



**West Virginia Cooperative Fish
and Wildlife Research Unit**

FY 2019

ANNUAL REPORT

1 October 2018 - 30 September 2019



COOPERATING AGENCIES:

U.S. Geological Survey
West Virginia Division of Natural Resources
West Virginia University
U.S. Fish and Wildlife Service
Wildlife Management Institute

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Introduction and History: The West Virginia Cooperative Fish and Wildlife Research Unit

The West Virginia Cooperative Fish and Wildlife Research Unit was formed at West Virginia University on July 1, 1986. The Coop Unit is housed in Percival Hall within the Division of Forestry and Natural Resources, Davis College of Agriculture, Natural Resources and Design. It is an integral part of the Wildlife and Fisheries program within the Division of Forestry and Natural Resources.

One mission of the West Virginia Coop Unit is to address the research and technical needs of the West Virginia Division of Natural Resources, U. S. Geological Survey, U. S. Fish and Wildlife Service, and other natural resource agencies and organizations. Research and technical needs goals are met by pursuing funding for research projects, collaborating with cooperators on research projects, publishing and presenting research results, and participating in short courses and workshops for cooperators when appropriate.

The Coop Unit's research program is focused on environmental impacts at the species and ecosystem levels. Wildlife research projects focus on the effects of anthropogenic disturbances (timber harvesting, mountaintop mining, and Marcellus shale gas development) in forested systems on wildlife populations. Fisheries research projects focus on contaminants in West Virginia watersheds, brook trout restoration, rainbow trout aquaculture, and systematics and ecology of West Virginia fishes.

Graduate education is also an important mission of the Coop Unit. Cooperating with West Virginia University, the Coop Unit contributes to the quality education and training of graduate students in fisheries and wildlife at West Virginia University. Coop Unit scientists achieve educational goals by chairing graduate committees, serving on graduate committees, teaching graduate level courses and delivering guest lectures and seminars. As of September 30, 2019, 126 students have completed their degree requirements; 93 Masters and 33 Ph.D. The Unit scientists are currently supervising 8 Master's students and 4 Ph.D. students.



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UNIT STAFF

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Ph.D., Memphis State University, 1989. Stress physiology, Toxicology.

ASSISTANT LEADERS

Petra B. Wood, Adjunct Professor of Wildlife
Ph.D., University of Florida, 1992. Wildlife/habitat relationships, raptor ecology and management.

Stuart A. Welsh, Adjunct Professor of Fisheries
Ph.D., West Virginia University, 1997. Fisheries ecology, zoogeography and systematics.

STAFF

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PROJECT COOPERATORS

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John Perez, National Park Service
Alan Temple, U.S. Fish & Wildlife Service
Dave Smith, U.S. Geological Survey
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Todd Fearer, Appalachian Mountains Joint Venture
Christopher Good, Freshwater Institute
Joe Hankins, Freshwater Institute
Jeff Larkin, Indiana University of Pennsylvania
Steve Latta, Pittsburgh National Aviary
Zachary Loman, University of Maine
Zac Loughman, West Liberty University
Pat Rakes, Conservation Fisheries, Inc.
Michael Schwartz, Freshwater Institute
J.R. Shute, Conservation Fisheries, Inc.
Steve Summerfelt, Freshwater Institute

STUDENTS

<u>STUDENT</u>	<u>DEGREE</u>	<u>GRADUATION DATE</u>	<u>ADVISOR</u>
Hannah Clipp	Ph.D.	Expected May 2022	Petra Wood/Chris Rota
Sara Crayton	M.S.	Completed Aug 2019	Petra Wood/Donald Brown
Sara Crayton	Ph.D.	Expected Dec 2023	Petra Wood/Donald Brown
Mack Frantz	Ph. D.	Completed May 2019	Petra Wood
Cory Hartman	M.S.	Expected Dec 2019	Pat Mazik
Brin Kessinger	M.S.	Expected May 2020	Stuart Welsh/Amy Welsh
Eric Margenau	Ph. D.	Expected Aug 2020	Petra Wood
Kyle Matt	M.S.	Expected May 2021	Stuart Welsh
Mark Matsche	Ph.D.	Expected May 2022	Pat Mazik
Gretchen Nareff	Ph. D.	Completed Dec 2019	Petra Wood
Lincoln Oliver	M.S.	Expected Dec 2020	Petra Wood/Chris Lituma
Nathaniel Owens	M.S.	Completed May 2019	Stuart Welsh
Lenza Paul	M.S.	Expected May 2020	Donald Brown
Brian Rolek	Ph. D.	Completed Dec 2018	Petra Wood/Cyndy Loftin
Joseph Siegel	M.S.	Expected May 2021	Stuart Welsh
Dustin Smith	Ph.D.	Completed Dec 2018	Stuart Welsh
Kristin Stockton	M.S.	Expected May 2020	Pat Mazik
MacKenzie Ullman	M.S.	Expected May 2021	Stuart Welsh
Emily Underwood	M.S.	Completed Dec 2018	Pat Mazik
Thomas Waldrop (NCTC)	M.S.	Expected Dec 2019	Pat Mazik
Heather Walsh	Ph. D.	Completed Dec 2018	Pat Mazik

UNIT STAFF COURSES TAUGHT

Patricia M. Mazik, Adjunct Professor of Fisheries

Aquatic Toxicology Fall 2018 3 credits

Stuart A. Welsh, Adjunct Professor of Fisheries

Advanced Ichthyology Fall 2018 3 credits

Petra B. Wood, Adjunct Professor of Wildlife

Wildlife and Fisheries Graduate Seminar Spring 2019 1 credit 29 Students

PROGRAM DIRECTION STATEMENT

The West Virginia Cooperative Fish and Wildlife Research Unit was established at West Virginia University on 1 July 1986. The Unit Leader began on 13 April 1987, and both Assistant Leaders were in place by 14 September 1987. The Unit is housed within the Division of Forestry and Natural Resources, College of Agriculture, Natural Resources, and Design. Offices and laboratories are located in Percival Hall.

In addition to involvement from WVU, coordination of the Unit is guided by cooperators from the WV DNR, Wildlife Resources Section Chief, the USFWS, USGS, and the Wildlife Management Institute. The mission of the Unit is: (1) to research problems affecting wildlife and fisheries; (2) to train the next generation of wildlife and fisheries biologists; and (3) provide training and technical assistance to cooperators, especially the state fish and wildlife agency.

The purpose of this document is to identify those general areas of fish and wildlife research that are most appropriate for study by the Unit. It is not a proposal for specific projects, but rather a definition of the types of areas of research most appropriate for the Unit given the expertise and facilities available.

The research mission of the Unit is to address fish and wildlife problems of mutual interest to all cooperators. Graduate education is also a mission. Studies will be accomplished by graduate research associates, research associates, technicians, non-thesis graduate students, graduate students working on separate thesis topics, or cooperating faculty members.

Most broadly interpreted, the cooperative agreement establishing the Unit provides access to expertise from among all segments of the University and other cooperators. However, most research will be directed by the Unit staff (Leader and Assistants) and those cooperating faculty members conducting research related to fish or wildlife resources.

There is a long-standing wildlife program in the Division of Forestry and Natural Resources, studying a broad range of terrestrial ecology problems, ranging from traditional population studies of wildlife species, to effects of forestry practices on wild animals, to social aspects of wildlife management. The Unit will enhance the wildlife and fisheries program by emphasizing research on wildlife/forestry issues inherent to West Virginia.

Research conducted through the Unit should stress functional responses of terrestrial and aquatic communities to management actions or environmental impacts. That is, we will attempt to determine how and why populations respond rather than simply to document or quantify responses. A study that evaluates management actions or examines ecological processes usually results in increased understanding of fish and wildlife community ecology and, thus has broader application than the immediate problem of concern.

Most of the Unit's research should be conducted within West Virginia or the bordering states. We will consider those occasional research opportunities that arise in areas remote to the state if they are of broad importance, or if they are logically undertaken most effectively by the West Virginia Unit.

COMPLETED PROJECTS

AQUATIC

DISTRIBUTION AND HABITAT USE OF BENTHIC FISHES OF THE KANAWHA RIVER, WEST VIRGINIA

Student Investigator: Nathaniel Owens

Principal Investigator: Stuart Welsh

Years Ongoing: 2017 - 2019

Degree Program: MS

Completed: May 2019

Funding Sources: West Virginia Division of Natural Resources

Objectives:

1. Model benthic habitat use of fishes in the Kanawha River
2. Determine benthic fish assemblages associated with island habitats in the Kanawha River
3. Document distribution data of benthic fishes within the Kanawha River

Summary:

The Kanawha River, a 6th order tributary to the Ohio River located in south-central West Virginia, is formed by the confluence of the New and Gauley rivers just above Kanawha Falls near Glen Ferris, WV. This river is a historically diverse system in terms of ichthyofauna although like many other large rivers, it has been subjected to a suite of anthropomorphic perturbations. These include this system being modified to allow the passage of commercial vessels for industry thus altering flow regimes, increased sedimentation, water depth, and channelization, and decreased interactions with its floodplain. Additionally, the fishes of this system have suffered from deleterious historical water quality issues caused by intensive timber operations, mining, and industrial waste. Post Clean Air Act, Clean Water Act, and Surface Mining Control and Reclamation Act water quality has substantially improved and assessments of pelagic fishes within this system indicate that they have responded positively. The distribution of benthic fishes within the Kanawha River, particularly in relation to island habitat, is not well understood. This study implemented benthic otter trawling with the Gerken Siamese Trawl (Innovative Net Systems) to document the distributions and habitat use of benthic fishes within the Kanawha River proper.

By implementing benthic otter trawling we were able to expand the known distributions of many of West Virginia's benthic fishes within the Kanawha River from collections taken during the summers and falls the years 2017 and 2018 (n = 328) throughout the Robert C. Byrd, Winfield, Marmet, and London Pools. These samples were collected in nine different habitats (Figure 1). A

total of 49,118 individuals were collected representing 53 species across 10 families. Seven of these species had previously been undocumented in the Kanawha River proper (Table 1; Figure 2) raising the species richness by 7% (e.g., from 94 to 101) for the Kanawha River proper.

Canonical correspondence analysis (CCA) was used to explore associations between benthic fishes (log-transformed catch per unit effort data from 16 species) and habitat parameters that were influenced by both physical factors and parameters influenced by water quality. After removing any variable pairs that exhibited high levels of correlation (i.e., $>|0.70|$) forward model selection forward model selection ($\alpha = 0.05$) the number of parameters were reduced from $K=25$ to $K=16$. After model fitting permutation Pseudo-F tests were conducted to evaluate statistical significance for the CCA model globally, for each axis respectively, and for each variable included in the model. Partial CCAs (pCCA) were conducted to determine the amount of variance associated with parameters consistent with physical variable and associated with water quality.

Environmental variables included in the final model explained 37.2% of the total variation in the species dataset $\log(\text{CPUE}+1)$ (i.e., 1.07 of the total inertia of 2.88). The first three axes of the CCA collectively contained 26.89 % of the total explained variation (e.g., constrained inertia) with 15.84%, 6.88%, and 4.16% for axis 1, 2, and 3, respectively. Axes 1, 2, and 3 consisted of eigenvalues of 0.456, 0.198, and 0.119, respectively, of the total 1.070 constrained inertia. Permutation tests of the constrained axes 1, 2, and 3 supported statistical significance (Pseudo – F statistics = 73.1, 31.8, and 19.2 for CCA axes 1, 2, and 3 respectively; p-values = 0.0009 for axes 1,2, and 3).

