freshwater mussel propagation facility located at Harrison Lake National Fish Hatchery. Since VFAWC's founding in 2007, the US Fish and Wildlife Service and the Virginia Department of Game and Inland Fisheries have jointly propagated 12 species of Atlantic slope freshwater mussels, and have released over 247,000 individuals back into the wild. During the 2017 propagation season, VFAWC produced over 1,152,000 juvenile mussels of 8 species of special concern, including the federally-endangered *Pleurobema collina* (James spinymussel) and the state-threatened *Lasmigona subviridis* (green floater). Of the mussels produced, approximately 228,000 were distributed to other hatcheries or researchers. A total of 45,421 mussels of 7 species were released during 2017 as part of population augmentation efforts. Future work will include mitigation projects utilizing both conventional and in-vitro propagation methods.

# REINTRODUCTION OF EPIOBLASMA AUREOLA (BIVALVIA: UNIONIDAE) IN THE UPPER CLINCH RIVER BASIN, TAZEWELL COUNTY, VIRGINIA.

Sarah L. Colletti, Tim W. Lane, Joseph F. Ferraro £ Tiffany C. Leach. Aquatic Wildlife Conservation Center, Virginia Department of Game and Inland Fisheries, Marion, Virginia 24354.

The critically endangered Golden Riffleshell mussel *Epioblasma aureola* (Jones and Neves 2010) is an Upper Tennessee River Basin (UTRB) endemic restricted to a single reproducing population in the Upper Clinch River. Previous population augmentation efforts using host fish were met with limited success and wild brood stock have become increasingly challenging to locate. In 2016, VDGIF biologists recovered three gravid females from Indian Creek, VA and transferred their glochidia to KDFWR's Center for Mollusk Conservation, Franklin, KY. There, using *in vitro* methods, 12,000 juveniles were successfully produced. At ~3 months of age, 1,200 remaining juveniles were split between 3 culture facilities with variable survival but were eventually transferred for final grow-out to VDGIF's Aquatic Wildlife Conservation Center (AWCC), Marion, VA in 2017. To initiate pre-release monitoring, 5 sites across the upper Clinch and Indian Creek were chosen in 2017 for silo deployment. Two silos with five mussels each were placed at all sites and monitored monthly for growth and survival. Survival was 100% across all sites, with mussels exhibiting variable growth among sites. The UTRB Mussel Recovery Group selected three out of the five sites for release, one being the native brood stock location in Indian Creek and two others in the main stem Clinch River. Pit-tagged *E. aureola* (N=100) were released in a 2.5 m² grid at each site and color-coded with Hallprint© tags to identify maternal pedigree. An additional 431 individuals were free-released across the chosen sites, for a total of 731 sub-adults released through this augmentation effort. Silos remain at the five sites and those 50 individuals will continue to be monitored. Mark-recapture surveys are planned to observe the growth and survival of the pit-tagged individuals. Individuals from the 2013 (N=4) and 2016 (N=252) cohorts, and a new 2017 (N=39) cohort, remain in captivity and are being program at AWCC. Future efforts to expand the species' range beyond the Clinch Riv

# PESTS, PREDATORS, PARASITES, AND RELATED PROBLEMS IN THE CULTURE OF FRESHWATER MUSSELS

Morgan Kern<sup>1</sup>, Louise Lavictoire<sup>1</sup>, Megan Bradley<sup>2</sup>, Nathan Eckert<sup>2</sup>, Beth Glidewell<sup>3</sup> & Chris Barnhart<sup>1</sup>. <sup>1</sup>Department of Biology, Missouri State University, Springfield, MO 65897; <sup>2</sup>Genoa National Hatchery, Genoa WI 54632; <sup>3</sup>Confederated Tribes of the Umatilla Indian Reservation, Pendleton, OR, 97801.