Axis one (15.8% of constrained inertia) was driven primarily (e.g., $r^2 \geq 0.2$) by distance to mouth (rkm) and distance to dams both upstream and downstream dams. Secondary explanatory gradients of axis 1 ($r^2 \geq 0.05$) consisted of physical habitat variables ISCB, IMCB, MC, depth, fine substrates, and water quality parameters of temperature and secchi depth (i.e., turbidity), and bottom water temperature. Axis 2 (6.88% of constrained inertia) primary being driven by distance to downstream dams and secondarily IMCB, rkm, and pH. Axis 3 (4.16% of constrained inertia) was most influenced by the average depth and average velocity secondarily it was influenced by habitats IH and SCB, distance to downstream dam, and distance to upstream dam, and pH.

Results of the canonical correspondence analysis indicated that physical habitat (not water quality) was the most influential factor regulating the distribution of fishes in the Kanawha River. Conversely, factors contributing to the physical habitat of the system are likely the most influential factors to lotic fishes in this system (i.e., 75.20 of the constrained variation was attributed to physical predictors). Evidence of longitudinally differing habitats exists along the Kanawha River, likely governed by the distances from upstream and distances from downstream dams as well as distance the Kanawha River's confluence with the Ohio River. Relative distance to mouth likely contributes to discharge (i.e., as you move further upstream there is less mean annual discharge). Dams have shown to have similar effects on fish assemblage composition and individual species' habitat use by being one of the major factors contributing to the construction of longitudinal substrate distribution as dominant fine substrates were positively correlated to distance from dams ($r = 0.478$, p-value = <0.0000).

In addition to distance to the river mouth (rkm) and relative distance to upstream and downstream dams, islands also had a strong influence on fish assemblages. My results, consistent with previous studies, suggest that habitats around islands provide unique functional process zones (i.e., areas of unique hydro and fluvial geomorphic characteristics) that promote habitat heterogeneity (i.e., variations in depth, flow, substrate, and vegetation) necessary for the life history of certain fishes (i.e., paddlefish, sturgeon, darters, catfishes, and others). All percid species (i.e., darters) that were collected in adequate frequencies (e.g., $\geq 10\%$ of all samples) were associated with islands in terms of their greatest relative abundance, suggesting that island areas should be of conservation focus. Island habitats provide “islands” of habitat heterogeneity within relatively homogenous navigable systems, where reduced access to respective floodplains results from channelization and altered natural flow regimes.

This study documented several state SGCN within lower Kanawha River proper that were previously undocumented (Table 1) through historic fish collection techniques (i.e., electrofishing, seining, rotenone, and gill nets). Because a high likelihood exists that small-bodied benthic fishes will be undetected by traditional collection techniques in navigable river systems it may be necessary to include a standardized benthic trawling method for an accurate biological assessment of river fish species richness. Additionally, because our data demonstrated an association with island habitats and speciose benthic fish assemblages, as well as more robust populations of several benthic fishes, managers should consider including the sampling of island habitats to complement previously developed large river Indices of Biotic Integrity. By adding benthic trawling to non-wadeable river health assessments, we may get a more holistic view of the fish assemblages and their interactions with the aquatic communities.

Table 1. Species collected representing new distribution and species richness records for the Kanawha River. SGCN = Species of Greatest Conservation Need.

Count	Common Name	Species Name	Abundance	SGCN
1	Western Sand Darter	<i>Ammocrypta clara</i>	16	Y
2	Tippecanoe Darter	<i>Etheostoma tippecanoe</i>	21	Y
3	Blue Catfish	<i>Ictalurus furcatus</i>	32	-
4	Gilt Darter	<i>Percina evides</i>	2	Y
5	Longhead Darter	<i>Percina macrocephala</i>	7	Y
6	Slenderhead Darter	<i>Percina phoxocephala</i>	15	Y
7	River Darter	<i>Percina shumardi</i>	7	Y

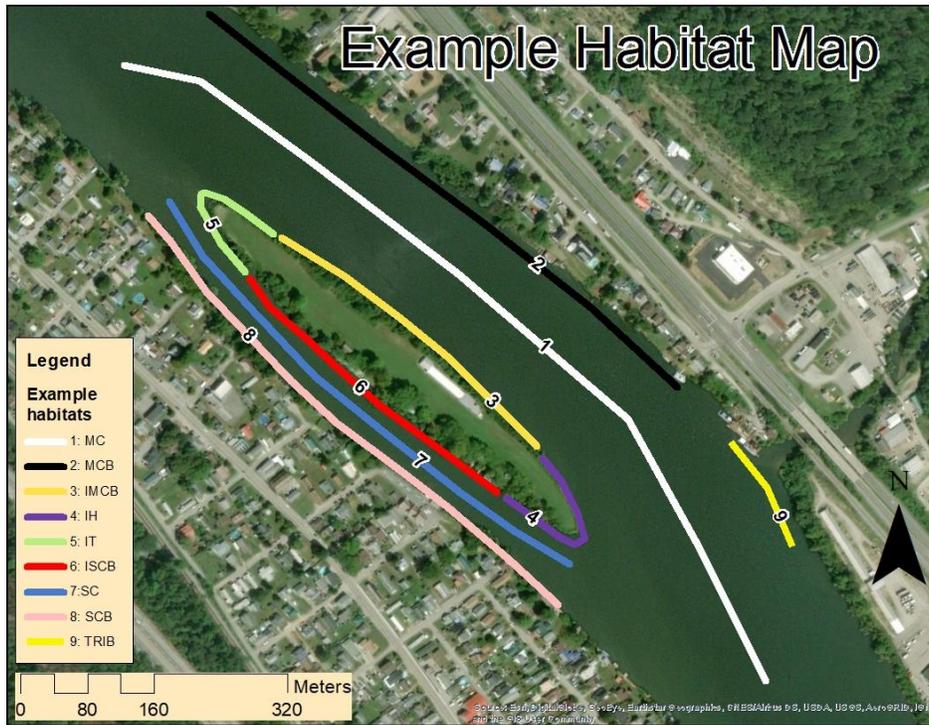


Figure 1. Map of various habitats sampled consisting of: 1. Main Channel (MC), 2. Main Channel Border (MCB), 3. Island Main Channel Border, 4. Island Head (IH), 5. Island Toe (IT), 6. Island Side Channel Border (ISCB), 7. Side Channel, 8. Island Side Channel Border (ISCB), and 9. Tributary (TRIB).



Figure 2. Blue Catfish (*Ictalurus furcatus*) from the 2018 benthic trawl study on the Kanawha River, West Virginia.

**AN INTERSPECIES INVESTIGATION OF THYROID PLASMA HORMONE CONCENTRATIONS,
HISTOLOGY, AND GENE EXPRESSION**

Student Investigator: Emily Underwood

Principal Investigators: Dr. Patricia Mazik

Collaborator: Dr. Vicki Blazer

Years Ongoing: 2017-2018

Degree Program: MS

Completed: December 2018

Funding Source: U.S. Geological Survey (RWO 68)

Justification:

A high prevalence of endocrine disrupting chemicals (EDCs) in Great Lakes Areas of Concern has raised concerns as to the health and fitness of fish and wildlife. It is not well understood to what extent existing contaminants, many with continuing inflows into the environment, may impact fish populations. This study provides an initial characterization of thyroid endocrine-related effects in two indigenous fish species sampled from Great Lakes AOCs. Biomonitoring was conducted on a pelagic, top predator species, smallmouth bass (*Micropterus dolomieu*) and benthic, omnivorous brown bullhead (*Ameiurus nebulosus*) at 7 sites in spring and fall of 2012. Thyroidal endpoints, such as plasma hormone levels (plasma thyroid stimulating hormone—TSH, thyroxine—T4, and triiodothyronine—T3), thyroid histology (thyroid epithelial cell height and colloid depletion), and expression levels of thyroid-related genes (Thyroid receptor alpha—THR α , Thyroid receptor beta—THR β , deiodinase type I—DIO1, and deiodinase type II—DIO2) were measured in both species, and relationships between the endpoints were evaluated to see if associations exist between thyroid endpoints at multiple biological levels.

Objectives:

1. Determine associations between thyroid-related endocrine disruption endpoints
 - a. Investigate histological features of thyroid follicles and to determine whether morphological changes are associated with the functional activity of the follicles
 - b. Investigate whether hepatic gene expression profiles can help explain histologic or hormonal responses.

2. Evaluate the potential of SMB and BBH as bioindicator species of thyroid disruption.

Results:

Fish were sampled in the Great Lakes AOC in the spring and fall (Figure 1). Histological evaluation of the thyroid tissue indicated hyperstimulation (as indicated by increased thyroid epithelial cell height and partially depleted colloid) in smallmouth bass and brown bullhead sampled in the spring. Despite observed histologic alterations, changes in thyroid gland histology did not coincide with changes in concentrations of circulating thyroid hormones. However, gene transcript abundance of THR α was negatively correlated with thyroid-stimulating hormone (TSH) and 3,5,3'-triiodothyronine (T3) while levels of deiodination enzymes were positively correlated with TSH and 3,5,3'-triiodothyronine (T3) in smallmouth bass, suggesting these genes are sensitive and stable indicators for thyroid status. The results demonstrate the importance of

using a multi-tiered approach to evaluate the potential risks of EDCs on the teleost thyroid system, as well as the importance of choosing sensitive species and accounting for seasonality.



Figure 1. Map of study sites in the Great Lakes basin. Sites were located in Lake Michigan (Fox River), Lake Erie (Detroit River, River Raisin), Lake Ontario (Rochester Embayment: Long Pond, Irondequoit), and Lake Superior (St. Louis Upper Estuary, St. Louis Middle Estuary).

DEVELOPMENT AND APPLICATION OF MOLECULAR PATHOLOGICAL METHODS TO ELUCIDATE MECHANISMS OF DISEASE IN WILD SMALLMOUTH BASS

Student Investigator: Heather L. Walsh

Principal Investigators: Dr. Patricia Mazik

Collaborators: Dr. Vicki Blazer, Dr. Luke Iwanowicz - USGS Leetown Science Center

Years Ongoing: 2014 - 2018

Degree Program: PhD

Expected Completion: December 2018

Funding Source: U.S. Fish and Wildlife Service (RWO 55, 61), U.S. Geological Survey (RWO 60)

Objectives

Objective 1. Since 2005, disease and mortality of young-of-year (YOY) smallmouth bass *Micropterus dolomieu* have been documented at numerous sites within the Susquehanna River basin, Pennsylvania. Smallmouth bass (SMB) is not a native species but have been established in the drainage since the 1870s and is important ecologically as a top predator. Prior to 2005, based on a State-wide, fixed-site sampling network which began in the late 1980s and conducted during the mid to late-July index period, signs of disease and mortality of YOY SMB had not been observed. However, mortality events since that time have resulted in low recruitment and a change in the population size structure shifting to older and larger fish. Initial observations suggested YOY disease and mortality resulting in reduced adult abundance were occurring in the

middle Susquehanna River (from Sunbury to York Haven, PA) and lower Juniata River from Port Royal to the confluence with the mainstem Susquehanna. However, since the initial observations, morbidity and mortalities have been documented in many other areas of the watershed, including smaller tributaries.

A number of avenues have been previously explored to determine the cause of disease and mortalities in YOY SMB. Culture-based diagnoses have isolated a variety of pathogens including *Flavobacterium columnare*, motile *Aeromonas* spp., *Pseudomonas aeruginosa* and largemouth bass virus. A re-described myxozoan parasite, *Myxobolus inornatus* has also been observed. Despite having knowledge of these findings, it is currently not known if one of these pathogens is more detrimental or more widespread; nor has the issue of co-infections been addressed.

Juvenile fish are especially susceptible to poor environmental conditions due to having immature immune systems. The multiple pathogens already identified suggest these fish may be exposed to environmental stressors and are unable to resist infections. Water quality factors such as increased temperature and low dissolved oxygen have been examined but do not consistently correlate with disease presence. Contaminant exposure has not, to date, been evaluated as a possible stressor. Based on previous findings, a more comprehensive examination into the effects of contaminant exposure and co-infections is needed. The objectives of this study were to 1) summarize the current state of knowledge; 2) describe and evaluate microscopic pathological changes observed over multiple years and 3) identify possible risk factors, including contaminant exposure and co-infections which may lead to decreased disease resistance.

Objective 2. In YOY SMB, one of the most frequently observed parasites is the myxozoan *Myxobolus inornatus*. This myxozoan infects the connective tissue of the muscle and plasmodium are often found below the dermis of the skin; however, large plasmodia can rupture the epidermis and create epidermal perforations. Previous studies have shown that primary infections with parasites that cause skin damage can lead to higher rates of subsequent infections with pathogenic bacteria. The presence of *M. inornatus* associated with subsequent bacterial infections remains unknown. One method that can be used to detect co-infections is in situ hybridization (ISH), which has been used in bacterial and myxozoan studies. More specifically, multiplex ISH can be used to detect multiple targets in one tissue sample. Performing multiplex ISH with probes designed to hybridize to bacteria, such as *Flavobacterium* spp. and motile *Aeromonads*, and *M. inornatus* may help identify whether this myxozoan is a cause of concern for subsequent bacterial infections.

Besides myxozoan-bacterial co-infections, many YOY SMB also had symptoms of a systemic infection in multiple tissues, including the liver, spleen, kidneys, gut, and muscle. Pathology of these infections included diffuse granulomatous inflammation with necrosis and resembled multifocal necrosis observed in laboratory reared YOY SMB exposed to largemouth bass virus. In wild YOY SMB, the causative agent has yet to be determined. In an effort to identify the pathogen in wild YOY SMB, these lesions can be cut out with a laser microscope and DNA sequencing can be conducted. Identification of the pathogen causing these systemic infections is necessary to determine its role in the disease and mortality events associated with YOY SMB.

One objective of this study is to determine whether co-infections of *M. inornatus* with *Flavobacterium* spp. and/or motile Aeromonads should be a cause of concern for disease in YOY SMB. A multiplex fluorescence ISH (FISH) assay will be developed to identify the presence of these co-infections. Additionally, tissues with areas of diffuse granulomatous inflammation will be cut out with laser capture microscopy (LCM) and sequenced to identify the presence of pathogen. Addressing these objectives will help to determine the pathogenicity of *M. inornatus* and the etiology of systemic infections and whether managerial actions can or should be enacted to help regulate these pathogens.

In addition to parasite infections, many YOY SMB also exhibit lesions and/or systemic, granulomatous infections. The cause of these infections remains unknown, but previous sampling has isolated *Aeromonas* spp., *Flavobacterium columnare*, and *Vibrio* sp. from bacterial swabs from both external and internal lesions. Largemouth bass virus (LMBV) has also been found in SMB from the Susquehanna River drainage, yet the effects of LMBV infection remains unknown. In order to determine the type of pathogen(s) present, sections of formalin fixed paraffin embedded (FFPE) tissue will be extracted for nucleic acids from sections cut out with a laser capture microdissection (LCM) microscope. However, optimization of LCM methods has not been worked out in our laboratory. Prior to using LCM for the detection of pathogens in YOY infections, determination of optimal extraction methods and amount of tissue to cut out for DNA and RNA extractions must first be conducted.

Objective 3. The types of parasites and pathogens involved in co-infections include trematodes in the muscle and liver, larval cestodes in the gut and liver, systemic infections, the myxozoan *Myxobolus inornatus* in the muscle connective tissue, pathogenic bacteria, and LMBV. In age-0 smallmouth bass, systemic infections and larval cestodes in the liver created the most damage to the infected tissues. Systemic infections consisted of diffuse granulomatous inflammation with necrosis in multiple tissues; similar to juvenile smallmouth bass experimentally exposed to LMBV. Liver parasites are a cause for concern since the liver is the main detoxifying agent in the body. In particular, larval cestodes (plerocercoids) have been observed to cause severe liver damage, including necrosis, inflammation, and tissue replacement. Additionally, cestode abundance has also been shown to respond positively to estrogenic exposure within the host. Similarly, age-0 smallmouth bass had a higher prevalence of larval cestodes in the liver at sites where more hormones were detected in the water.

As it turns out, location within the Susquehanna River drainage (i.e. land use effects) has an impact on co-infections in Age-0 smallmouth bass. There have been multiple studies documenting the effects of land use on parasitism in fish, particularly in regards to water quality. The diversity of parasite communities is also shown to vary based on the surrounding land use. In the Susquehanna River drainage, the dominant type of land use is forest, followed by agriculture and urban. It has been previously shown that prevalence in age-0 smallmouth bass in the Susquehanna River drainage was negatively correlated with urban land use and positively correlated with agriculture at the local scale. This study concluded that agricultural settings may provide more appropriate habitat for the intermediate host of the myxozoan and pesticides may have immunomodulatory effects on the fish. The objective of the current study is to conduct a similar modeling analysis (Bayesian hierarchical logistic regression model) to determine the influence of agriculture and urban land use variables on liver parasite and co-infection

prevalence. Since the liver parasites also have intermediate hosts that respond positively to agricultural settings, they may follow the same trends as observed with *Myxobolus inornatus*. The same could be likely with co-infections considering the same types of parasites with multiple hosts are included.

Objective 4. The use of formalin fixed, paraffin-embedded (FFPE) tissue has been regarded as a valuable reservoir of preserved nucleic acids. Many studies have utilized FFPE tissues for exploratory and diagnostic research in cancer, cellular mutations, parasite infections, and hormone regulation. Although FFPE tissues provide a vast source of pathologically diverse types of genetic material it has its drawbacks compared to other tissue preservation methods. Formalin fixation causes nucleic acids to fragment, degrade, and cross-link, which is less problematic for frozen tissues or tissues specifically preserved for downstream nucleic acid applications. Despite these difficulties, nucleic acids extracted from FFPE tissue have proven to be suitable for use in end-point PCR, real-time qPCR, and Next-generation sequencing. Optimization of FFPE tissues for downstream nucleic acid applications has been attempted in multiple studies by evaluation of different fixation methods, tissue handling and processing times, and extraction methods. Additionally, FFPE tissue has practicality especially when used with laser capture microdissection (LCM). Laser capture microdissection is the use of a microscope equipped with a laser which can be used to target specific cells from a heterogeneous population of cells. Single cells that are malignant, neoplastic, abnormal, or parasites can be microdissected while the surrounding normal tissue remains on the slide. The benefit of this is being able to directly analyze the biomolecules from specific populations of cells which is extremely useful in gene expression studies, transcriptome development, or parasite identification.

Laser capture microdissection has been used extensively for cancer, genetic biomarker identification, forensics, and plant cell research; however, it has been utilized less frequently in fish-related studies. Snap-frozen tissue is the best to use with LCM for the downstream recovery of nucleic acids. However, the use of snap-frozen tissue is not always applicable, particularly in wild fish studies where removal and fixation of the organs occurs in the field and can be days before they are returned to the laboratory. Optimal time for fixation has been shown to be around 24 hrs and longer fixation times, such as one week, have been shown to cause significant degradation to nucleic acids. This degradation results in lower quality and quantity nucleic acids which are significantly fragmented and less likely to be efficiently extracted.

In our laboratory, it can be up to two weeks before fixed tissue from wild fish is processed for histology. Smallmouth bass are often utilized in our studies as indicator species of contaminant exposure and are non-model species, so results are often novel. The aim of this study was to determine how fixative type and fixation time affects nucleic acids by using FFPE tissue cut with LCM. In an effort to address this question, smallmouth bass liver was sampled and fixed for multiple time periods in the fixatives 10% neutral buffered formalin (NBF) and Z-Fix™ (ZF), and the non-formalin fixative PAXgene (PG) in order to determine which fixation duration and which fixative type resulted in the best quality and quantity of nucleic acids. PAXgene was created to eliminate the effects of cross-linking and to improve nucleic acid integrity for downstream molecular analyses. Similarly, ZF (a type of zinc based formalin) was chosen since samples have been shown to produce higher yields of DNA and RNA when compared to samples

fixed in NBF. In addition to quantifying DNA and RNA, downstream analyses which included Nanostring nCounter® analysis and nested qPCR were used to determine if nucleic acids extracted from LCM tissue would have utility in our future studies.

Objective 5. Intersex, the presence of testicular oocytes, was first described in male smallmouth bass from the Potomac River basin in 2003 and from the Susquehanna River basin in 2007. Intersex is widely studied in fish and is used as a biomarker of exposure to endocrine disrupting chemicals (EDCs) released in wastewater treatment plant effluent, agricultural runoff, and industrial effluent. Reproductive effects such as reduced sperm motility and abundance have been observed in smallmouth bass; however, population-level effects and time of induction remain unknown. Additionally, smallmouth bass are the most commonly reported species of fish with intersex and it occurs more often in bass which inhabit waters with agricultural and industrial landuse.

Historically, histology has been used as the gold standard to visually identify the presence testicular oocytes; however, unless the target species is very small, histological preparation of the entire testes is not feasible. This is particularly true for mature smallmouth bass and therefore only a small number of tissue cross-sections are scanned microscopically. In order to provide more accuracy, techniques which incorporate molecular identification of genes associated with intersex are becoming more widely accepted. Such techniques include microarray analysis, next-generation sequencing, Sanger sequencing, and quantitative real-time PCR (qPCR).

Microarray analysis is oftentimes used in gene expression studies of fish however, the scope of this technique is limited since novel genes cannot be identified. The use of NGS, particularly RNA-Sequencing (RNA-Seq), on non-model fish for transcriptome development and biomarker gene discovery is highly valuable since existing sequence data is scarce. Unlike the abundance of sequencing data available for model species, such as zebrafish *Danio rerio*, fathead minnow *Pimephales promelas*, and medaka *Oryzias latipes*, there is limited data available for smallmouth bass. Currently, sequencing data exists for smallmouth bass liver and largemouth bass *Micropterus salmoides* gonad. Although gonad sequence data exists for largemouth bass, use of this data for smallmouth bass could lead to the exclusion or lack of identification of genes unique to intersex in smallmouth bass.

In this study, intersex and non-intersex males were compared with NGS from sites within the Potomac and Susquehanna River drainages (tributaries of the Chesapeake Bay) and from out-of-basin sites in the Presque Isle Bay drainage. Additionally, Nanostring nCounter® technology was used to analyze transcript abundance from specific genes that could be associated with intersex. The goal of this study was to 1) create a partial testes transcriptome to identify intersex biomarker genes for transcript abundance analysis 2) quantify reproductive endpoints, such as vitellogenin (VTG) and the gonadosomatic index (GSI), and 3) to analyze associations between intersex biomarker genes, reproductive endpoints, and intersex severity.