# Poster 20

A wide variety of organisms impact the captive culture of freshwater mussels. We provide an overview of some of these organisms, their effects, and mitigation methods. Parthenogenic midge larvae (*Paratanytarsus*) immobilize juveniles and foul screens by case building. Midge can be controlled with entomopathic bacteria products such as Gnatrol<sup>®</sup>. Suspension feeding Cladocera and Ostracoda similar in size to juvenile mussels may bloom in culture. If removal by filtration is impractical, these can be reduced by decanting as they swim above the substrate. Colonial ciliates and rotifers can foul surfaces including juveniles as well as competing for suspended food. Routine control involves cleaning culture vessels, screens and substrate. Fouling organisms can be removed from juveniles by brief drying. Predators on early juveniles include oligochaete annelids (*Chaeotgaster*) and rhabdocoel flatworms (*Macrostomum*, *Microstomum*). Rhabdocoels can be removed from host fish with formalin. Water molds, probably mainly *Saprolegnia*, are occasionally observed in dead or moribund juveniles and adults. Sterilizing larval trematodes (Bucephalidae) occur sporadically in Unionidae and may affect from 0-25% of wild adult mussels. Completion of the trematode life cycle requires host fish, so that reinfection is unlikely but possible in pond-based culture operations. Diseases that commonly affect host fish carrying mussels include *Flavobacterium columnaris*, which responds to Kanamycin sulfate and NaCl. The ciliate *Ichthiopthirius* can be controlled with NaCl, which excysting juvenile mussels can tolerate briefly up to 4.5 g/L. Praziquantel can control Monogenea in host fish without harm to juveniles.

# A COMPARISON OF SUBSTRATE PREFERENCES FOR NATIVE AND INVASIVE MUSSEL POPULATIONS IN THE KISHWAUKEE RIVER Sophie C. McComb & Madeline M. McCormick. Students, Sycamore High School, Sycamore, IL 60178.

# Poster 21

Freshwater mussels (family Unionidae) are particularly important for the preservation of local freshwater aquatic habitats and environments. Of increasing priority is examining the relationship between the growing population of non-native *C. fluminea* and the survival of the species in aquatic environments, as well as the discovery of any substrate preferences. Many of the articles reviewed indicate that the scientific community studying freshwater mussels has paid great attention to the latter, but very few studies focus on a relationship between non-native *C. fluminea* and native mussel populations. Two sites were surveyed within the East Branch of the South Branch of the Kishwaukee River sub-watershed. A total of 64 randomly selected quadrats were surveyed for substrate composition, native mussel species, and non-native mussel populations. Significant correlations were found between both native and nonnative populations to the percentage of sand in the substrate, as well as a strong positive correlation between *C. fluminea* populations and the presence of native species. Important in the consideration of these results is the late period of survey in the freshwater mussel activity season, and the relatively small sample size of the study. The results, with consideration to the limitations of the study, suggest the dominance and strong adaptive ability of *Corbicula* (Sousa, et al. 2009). This also presents a need for further examination of the relationships between native and non-native species, and the containment of invasive population growth.

### CONSERVATION OF MARGARITIFERA MARGARITIFERA IN SPAIN: AN OVERVIEW OF THE MARGALULLA PROJECT

Varela, C.¹, <u>CASTRILLO</u>, P.A.², Outeiro, A.¹, Amaro, R.¹, San Miguel, E.¹, Castro, J.¹, Ondina, P.¹. ¹ Dpto. de Zoología, Genética y Antropoloxía Física; ²Dpto de Anatomía Patológica y Ciencias Clínicas Veterinarias. Facultad de Veterinaria. Universidad de Santiago de Compostela. 27002 Lugo. España.

# Poster 22

The naiad M. margaritifera is an endangered species throughout its holartic distribution area. Galician river basins (NW Spain, on the southwestern limit of its European range) are a core area that houses 80% of Iberian populations. However, Galician rivers have suffered an increased number of pressures during the last century, which have significantly decreased their quality and ecological function. As a consequence, most of the mussel populations are now fragmented, with low abundance and little or no recruitment currently. The European Life funds provides financing for environmental protection and nature conservation projects with the aim of implementing and developing the EU's environmental policies. MargalUlla was conceived as a long-term species conservation project, dealing both with captive breeding and habitat restoration in the Ulla basin. Accordingly, a breeding program has been launched in 2013 in the rearing facility "O Veral" (the only one on the Iberian Peninsula) with the aim of obtaining juveniles to reinforce populations.