Results:

Objective 1. Young-of-the-year smallmouth bass that were included in this study were collected in the summer of 2007-2012. To better understand contributing factors of disease and mortality, fish collected from multiple areas throughout the watershed as well as reference populations were examined grossly and histologically for abnormalities. Tissue contaminant concentrations were determined from whole body homogenates and water contaminant concentrations were estimated using time integrated passive samplers at selected sites. Observed or isolated pathogens included bacteria, predominantly motile *Aeromonas* spp. and *Flavobacterium columnare*; largemouth bass virus, and parasites, including trematode metacercariae and the myxozoan *Myxobolus inornatus*. Although these pathogens were found in YOY SMB from multiple sites, no one pathogen was consistently associated with mortality. Chemicals detected in tissue included PCB congeners, organochlorine and current-use pesticides. The findings support the hypothesis that there is no single cause for disease of YOY SMB, rather the cumulative effects of co-infections and potential immunomodulation by environmental stressors during a sensitive developmental life stage may lead to mortality. Identifying the most important risk factors will be necessary for better management of the habitat and fish populations. This chapter has been published in the Journal of Aquatic Animal Health (Walsh, H.L., Blazer, V.S., Smith, G.D., Lookenbill, M., Alvarez, D.A., and Smalling K.L. 2018. Risk Factors Associated with Mortality of Age-0 Smallmouth Bass in the Susquehanna River Basin, Pennsylvania. Journal of Aquatic Animal Health 30: 65-80).

Objective 2. For in situ hybridization (ISH), probes specific for *M. inornatus* with a digoxigenin label were used in an assay that was successfully optimized to work for this parasite/probe pair. Fish preserved in Z-fix and PAXgene were used to determine which preservative was optimal for hybridization. It was revealed that Z-fix samples decaled with a hydrochloric acid solution are unsuitable for ISH since it causes extensive DNA damage and were therefore decaled with EDTA. With the use of ISH, early infections of *M. inornatus* or areas where no mature spores are present (inflammation only) were able to be identified. In addition to early-stage detection of *M. inornatus*, ISH was also used to identify co-infections with bacteria. Fluorescently labeled probes were made for *M. inornatus*, *Flavobacterium* spp. and *Aeromonas* spp. and tested as positive controls on tissue sections of fish that were used in exposure studies. Using YOY SMB from 2014, 139 fish were used in a multiplex FISH with *M. inornatus*, *Flavobacterium* spp., and *Aeromonas* spp. probes Only 4 (2.9%) exhibited co-infections of *M. inornatus* with *Flavobacterium* spp. or *Aeromonas* spp. These findings indicate that *M. inornatus* does not seem to contribute to secondary *Flavobacterium* spp. or *Aeromonas* spp. infections in YOY SMB from the Susquehanna River drainage.

Objective 3. In 2013-2016 up to 20 YOY SMB were sampled with backpack electroshockers at multiple sites within the Susquehanna River, Allegheny River, and Delaware River drainages in Pennsylvania. Subbasins within the Susquehanna River drainage included the West Branch, North Branch, Lower, and Juniata. Out-of-basin sites were located in the Allegheny and Delaware drainages and were originally included in previous studies due to the lack of observable disease in YOY SMB. In Maryland and West Virginia fish were sampled at sites located within the Potomac River drainage. Unlike previous sampling for YOY SMB that targeted only diseased fish, normal and diseased fish were collected. Fish were examined with

histopathology for liver parasite prevalence and co-infections (parasites and/or systemic infections observed in multiple organs). A Bayesian hierarchical logistic regression modeling approach was conducted to determine the impact of land use (agricultural or urban) on liver parasites and co-infections. The results showed that coinfection prevalence was positively predicted by agriculture and negatively predicted by development at the local NHD catchment scale. The best predicting variable for liver helminths was development at the local NHD catchment scale (probably negative) and it was weakly predicted to be positive with agriculture at both catchment scales. It is suggested that agricultural land use can increase the intermediate hosts of YOY SMB parasites, influence water quality (such as temperature and nitrification), and contribute to agriculture-associated contaminant runoff. Additionally, out-of-basin sites in Pennsylvania had lower rates of coinfections than sites in the Susquehanna and Potomac River basins. This type of data is useful for management decisions and can help guide future SMB conservation efforts to focus on land use at the local catchment scale rather than at a large scale.

Objective 4. In order to determine the amount of tissue necessary to acquire a quantifiable and usable amount of nucleic acids, pieces of liver were necropsied from adult smallmouth bass and fixed in 3 fixatives: Z-Fix®, PAXgene®, and 10% neutral buffered formalin. Tissues were fixed for 24hr, 48hr, 1 week, and 2 weeks in order to determine if differences in fixation times had an effect on nucleic acid quantity and quality. Sections were microtomed at a thickness of 8-10µm and cut with a laser on a laser capture microscope. DNA was extracted with TaKaRa DEXPAT™ Easy by incubating the samples at 95°C for 10 min and quantified on a Qubit® 2.0 Fluorometer using the Qubit™ dsDNA HS Assay Kit. RNA was extracted with an Omega Bio-Tek E.Z.N.A.® FFPE RNA Kit and quantified with a Qubit™ RNA HS Assay Kit. An Agilent 2100 Bioanalyzer was used to determine quality of DNA and RNA samples with the High Sensitivity DNA Kit and Agilent RNA 6000 Pico Kit, respectively. The concentration of extracted RNA and DNA was calculated by obtaining total RNA or DNA (quantity of DNA or RNA * volume of sample; ng) and dividing it by the amount of tissue cut out (mm³). Results showed that at 48hrs, the greatest amount of nucleic acids can be obtained from each of the three fixative types. Samples fixed with PAXgene® produced the greatest amount of nucleic acids. The quality of nucleic acids (as assessed by RIN scores for RNA and average size (bp) of DNA fragments) was similar amongst each fixative type for each fixation time. Additionally, nested PCR and Nanostring analyses were successfully conducted and showed little differences between fixative types.

Objective 5. In order to identify intersex biomarker genes, Next-generation Sequencing was run on pooled, normalized samples of intersex males, non-intersex males, and immature females. The addition of immature females was to include female reproductive genes in the transcriptome which might also be identified in intersex males. The data was checked for quality with FastQC and assembled with the de novo assembly program, Trinity. The reads were aligned back to the assembly with Bowtie to check for quality of assemblage. Since Trinity creates many false positives, the program Evigenes was used to remove redundant transcripts and dropped 81,851 transcripts, resulting in a final total of 50,892 transcripts. Transcripts were annotated with the program Diamond with a RefSeq protein database consisting of medaka, zebrafish, smallmouth bass, barramundi, and rainbow trout. Next-generation sequencing was also run on 16 individual samples consisting of eight intersex and eight non-intersex males. These reads were mapped to

the transcriptome and a count table of genes was generated in Blast2Go. Differential gene expression was done with the Bioconductor package, EdgeR. A total of 690 genes were found to be differentially expressed between intersex and non-intersex males. The number of differentially expressed genes (DEG) was narrowed down by excluding transcripts with a fold change of ≤ 1 and furthermore by excluding transcripts with counts lower than 50. In addition to DEG that represented female and male reproductive genes, intersex biomarker genes found in the literature for other fish species were identified in the transcript data and used to create a list of genes for a Nanostring codeset. The final codeset consisted of 40 potential intersex biomarker genes. Nanostring nCounter technology was used to create count data for each of the genes in the CodeSet from male smallmouth bass from integrator sites in the Potomac and Susquehanna River basins and from other sites in 2015-2017. Fourteen genes were found to be differentially regulated in intersex fish, including multiple upregulated genes that were associated with oocyte development and downregulated genes associated with male spermatogenesis and steroidogenesis. Intersex severity comparisons showed an increase in the number of transcripts that were differentially expressed between non-intersex fish and fish with more severe intersex. Additionally, there were 30 genes that were found to be correlated with intersex severity; 20 genes positively correlated and 10 genes negatively correlated. Again, many of the genes positively correlated with intersex severity were oocyte-related genes and many that were negatively associated with severity were involved in male reproductive function. Future laboratory exposure studies with various endocrine disrupting chemicals will need to be conducted to further validate these genes. Additionally, these biomarker genes can be used to determine when smallmouth bass are induced with intersex and to determine if they are associated with environmental contaminants.



WILDLIFE

STREAM SALAMANDER AND BENTHIC MACROINVERTEBRATE COMMUNITY RESPONSES TO IMIDACLOPRID EXPOSURE

Student Investigator: Sara Crayton

Principle Investigators: Petra Wood, Donald Brown, Yong-Lak Park

Cooperators: John Perez, Christine Arnott, Alice Millikin, Kang Mo Ku, Tyler Simpson, Kevin Eliason

Years Ongoing: 2017-2019

Degree Program: MS

Completed: September 2019

Funding Sources: USGS/NPS Water Quality Partnership Program, the West Virginia University Program to Stimulate Competitive Research, and the Foundation for the Conservation of Salamanders

Objectives:

1. Determine whether stream salamanders and stream invertebrates bioaccumulate imidacloprid that is applied for hemlock conservation or its metabolites and whether exposure to imidacloprid is related to sublethal effects in salamanders.
2. Relate abundances of adults and larval salamanders with imidacloprid presence in the environment.
3. Compare predictors of imidacloprid exposure to metrics of benthic macroinvertebrate abundances, diversity, biomass, and functional traits.

Background:

The greatest threat to both eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) in the United States and Canada is hemlock woolly adelgid (HWA, *Adelges tsugae*), an invasive aphid-like insect native to Asia. The most common control for HWA is the neonicotinoid insecticide imidacloprid. Imidacloprid is widely used for hemlock conservation despite a deficit of knowledge about the long-term impact imidacloprid may have on aquatic resources and the persistence of imidacloprid in soil and aquatic environments.

We conducted this study in the Monongahela National Forest (MNF) and two units of the National Park Service (NPS): Gauley River National Recreational Area (GARI) and New River Gorge National River (NERI) in West Virginia, USA. In April – July of 2017 and 2018, we sampled 48 headwater streams; 27 were exposed to imidacloprid treatments and 21 were not. We used ultra-performance liquid chromatography-tandem mass spectrometry ([UP] LC-MS/MS) to quantify the concentration of imidacloprid in stream water for all 48 sites, the concentration of two imidacloprid metabolites (imidacloprid-urea and imidacloprid-olefin) for 24 sites, and the presence/absence of imidacloprid and the two metabolites in the stream sediment of all 48 sites.

We sampled stream salamanders (Fig. 1) 5 – 7 times at all 48 sites from April – July of 2017 and 2018 using active searching methods. We weighed and measured all captured salamanders and calculated a body condition index (BCI) for each; a positive BCI indicated a salamander had higher-than-average weight per their length. After salamander sampling was completed in the NPS sites, we also collected and euthanized 168 salamanders of the genus *Desmognathus* from seven streams directly adjacent to HWA treatments and four streams not directly adjacent to HWA treatments. We quantified the concentrations of imidacloprid and the two metabolites within the tissues of 107 of these salamanders and concentrations of the stress hormone, corticosterone, in the tissues of 119 of these salamanders. Studies have documented increases in corticosterone levels in salamanders due to environmental stressors, and long-term elevation of corticosterone levels causes negative physiological effects such as immune suppression.



Figure 1. Two-lined salamanders captured during salamander sampling. Photo by Sara Crayton

Finally, we sampled benthic macroinvertebrates at the 48 sites using D-nets during 2017 – 2018. All macroinvertebrates were identified to genus or family and measured. We calculated metrics of biotic integrity, including GLIMPSS (Genus Level Indicator of Most Probable Stream Status), WVSCI (West Virginia Stream Condition Index), and biomass. After we completed benthic macroinvertebrate sampling, we also collected benthic macroinvertebrates and small crayfish from 16 sites adjacent to HWA treatments and quantified the concentration of imidacloprid and the two metabolites in invertebrate samples.

Results:

Imidacloprid was detected in stream water at 24 sites, with a mean concentration of 49.8 ± 20.2 ng/mL (range = 6.5 – 489.6). We detected imidacloprid-olefin in the water at two sites, both of which had detectable levels of imidacloprid. Imidacloprid-urea was not detected in the stream water at any site. Imidacloprid was detected in sediment at eight sites, five of which were adjacent to HWA treatments. We did not detect imidacloprid-olefin or imidacloprid-urea in the sediment at any site.