In this work we present the results obtained in different experiences carried out over four years. The publication of this kind of data is essential to understand conservation measures on wild populations and to improve the efficiency of applied methods of rearing. This work has been funded by the *MarMaCul* project from the Fundación Biodiversidad (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente). The experience was carried out at Veral rearing facility (Consellería de Medio Ambiente, Xunta de Galicia).

# ASSESSING THE IMPACT OF CONTAMINANTS OF EMERGING CONCERN ON THE HEALTH AND REPRODUCITON OF FRESHWATER MUSSELS: TOOLS TO INFORM RESOURCE MANAGEMENT

Mandy Annis<sup>1</sup> & <u>Daelyn A. Woolnough</u><sup>2</sup>. <sup>1</sup>U.S. Fish and Wildlife Service, Michigan Ecological Services Office, East Lansing MI 48823. <sup>2</sup>Department of Biology and Institute for Great Lakes Research, Central Michigan University, Mt. Pleasant MI, 48859.

# Poster 23

Contaminants are considered a factor in freshwater mussel decline with increasing interest in the impacts of contaminants of emerging concern (CEC). CECs including pharmaceuticals, personnel care products, manufacturing by-products, and new agricultural chemicals are found in complex mixes in all landscapes including mussel habitats that support species of concern, yet the impacts of CECs are unknown. Research often does not accurately reflect real world exposure of mussels with complex lifecycles and long lives. Further, research regarding sub-lethal, populationlevel impacts of CECs found at non-lethal levels are limited. Understanding and non-lethally assessing the impacts of CECs on declining populations is vital to species conservation. With funding through the Great Lakes Restoration Initiative, the US Fish and Wildlife Service and Central Michigan University, among others, have partnered to develop tools which begin to address these knowledge gaps. Studies include mussel and host fish surveys, stream side exposures of mussels to naturally experienced CEC gradients, and laboratory exposures of mussels and host fish to ecologically relevant chemical mixes through brooding, host fish interaction, and transformation. Biological, behavioral, and contaminants data have been collected and will be evaluated to assess mussel health in relation to CECs. Non-lethal tool development includes adapted biological sampling, and population and hazard assessment modeling. These studies will provide information regarding potential population-level impacts of CEC mixtures enabling managers to select optimal conservation and management practices in order to avoid further population declines, future listing of imperiled species, and loss of trust resources.