We detected imidacloprid bioaccumulation in the tissues of 14 *Desmognathus* spp. and the metabolite imidacloprid-olefin in the tissues of 19 *Desmognathus* spp. for a total of 29 salamanders with one or both chemicals. We detected imidacloprid in all 15 benthic macroinvertebrate/crayfish samples, with a mean concentration of 26.6 ± 3.7 ng/mL (range = 12.1 – 68.8) and imidacloprid-urea in 13 samples. We did not detect imidacloprid-olefin in any invertebrate samples. We identified sublethal effects in salamanders from imidacloprid exposure. Corticosterone in *D. monticola* and *D. fuscus* was positively associated with number of imidacloprid applications, and this effect was strongest for non-gravid females ($\beta = 0.0013$, 95% CI: 0.0005–0.0021; Fig. 2a,b). Salamander BCI decreased as concentration of imidacloprid in stream water increased ($\beta = -0.0009$, 95% CI: -0.0017 – -0.0001; Fig. 2c).

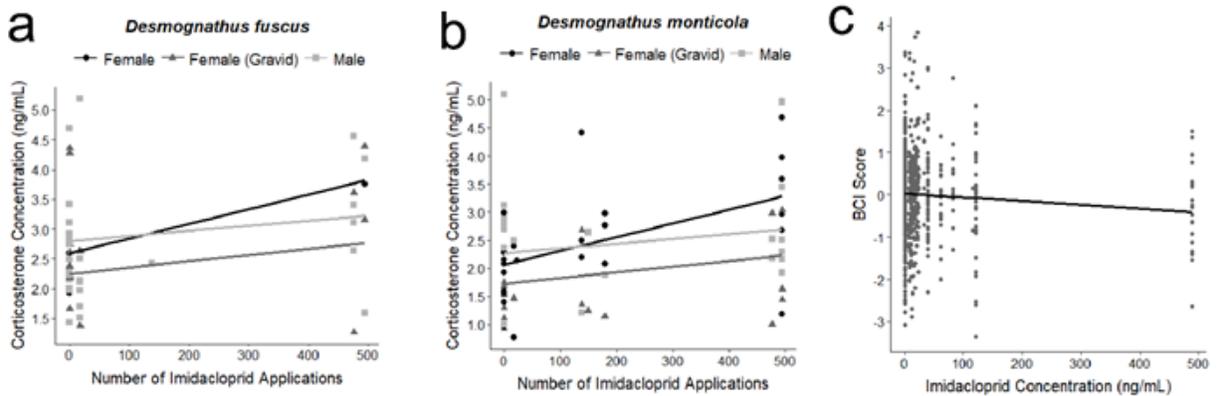


Figure 2. Model-estimated relationship between concentration of the hormone corticosterone and total number of imidacloprid applications for male, non-gravid female, and gravid female (a) *Desmognathus fuscus* ($n = 46$) and (b) *Desmognathus monticola* ($n = 69$). (c) Model-estimated relationship between standardized salamander body condition index (BCI) score and concentration of imidacloprid in stream water ($n = 802$ individuals representing 5 species).

The presence of environmental imidacloprid and presence of treated trees were influential predictors of multiple indicators of biotic integrity for benthic macroinvertebrates but the directional effects of presence of environmental imidacloprid differed depending on locality (MNF or NPS; Fig. 3). These differences in community responses may be due to historical land uses, such as differences in historical mining and logging in the two localities.

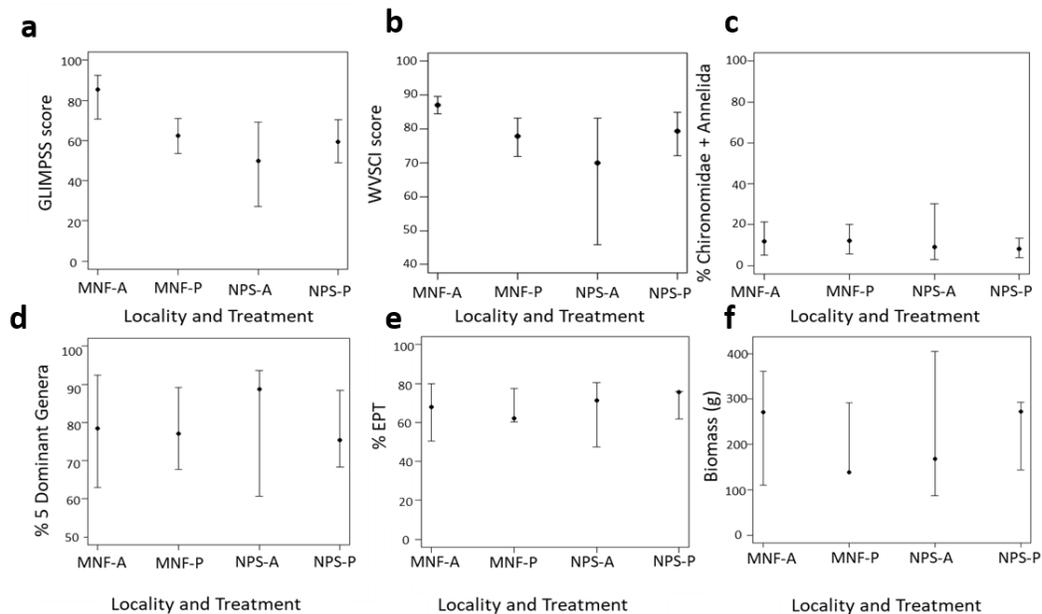


Figure 3. Model-estimated predictions and 95% confidence intervals (CI) for six benthic metrics for Monongahela National Forest sites without imidacloprid exposure (MNF-A; $n = 8$) and with imidacloprid exposure (MNF-P; $n = 16$) and for National Park Service sites without imidacloprid exposure (NPS-A; $n = 6$) and with imidacloprid exposure (NPS-P; $n = 17$).

Additionally, our study provides evidence that adult *Desmognathus* spp. and larval *Gyrinophilus porphyriticus* abundances may be negatively affected by HWA treatments, particularly number of imidacloprid applications and presence of treated trees. However, we did not detect negative effects of imidacloprid exposure on adult *Eurycea* spp.

**LOUISIANA WATERTHRUSH DEMOGRAPHIC, SPATIAL, AND EPIGENETIC RESPONSE TO
MARCELLUS SHALE GAS DEVELOPMENT IN WEST VIRGINIA FOREST HABITAT CREATION**

Student Investigator: Mack Frantz

Principal Investigator: Petra B. Wood

Cooperators: Amy Welsh, Steve Latta, George Merovich

Years Ongoing: 2013–2019

Degree Program: PhD

Completed: May 2019

Funding Sources: WV Division of Natural Resources, US Department of Energy, Pittsburgh National Aviary, Eastern Bird Banding Association, FCSL Association, West Virginia University, Appalachian Stewardship Foundation, USDA National Institute of Food and Agriculture

Objectives:

1. Determine how shale gas well development activities influence Louisiana Waterthrush (*Parkesia motacilla*) territory density and length, nest survival and productivity, nest abandonment, riparian habitat quality, apparent survival, site fidelity, and site fidelity factors.
2. After accounting for spatial and non-spatial sources of variability, examine how shale gas development influenced demographic response of waterthrush to aquatic prey changes in 2013–2014.
3. Using spatial stream network models, explore relationships between observed areas of waterthrush foraging and a) water chemistry, b) a waterthrush foraging score based on stream channel data, c) shale gas land use based on reach contributing area (i.e. catchment area), and d) multi-metric indices of biotic stream integrity at the family and genus level.
4. Examine how shale gas development may influence DNA methylation variation, hereafter differential methylation, of waterthrush between shale gas undisturbed and disturbed areas at the territory scale, and b) determine if differential methylation of DNA fragments or loci, hereafter restriction sites, varied by demographic characteristics such as sex and age.

Background:

The Louisiana Waterthrush (*Parkesia motacilla*), hereafter waterthrush, is a forested headwater stream specialist known for its ability to respond to changes in ecological conditions (Fig 1). Many of the forested stream ecosystems in which the waterthrush primarily breed have undergone unconventional shale gas development. Species with specialized terrestrial or aquatic habitat needs that overlap forested areas undergoing shale gas development may be the most vulnerable to disturbance. We examined how shale gas development influenced demographic, spatial, and epigenetic response of waterthrush during 2009–2011 and 2013–2015 on 58.1 km of 14 predominately forested headwater streams. Forest cover across the study area decreased from 95.3% in 2008 to 90.8% in 2015 while area affected by shale gas development increased from 0.4% to 2.4%.



Fig. 1. Louisiana Waterthrush, photo by Mack Frantz

Results:

For objective 1, we manually digitized areas of forest canopy disturbance within the study area for each year of the study. All forest canopy disturbances were classified as shale gas development related, unrelated, or pre-existing to examine how shale gas development influenced demographic response of waterthrush. We quantified nest survival, abandonment, and productivity, a source-sink threshold, riparian habitat quality, territory density and length, apparent survival, site fidelity, and site fidelity factors. Across years, we saw annual variability in nest survival with a general declining trend over time. After accounting for temporal effects, shale gas development had negative effects on nest survival. Population-level nest productivity

declined, and individual-level productivity was lower in areas disturbed by shale gas. A source-sink threshold suggested these areas are more at risk for being sink habitat (Fig 2).

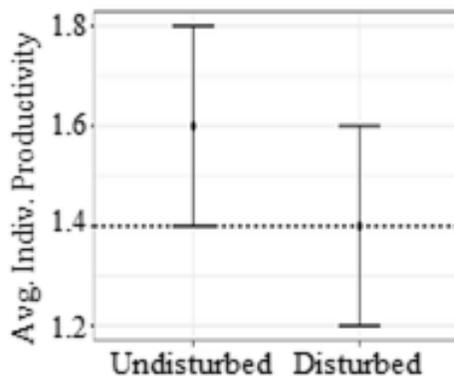
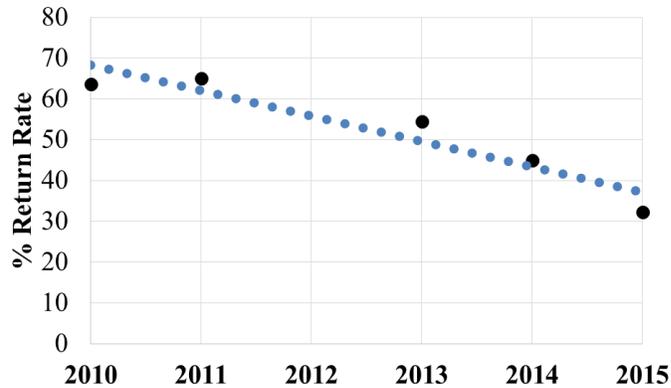


Fig 2. Average individual productivity (avg. # fledglings per successful nest per male * nest survival) \pm 95% CIs in areas undisturbed and disturbed by shale gas to a source-sink threshold (gray bracketed line) of 1.4 fledglings.

Riparian habitat quality scores, as measured by a US Environmental Protection Agency index (EPA) and a waterthrush Habitat Suitability Index (HSI), differed by year and were negatively related to the amount of each territory disturbed by shale gas development. Territory density was not related to the amount of shale gas disturbance on streams, but decreased over time as territory lengths increased. Waterthrush initially exhibited high site fidelity that declined over time. At the same time, the number of unpaired males defending territories increased as did natal fidelity. Females in shale gas disturbed areas had a higher number of breeding attempts and lower individual productivity. We saw a general nonsignificant trend in declining apparent survival over time (Fig. 3). Overall, our results suggest a decline in waterthrush site quality as



shale gas development increased, despite relatively small site-wide forest loss. Since waterthrush can maintain pair bonds from the previous year and females must pick a nest site within the defended male's territory, there are potential conflicts between factors that influence adult survival and site fidelity that may affect long-term population persistence.