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Attend	

Aloisi	Doug	Genoa National Fish Hatchery- USFWS	doug_aloisi@fws.gov
Baran Dagendesh	Angela	Genoa National Fish Hatchery- USFWS	angela_baran_dagendesh@fws.gov
Boedeker	Nancy	Indiana Department of Natural Resources	nboedeker@dnr.in.gov
Bradley	Megan	USFWS- Genoa National Fish Hatchery	megan_bradley@fws.gov
Buntin	Michael	Alabama Aquatic Biodiversity Center	michael.buntin@dcnr.alabama.gov
Butitta	Vince	Center for Limnology	vincent.butitta@wisc.edu
Byrne	Caitlin	OSU	byrne.88@osu.edu
Carey	Caitlin	Virginia Tech, CMI/FiW	cscarey@vt.edu
Carlson	Brian	AllStar Ecology	brian@allstarecology.com
Carnegie	Ryan	Virginia Institute of Marine Science	carnegie@vims.edu
Carney	Joseph	Lakehead University, Biology Department	jcarney@lakeheadu.ca
Castrillo Arias	Pedro Antonio	Universidade de Santiago de Compostela	pedroantonio.castrillo@usc.es
Chance	Stephanie	USFWS - TN Ecological Services Field Office	stephanie_chance@fws.gov
Christian	Alan	University of Massachusetts Boston	alan.christian@umb.edu
Ciparis	Serena	US Fish and Wildlife Service	serena_ciparis@fws.gov
Clayton	Janet	WV Division of Natural Resources	janet.l.clayton@wv.gov
Colletti	Sarah	VA Dept. of Game and Inland Fisheries	sarah.colletti@dgif.virginia.gov
Cope	W. Gregory	North Carolina State University	greg_cope@ncsu.edu
Dahlberg	Angelique	St. Croix River Association	angeliqued@scramail.com
Dare	Jason	Dare Ecosystem Management	jason_dare@hotmail.com
Davis	Ben	USFWS	benjamin_davis@fws.gov
DeMartini	Jessi	Forest Preserve Dist. DuPage County II.	jdemartini@dupageforest.org
Dorman	Rebecca	USGS, Columbia Environmental Research Center	rdorman@usgs.gov
Douglas	Barbara	USFWS	barbara_douglas@fws.gov
Douglass	Sarah	Illinois Natural History Survey	sabales@illinois.edu
Dunn	Christopher	UW-Madison	cddunn2@wisc.edu
Dunn	Heidi	EcoAnalysts, Inc.	hdunn@ecologicalspecialists.com
Eckert	Nathan	US Fish & Wildlife Service	nathan_eckert@fws.gov
Erickson	Sara	U.S. Fish & Wildlife Service	sara_erickson@fws.gov
Everhart	Michael	West Virginia Division of Natural Resources	mike.e.everhart@wv.gov
Faiman	Scott	MDC	scott.faiman@mdc.mo.gov
Finney	Sam	USFWS	sam_finney@fws.gov
Ford	David	Ecological Specialists, Inc.	dford@ecologicalspecialists.com
Fritts	Andrea	U.S. Geological Survey	afritts@usgs.gov
Ganser	Alissa	Virginia Tech	alissamganser@gmail.com
Glidewell	Elizabeth	(CTUIR) Confederated Tribes of the Umatilla Indian Reservation	ElizabethGlidewell@CTUIR.org
Goldberg	Tony	University of Wisconsin-Madison	tony.goldberg@wisc.edu
Grossman	Emily	EcoAnalysts, Inc.	egrossman@ecologicalspecialists.com
Haag	Wendell R	U.S. Forest Service	whaag@fs.fed.us
Haas	Jeremiah	Exelon	jeremiah.haas@exeloncorp.com
Halmbacher	Jacqualyn	The Ohio State University	halmbacher.2@osu.edu
Hampton	Dave	FWS	dave_hampton@fws.gov
Hazelton	Peter	Massachusetts Division of Fisheries & Wildlife	peter.hazelton@state.ma.us
Hern	Tyler	United States Fish and Wildlife Service	tyler_hern@fws.gov
Hoch	Rachael	North Carolina Wildlife Resources Commission	rachael.hoch@ncwildlife.org
			<u> </u>

Attendee List Intihar	Jim	Forest Preserve District of DuPage County	jintihar@dupageforest.org
Jackson	Katelyn	EA Engineering, Science, and Technology, Inc, PBC	kjackson@eaest.com
Jenkinson	John	FMCS Member	jjjenkinson@hotmail.com
Johnson	Jennifer	Michigan DNR Fisheries	johnsonj17@michigan.gov
Kenney	Aleshia	USFWS	aleshia_kenney@fws.gov
Kern	Morgan	Missouri State University	mkern03@gmail.com
Kitchel	Lisie	WDNR - BER	lisie.kitchel@wisconsin.gov
Knowles	Susan	National Wildlife Heath Center	sknowles@usgs.gov
Kunz	James	Columbia Environmental Research Center	jkunz@usgs.gov
Lane	Tim	Virginia Department of Game and Inland Fisheries	tim.lane@dgif.virginia.gov
Lavictoire	Louise	Missouri State University	louiselavictoire@hotmail.co.uk
Lawlis	Clarissa	Lewis Environmental Consulting, LLC	clarissa_lawlis@yahoo.com
Lennon	Tiernan	USFWS	tiernan_lennon@fws.gov
Leis	Eric	USFWS Midwest Fisheries Center	eric_leis@fws.gov
Lewis	Chad	Lewis Environmental Consulting, LLC	lewis_environmental@yahoo.com
Lewis	Teresa	USFWS Midwest Fisheries Center	teresa_lewis@fws.gov
Limpers	Joe	Forest Preserve District of DuPage County	jlimpers@dupageforest.org
Matthews	Mickey	Arkansas Dept of Transportation	mickey.matthews@ahtd.ar.gov
Maynard	Amy	Virginia Department of Game and Inland Fisheries	amy.maynard@dgif.virginia.gov
Mazzacano	Celeste	CASM Environmental, LLC	cmazzacano@gmail.com
McComb	Sophia	Sycamore High School Watershed	mccombsoph@gmail.com
McCormick	Madeline	Sycamore High School/Student	madeline6520@gmail.com
McElwain	Andrew	SUNY Oswego	andrew.mcelwain@oswego.edu
McMurray	Stephen	MO Dept. of Conservation	stephen.mcmurray@mdc.mo.gov
Miller	Phil	Department of Veterinary Population Medicine	
Millsap	Deborah	US FWS	deborah_millsap@fws.gov
Minerich	Ben	Minnesota Zoo	ben.minerich@state.mn.us
Molloy	Daniel	Molloy & Associates, LLC	dan@danielpmolloy.com
Monroe	Emy	US Fish & Wildlife Service	emy_monroe@fws.gov
Morrison	Patricia	USFWS Retired	pearlymussel@gmail.com
Mosier II	Dan	Kansas Dep. of Wildlife, Parks & Tourism	dan.mosier@ks.gov
Moss	Lindsey	TRC	lmoss@trcsolutions.com
Nemeth	Doug	Idaho Fish and Wildlife Conservation Office	douglas_nemeth@fws.gov
Newton	Teresa	USGS	tnewton@usgs.gov
Ohlman	Lindsay	University of Nebraska	lohlman@unl.edu
Ostby	Brett	Daguna Consulting, LLC	ptychobranchus@gmail.com
Parsons	Edward	Greensboro Science Center	eparsons@greensboroscience.org
Patterson	Matthew	USFWS	matthew_patterson@fws.gov
Penn	Michael	USFWS - Lamar Fish Health Center	michael_penn@fws.gov
Phelps	Nick	Minnesota Aquatic Invasive Species Research Center	phelp083@umn.edu
Primus	Alex	Department of Veterinary Population Medicine	primu012@umn.edu
Riccardi	Nicoletta	CNR Institute of Ecosystem Study	n.riccardi@ise.cnr.it
		• • •	

US Fish and Wildlife Service

Richard

Jordan

 $jordan\_richard@fws.gov$ 

# Attendee List

Attenuce List			
Rosenberger	Amanda	USGS Missouri Cooperative Fish and Wildlife Research Unit	rosenbergera@missouri.edu
Roznere	Ieva	The Ohio State University	roznere.1@osu.edu
Runstrom	Ann	U.S. Fish and Wildlife Service	ann_runstrom@fws.gov
Sasson	Anthony	The Nature Conservancy	asasson@aol.com
Schmuecker	Sara	U.S. Fish and Wildlife Service	sara_schmuecker@fws.gov
Scoggin	Dan	Ecoanalysts, Inc.	scogdw@hotmail.com
Seagraves	Sara	US Fish and Wildlife Service	sara_seagraves@fws.gov
Secrist	Zebulin	Dept. of Natural Resources	zeb.secrist@state.mn.us
Sietman	Bernard	Minnesota DNR	bernard.sietman@state.mn.us
Sneed	Lesley	Kentucky State University	lesley.sneed@kysu.edu
Taskinen	Jouni	University of Jyväskylä	jouni.k.taskinen@jyu.fi
Tiemann	Jeremy	Illinois Natural History Survey	jtiemann@illinois.edu
Urbańska	Maria	Poznań University of Life Sciences, Institute of Zoology	urbanska@up.poznan.pl
Vaughn	Chris	San Antonio River Authority	cvaughn@sara-tx.org
Waller	Diane	USGS-UMESC	dwaller@usgs.gov
Watson	Brian	VA Dept of Game & Inland Fisheries	brian.watson@dgif.virginia.gov
Weinzinger	Jesse	Wisconsin DNR	jesse.j.weinzinger@gmail.com
Wengstrom	Niklas	University of Gothenburg	niklas.wengstrom@bioenv.gu.se
Wolf	Tiffany	Department of Veterinary Population Medicine	wolfx305@umn.edu
Woolnough	Daelyn	Central Michigan University	daelynw@gmail.com

From the City of La Crosse and the 2018 Workshop Planning Committee:

# Thank you for making the2018 Workshop a Success!