Fig 3. Annual return rates of first year adults.

For objective 2, we related waterthrush demographic response and nest survival to benthic macroinvertebrate aquatic prey and to shale gas development parameters using models that accounted for both spatial and non-spatial sources of variability. In 2013, aquatic prey density and pollution intolerant genera decreased statistically with increased waterthrush territory length but not in 2014 when territory densities were lower. In general, most demographic responses to aquatic prey were variable and negatively related to aquatic prey in 2013 but positively related in 2014. Potential hydraulic fracturing runoff decreased nest survival both years and was statistically significant in 2014. EPA and HSI habitat quality scores were higher when aquatic prey metrics were higher, but EPA was more strongly linked than HSI and varied annually. While potential hydraulic fracturing runoff in 2013 may have increased Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, in 2014 shale gas territory disturbance decreased EPT richness. In 2014, intolerant genera decreased at the territory and nest level with increased shale gas disturbance suggesting the potential for localized negative effects on waterthrush. Loss of food resources does not seem directly or solely responsible for demographic declines where waterthrush likely were able to meet their foraging needs. However collective evidence suggests there may be a shale gas disturbance threshold at which waterthrush respond negatively to aquatic prey community changes. Density-dependent regulation of the birds' ability to adapt to environmental change through acquisition of additional resources may also alter demographic response.

For objective 3, we demonstrated use of SSNMs for the first time on an aquatic-terrestrial organism to explore relationships between waterthrush and stream monitoring data in an area undergoing shale gas development. SSNMs allowed us to account for spatial autocorrelation inherent to stream monitoring data and properties that traditional modeling approaches cannot capture to elucidate factors that affect waterthrush foraging locations. While monitoring waterthrush, we sampled benthic macroinvertebrates in waterthrush territories, and collected wetted perimeter stream channel and water chemistry data. Spatial models outperformed traditional regression models and made a statistical difference in whether stream covariates of interest were considered relatable to waterthrush foraging. Waterthrush foraging probability index (FPI) was greater in areas where family (West Virginia Stream Condition Index, WVSCI) and genus-level (Genus Level Index of Most Probable Stream Status, GLIMPSS) multi-metric

indices of biotic stream integrity were higher. Waterthrush were found foraging both where stream locations were hydrologically connected and unconnected by stream flow. These stream foraging locations were relatively closer to undisturbed headwaters where WVSCI and GLIMPSS were predicted to be highest. While there was no significant relationship between FPI and shale gas land use on a catchment area scale, further information on bioaccumulation of contaminants in benthic macroinvertebrates of shale gas disturbed areas is needed before establishing the extent to which waterthrush foraging may be affected by shale gas development.

Finally, for objective 4 we examined an epigenetic mechanism, DNA methylation, which may vary in response to environmental stressors and influence gene expression within and among wild bird populations. We examined the association between DNA methylation and waterthrush demographic characteristics and the potential of differential methylation from shale gas development during 2013–2015. We also evaluated the degree to which an individual's methylated state changed across years in recaptures. Overall population methylation differed between adult male and female waterthrush where adult males generally had fewer methylated restriction sites. Differential methylation also occurred between adult females and nestlings. Age influenced differential methylation in both adult males and females where there was generally decreased methylation with age. While adult male recaptures had decreased methylation with age, adult female recaptures had increased methylation with age. Adult males were differentially methylated between shale gas undisturbed and disturbed areas at a population and restriction site level, where restriction sites were predominately less methylated in shale gas disturbed areas, a trend consistently seen year to year in adult male recaptures. Barium (Ba) and Strontium (Sr) data were collected in 2013 feather samples and adult males had fewer methylated sites at higher concentrations of Ba and Sr (Fig. 4), while nestlings displayed no correlation of methylation to Ba and Sr concentrations. Adult females displayed increased methylation with increased Sr (Fig. 4), a trend also seen year to year in adult female recaptures. Overall, results of our study suggest sex-specific influences of shale gas development on gene expression that may affect long-term population survival and fitness.

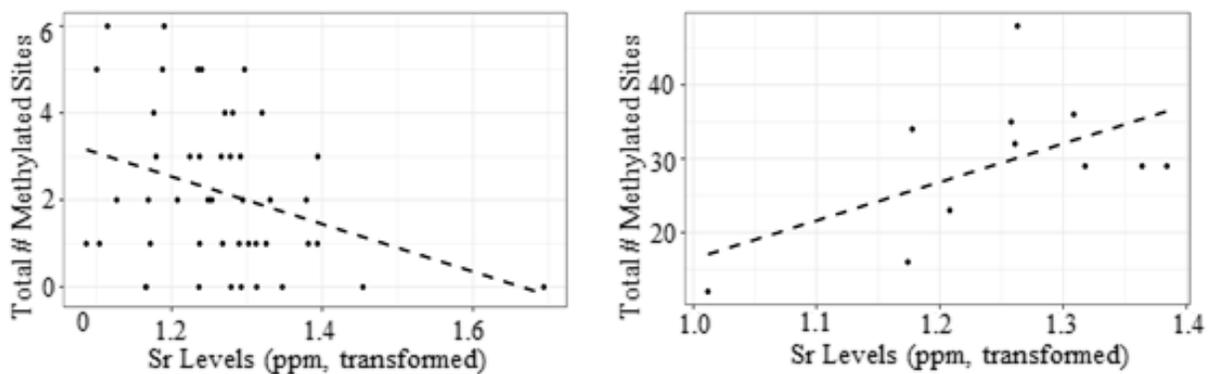


Fig 4. Adult males had fewer methylated sites with increasing Sr (left panel) whereas adult females had an increased number of methylated sites with increasing Sr (right panel).

**CERULEAN WARBLER AND ASSOCIATED SPECIES RESPONSE TO OPERATIONAL
SILVICULTURAL IN THE CENTRAL APPALACHIAN REGION**

Student Investigator: Gretchen E. Nareff

Principal Investigator: Petra Wood

Cooperators: Todd Fearer, Mark Ford, Jeff Larkin, Scott Stoleson

Years Ongoing: 2013-Present

Completed: September 2019

Funding Sources: U.S. Geological Survey (WV RWO 62), WV Division of Natural Resources, Pennsylvania Game Commission, Virginia Game and Inland Fisheries, Science Support Partnership Grant to University of Maine

Objectives:

1. Quantify and compare broad-scale influence of timber harvests on relative avian abundance and community composition pre- and post-harvest across four states and territory density at two sites in West Virginia.
2. Examine how silvicultural practices affect habitat use of a breeding interior-forest passerine, the cerulean warbler, in West Virginia, pre- and post-harvest.
3. Examine the response of 5 focal species to silvicultural harvest mosaics and assess using the cerulean warbler as an umbrella species for songbird management.

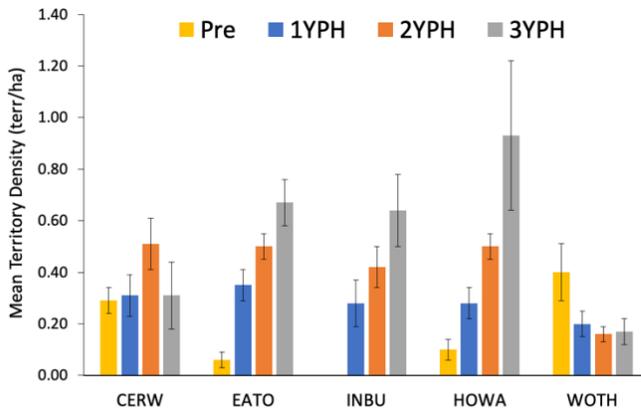
Background:

The Cerulean Warbler (*Setophaga cerulea*; hereafter, “cerulean”) is a severely declining, late-successional songbird species whose core breeding range is in the hardwood forests of the central Appalachian Mountains. It requires heavily forested landscapes with heterogeneous vegetation structure. Because of its status as a species of conservation concern, the cerulean has been a focus of research in the central Appalachian region where it reaches some of its highest abundances. In 2013, some of this research led to the production of the *Cerulean Warbler Management Guidelines for Enhancing Breeding Habitat in Appalachian Hardwood Forests* (Wood et al. 2013; hereafter, “Guidelines”). This follow-up study was designed to assess cerulean response to harvests recommended in the Guidelines, but implemented by means of more typical silvicultural prescriptions in the central Appalachian region and across a broad range of topographic conditions. The harvests at my study areas encompassed all available slope positions (i.e., lower to ridge) and aspects (i.e., Beers aspects 0–2). Two study areas were within the Ridge and Valley physiographic region, not included in the original study. I collected abundance and territory density data for the avian community and measured key vegetation characteristics.

Results:

I used the point count data to model cerulean abundance pre- and post-harvest at 5 study areas (Kentucky [n=1], Virginia [n=2], West Virginia [n=2]) and post-harvest at an additional 2 study areas (West Virginia [n=2]). I analyzed territory data from 2 of the 4 study areas in West Virginia. I compared 3 point types—harvest interior, harvest edge, and reference—to assess cerulean change in abundance pre- and post-harvest and by years-post-harvest with dynamic,

open population N -mixture models and accounted for detection bias. Using only the post-harvest data from the 7 study areas, I used static N -mixture models to estimate modeled abundance and change in abundance among years-post-harvest. The primary results of these analyses indicated that point type, basal area of preferred tree species (i.e., white oak species [*Quercus* spp.], sugar maple [*Acer saccharum*], hickories [*Carya* spp.]), and basal area of large diameter trees (≥ 40.6 cm dbh) were the most important drivers of cerulean abundance. Models including slope position and Beers aspect had limited support for the data indicating that timber harvests implemented on less preferred aspects and slope positions can still improve habitat for ceruleans. Territory



density increased 100% between pre-harvest and 2 years post-harvest (Fig. 1). The greatest increases in abundance and territory density occurred where pre-harvest numbers were low.

Figure 1. Mean territory density (territory/ha) and standard error of Cerulean Warbler (CERW), Eastern Towhee (EATO), Indigo Bunting (INBU), Hooded Warbler (HOWA), and Wood Thrush (WOTH) at 4 study areas ($n=10$ plots) in West Virginia, pre-harvest and 1, 2, and 3 years-post-harvest (YPH).

I analyzed the response of 5 additional focal species to the harvests at the same 7 study areas I used for cerulean analysis, plus 2 additional study areas in Pennsylvania. The focal species, Eastern Towhee (*Pipilo erythrophthalmus*), Indigo Bunting (*Passerina cyanea*), Hooded Warbler (*S. citrina*), Scarlet Tanager (*Piranga olivacea*), and Wood Thrush (*Hylocichla mustelina*), use a range of seral stages available in the central Appalachian region or that can be created and maintained through operational silviculture (i.e., early successional to mature forest). I again used static N -mixture models to estimate abundance of the 5 focal species, pre- and post-harvest, and among years post-harvest. Eastern Towhee and Indigo Bunting abundance increased most at harvest interior points followed by harvest edge points and both decreased in abundance at reference points (Fig. 2). Hooded Warbler abundance increased at all 3 point types, suggesting an overall positive response to the harvest mosaics rather than just to the reduction of basal area or opening of the canopy. Scarlet Tanager abundance initially decreased post-harvest, but subsequently increased at harvest edge and reference points. Wood Thrush abundance increased at reference points but decreased at harvested points, particularly harvest interior. Although Wood Thrush remained on the study areas, their territory density declined by 50% during the first year post-harvest (Fig. 1). Eastern Towhee, Indigo Bunting, and Hooded Warbler territory density increased post-harvest and continued to increase 2 and 3 years-post-harvest (Fig. 1).

I used logistic regression and resource selection functions to assess male cerulean territory habitat selection with matched used-available habitat data. I used vegetation points at male cerulean singing locations as “used” locations, which I delineated from detections during 7–8 territory mapping visits, and systematically sampled vegetation points within the territory mapping plot grids as “available” locations. Based on AIC model selection of pre-harvest data, male ceruleans selected for increasing Beers aspect, whereas tree species composition was not important for territory habitat selection. Post-harvest, Beers aspect was the least important

variable to male cerulean habitat selection but they selected for increasing percentage of basal area that was trees intermediate to or overtopped by the canopy trees (i.e., midstory vegetation). Post-harvest, males selected breeding habitat across a wider range of available vegetation and topographic conditions than they did pre-harvest. Territory size decreased post-harvest, suggesting an improvement in quality of breeding habitat.

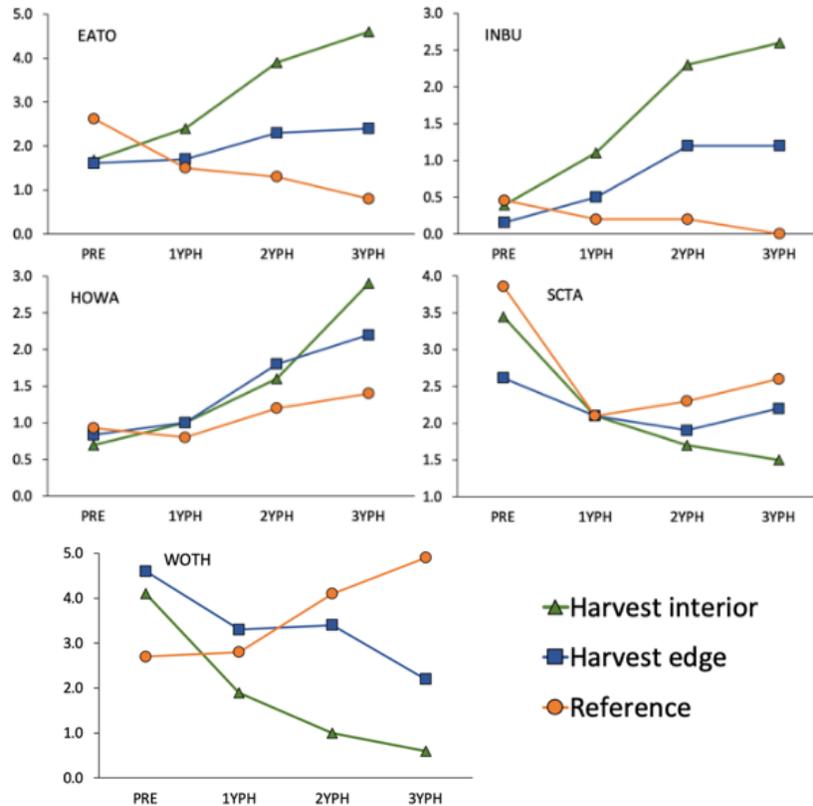


Figure 2. Abundance (birds/point) of 5 focal songbird species pre-, 1 year (1YPH), 2 years (2YPH), and 3 years (3YPH) post-harvest. Species are Eastern Towhee (EATO), Indigo Bunting (INBU), Hooded Warbler (HOWA), Scarlet Tanager (SCTA), and Wood Thrush (WOTH).

In summary, findings corroborate the recommendations of the Guidelines, but show that harvests designed with ceruleans in mind can create breeding habitat on otherwise less desirable topographic conditions. Further, topographic conditions were not important influences on abundance of the five focal species. Where mature forests are not available, silvicultural prescriptions can be used to enhance forest stands for ceruleans and a broad suite of other birds, particularly those that breed in shrubby early successional habitat, young forest, or mature forest gaps. Benefits to closed canopy, mature forest species may be limited, but they were not extirpated from harvest mosaics that retain reference stands or at least light harvests with higher basal area.

CONTINUING PROJECTS

AQUATIC

AN EVALUATION OF FISH USE OF ARTIFICIAL HABITAT STRUCTURE IN CHEAT LAKE

Student Investigator: Kyle Matt

Principal Investigator: Stuart Welsh

Years Ongoing: 2019

Degree Program: MS

Expected completion date: December 2020

Funding Sources: Cube Hydro

Objectives:

1. Document the timing of spawning, as well as examine variables associated with spawning, i.e., water temperature, water depth, distance from shoreline, and water turbidity.
2. Examine water level fluctuation as a variable of influence on the timing of spawning, as well as its role in the potential for egg dewatering.
3. Document benthic habitat use by young-of-year Yellow Perch.

Progress:

Water level fluctuations of Cheat Lake, a 700 hectare (1730 acre) hydropower reservoir on Cheat River, result in part from the storage and release of water for power production. The reservoir also provides boat and bank anglers with a diversity of recreational fishing opportunities, including fisheries for Black Bass, Black Crappie, Channel Catfish, Bluegill and Pumpkinseed Sunfish, Walleye, White Bass, and Yellow Perch. The West Virginia Division of Natural Resources (WVDNR) manages the recreational fisheries of Cheat Lake, and sets regulations based, in part, on fishery dependent and fishery independent data. Lake elevations are held between 868 and 870 ft from May through October to enhance recreation. Elevations fluctuate between 857 and 870 ft from November through March. During April, elevation fluctuations are restricted to between 863 and 870 ft, a regulation that may reduce impacts on early spawning of fishes, particularly Walleye and Yellow Perch.

Yellow Perch typically spawn in nearshore littoral zones, where egg skeins are draped across vegetation or woody debris. Once Yellow Perch eggs hatch, the larvae are expected to leave the littoral zone and live in the water column of the limnetic zone for about 30–40 days. In the limnetic zone, the larvae forage on Zooplankton. Individuals grow to about one inch in length, before returning to near-shore littoral habitats. Currently, we have little information on Yellow Perch spawning in Cheat Lake, so information on when Yellow Perch spawn, as well as data on spawning water depths and distances from the shoreline are needed to understand the potential

for egg dewatering. Also, we know very little about the near-shore habitats that young-of-year Yellow Perch use following their larval stage.

During spring 2019, 40 artificial spawning structures were placed (submerged) in near-shore areas at two sites on Cheat Lake; 20 structures at Crammys Run and 20 at Canyon Bend (Figure 1). The 40 spawning structures were checked daily (removed from the water) for the presence of egg skeins during the expected spring spawning period. We analyzed presence/absence data of egg skeins using logistic regression analysis. Model selection was based on 21 candidate models (i.e. alternative hypotheses) including single or fixed-effects of six single covariate models (Secchi disk depth, Distance to the shoreline, Water temperature, Water depth, Lunar illumination, and Lake level fluctuation). For analysis on water level fluctuations included the estimation of the proportion of egg skeins located in potential dewatering areas based on two scenarios. In the first scenario, we assumed that eggs skeins were deposited on or near the lake bottom. In the second scenario, the assumption was that egg skeins were deposited onto a structure at a position of 3 ft above the bottom. These scenarios were based on the literature and our unpublished findings that Yellow Perch generally spawn on structure, with egg placement rarely exceeding three feet above the lake bottom.

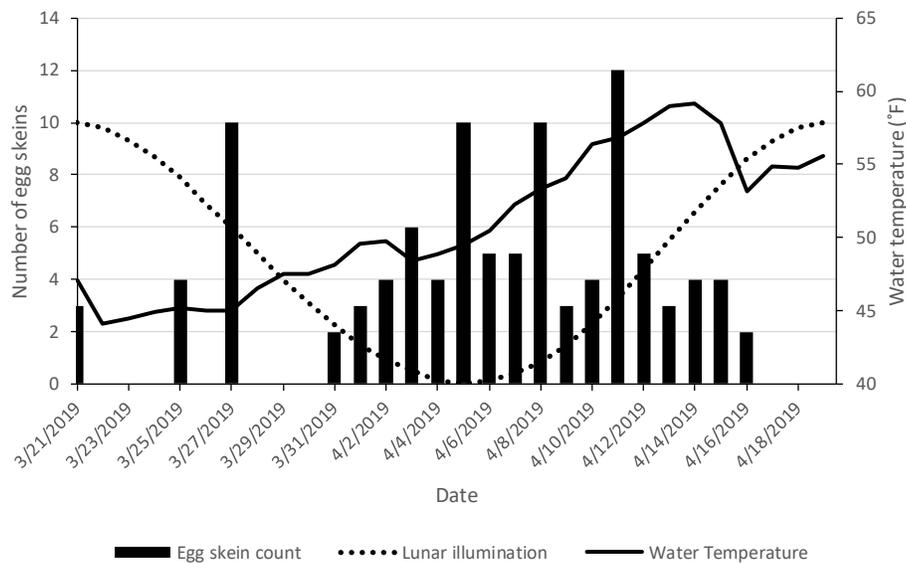


Figure 1. Daily counts of Yellow Perch egg skeins on 40 artificial habitat units, Cheat Lake, West Virginia. Water temperature and lunar illumination are plotted for the spawning duration period, which started on 21 March and ended on 16 April.

Artificial spawning structures were deployed for 51 days (11 March to 30 April) at the Crammys Run and Canyon Bend study sites, Cheat Lake, West Virginia. The presence of egg skeins of Yellow Perch on the spawning structures were documented during a 27-day time period (21 March to 16 April; Figure 1). The presence of egg skeins was documented 46 and 35 times on spawning structures at Crammys Run and Canyon Bend, respectively. Typically, a single egg skein was present on a spawning structure, but multiple skeins were found occasionally on

spawning structures. Egg skeins were generally attached to the spawning structures in two ways: spiraled or draped (Figure 2). The lengths of egg skeins varied considerably, but the longest skein measured was approximately 1.4 m in length.



Figure 2. Artificial spawning habitat structures as viewed from two angles (top panels), and with presence of yellow perch egg skeins (spiraled on left or draped on right). Artificial habitat structures included reelweeds by LaDredge Outdoors; <https://www.reelweeds.com/>.



Yellow Perch spawned on the artificial structures when surface water temperatures ranged from 44 to 60 °F, and within the full range of lunar values, from new moon to full moon (0 to 100% illumination) (Figure 1). Spawning occurred on artificial structures in 3 ft to 20 ft depths. Yellow Perch spawned on artificial structures that were located at distances ranging from 3–39 m from the current water level of the shoreline. For the logistic regression

analysis, the secchi disk model was the only model supported by the data. Egg skeins on spawning structures were rarely present when the secchi disk reading was below 185. We do not know if yellow perch delayed spawning during periods of high water turbidity, or if the fish were just not able to locate the spawning structures. For a scenario based on the assumption that all egg skeins were deposited onto the lake bottom, Crammys Run was estimated to have 31% of egg skeins (14 of 46) in potential dewatering areas, whereas Canyon Bend had 11% of egg skeins (4 of 35) in potential dewatering areas. With the two sites combined, then estimates were 23% of egg skeins (18 of 81) in potential dewatering areas (Figure 3). For a scenario where egg skeins are deposited onto structures at 3 ft above the lake bottom, then estimates of egg placement in potential dewatering areas were 59% (27 of 46), 41% (14 of 35), and 51% (41 of 81) for Crammys Run, Canyon Bend, and the two sites combined, respectively (Figure 3).

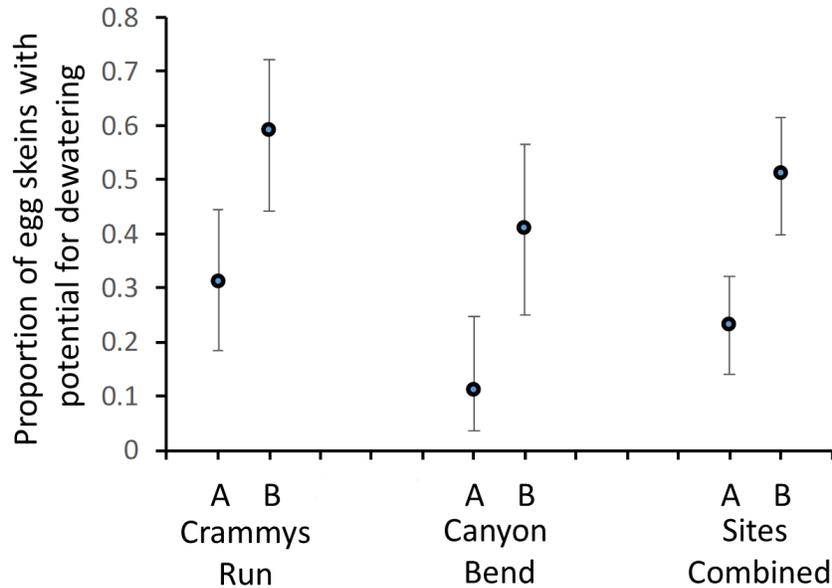


Figure 3. Estimation of the proportion of egg skeins with potential for dewatering at Crammys Run and Canyon Bend, Cheat Lake, West Virginia. Estimates are based on two scenarios, where egg skeins are deposited directly onto the lake bottom (A), or egg skeins are deposited onto structures at 3 feet above the lake bottom (B). Error bars are 95% profile likelihood confidence intervals.

**EFFECTS OF DAM PASSAGE AND SEASONAL MOVEMENT PATTERNS
OF A RESTORED PADDLEFISH POPULATION**

Student Investigator: MacKenzie A. Ullman

Principal Investigator: Stuart A. Welsh

Cooperators: Katie Zipfel, Quinton Phelps

Years Ongoing: 2019 – 2022

Degree Program: M.S.

Completion: Dec 2022

Funding Source: West Virginia Division of Natural Resources, Ohio River Endowment.

Objectives:

This project is the earliest stage of ten-year program to evaluate the population status, determine movement patterns and assess reproductive status of paddlefish populations in West Virginia.

The project includes the following objectives:

- Evaluate paddlefish population age, growth, and mortality rates through mark-recapture analyses.
- Estimate proportion of paddlefish population with coded wire tags in the Robert C. Byrd pool.

- Determine dam passage frequency in the Robert C. Byrd pool of the Ohio River using ultrasonic telemetry.
- Monitor seasonal movements of individuals implanted with transmitters using passive ultrasonic telemetry from stationary receivers and using active manual tracking with a Vemco VR100 acoustic receiver from the beginning of March to the end of May or until areas of aggregation are located.
- Use telemetry data to determine areas of high paddlefish concentrations to collect fine scale habitat information and identify potential spawning locations.

Progress:

The study area of this project is the R.C. Byrd pool of the Ohio River. Several potential sites have been selected throughout the pool for future overnight gill net sets. The sites were selected using GIS technology to locate points along the shoreline with immediate, dramatic drops in depth. Sites along the locks and dam, and sites near the mouth of tributaries and creeks were also selected. Sites with these features were chosen based on previous high proportions of netted paddlefish in similar site locations at different pools of the Ohio River.



Figure 1: MacKenzie Ullman with paddlefish captured during gill netting survey.

Gillnetting will be conducted from October through May during lower water temperatures to reduce stress. 2-4 nets will be deployed at each site before dark and soak for 18 hours overnight. All paddlefish collected from the samples will be

counted, weighed, measured, sexed, and checked for coded wire tags. Live individuals will also be jaw tagged for mark-recapture analyses. Deceased individuals will have jaw bones removed (and coded wire tags if present) for additional analyses. In previous sample sites along the Ohio River from December 2018 until August 2019, 26 paddlefish have been jaw tagged, 18 living individuals were detected with coded wire tags, and 7 coded wire tags were recovered from deceased individuals as well as corresponding jaw bones. The jawbones and coded wire tags recovered from deceased individuals are currently being analyzed.

Future:

A sub sample of paddlefish will be implanted with ultrasonic transmitters. Once paddlefish are fitted with transmitters their movements throughout the pool and passage through the R.C. Byrd dam will be documented. Stationary receivers are currently in place in the R.C. Byrd pool for passive tracking techniques. Manual tracking of transmitted individuals will also take place using active telemetry techniques.



Figure 2: Paddlefish with inserted jaw band

**BLUE CATFISH AND FLATHEAD CATFISH POPULATION DEMOGRAPHICS AND MOVEMENT IN
THE ROBERT C. BYRD POOL OF THE OHIO AND KANAWHA RIVERS**

Student Investigator: J. Vincent Siegel

Principal Investigator: Stuart A. Welsh

Cooperators: Nate Taylor, Quinton Phelps

Years Ongoing: 2016-2019

Degree Program: M.S.

Expected Completion: Spring 2021

Funding Source: West Virginia Division of Natural Resources

Objectives:

1. Document population demographics, including size and age data, and diet information
2. Examine movements using telemetry

Starting in 2016, the West Virginia Division of Natural Resources have intensely monitored the Robert C Byrd Pool's Blue and Flathead Catfish populations due to increasing angler interest (Figure 1). Blue Catfish were reintroduced to this pool in 2004 and one of this study's aims is to determine if continued stocking is necessary. The WVDNR has been collecting catch data and tagging fish during spring electrofishing and fall trotline surveys to monitor both species. In addition, WVDNR biologists initiated a movement study in the pool, using the existing USFWS Asian Carp acoustic receiver network. In 2016, the WVDNR implanted a total of 24 acoustic tags both catfish species.

This project builds on the WVDNR's work, collecting otoliths and stomach contents during the Spring 2019 electrofishing survey for deeper analysis on both populations. The telemetry section is expanded as well, increasing numbers of fish tagged and receiver coverage around the pool. The goal of this study is to provide the necessary population data to make informed management

decisions regarding these highly regarded fisheries and to make inferences into the ecology of these large river predators.



Figure 1. Examples of Blue Catfish and Flathead Catfish collected during the study

Progress:

Spring boat electrofishing surveys utilize low-frequency DC output of ~200V, 2-3 amps, 15 pulses/second and a chase boat at ten standardized locations. This technique has been significantly more efficient at capturing Flatheads (mean CPUE 35.1 ± 6.35 fish/hour) than Blues (2.3 ± 0.9 fish/hour), with zero transects capturing more Blues than Flats. Catches represented all size classes for Flatheads (Figure 2), with sub stock fish comprising 48.5% of the total Flathead catch. Memorable fish have been collected every year, and trophy fish collected in the 2016, 2017 and 2019 surveys. Blue Catfish size

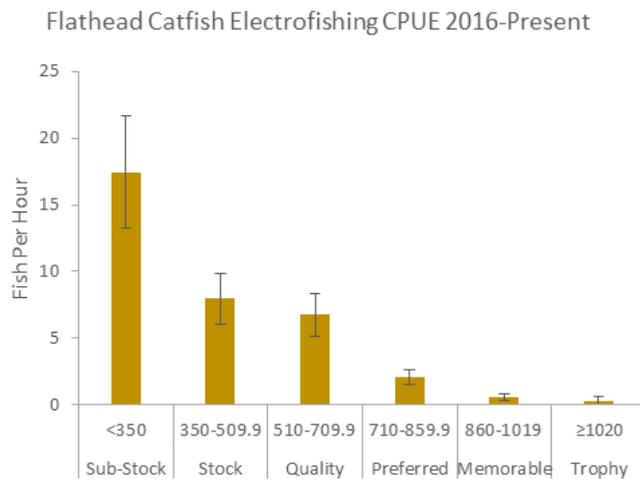
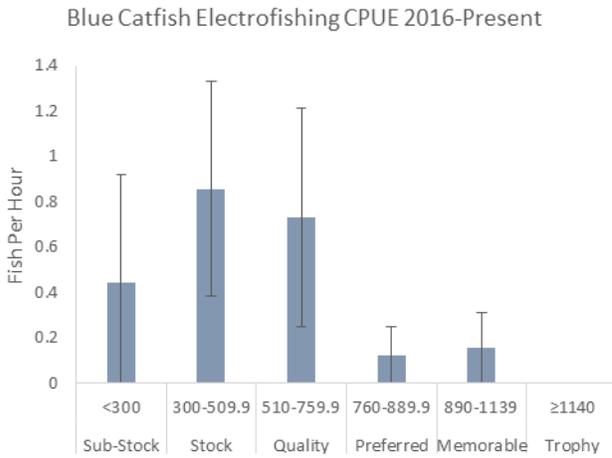


Figure 2. CPUE by Size Class of Flathead Catfish during RC Byrd Pool Spring Electrofishing Surveys 2016-2019.



distributions were highly variable between years (Figure 3), with stock and quality fish being most common, making up 70% of the total catch. Memorable fish were collected in 2017 and 2019 but no trophy Blues have been collected so far using this gear. During the 2019 Electrofishing survey, otoliths and stomachs were collected from 154 Flatheads, and 31 Blues. Analysis is ongoing.

Figure 3. CPUE by Size Class of Blue Catfish during RC Byrd Pool Spring Electrofishing Surveys 2016-2019

Fall trotline surveys employ five lines at the same ten locations. Each line uses twenty 7/0 circle hooks baited with live Gizzard Shad of all sizes. Flathead and Blue Catfish catch rates have been similar (Figure 4), averaging 1.26 ± 0.55 and 1.33 ± 0.45 fish/line, respectively. Trotlines are selecting larger fish and more Blue Catfish than electrofishing. All flatheads collected have been quality size or larger, with an average of 46% being preferred size. 96% of Blue Catfish have been over stock size with quality blues averaging 49% of the yearly catch for this species. Trophy Flatheads and Blues were not common, averaging 4.4% and 1.2% of the yearly catch for flats and blues respectively.

The acoustic telemetry section of this study ramped up during the 2018 trotline survey, implanting tags in 21 of 37 flatheads and 24 of 26 blues collected. Fish were implanted to get even sex ratios and ensure fish were tagged from different locations throughout the pool. A total of 45 of 60 tags have been deployed, with the remaining being implanted during the 2019 trotline survey. The receiver network has been setup and currently includes 14 receivers distributed evenly throughout the pool.

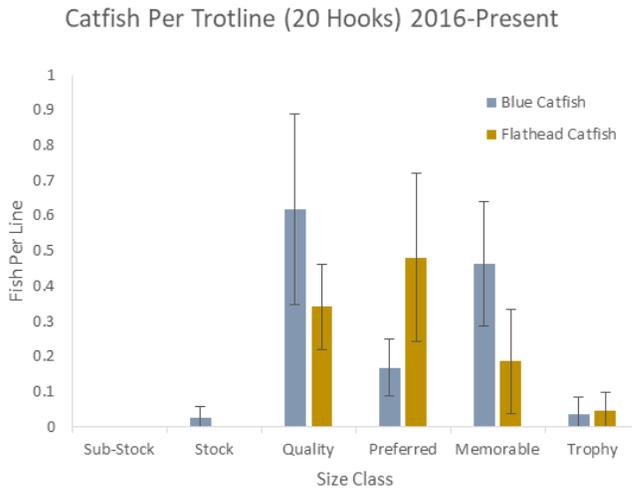


Figure 4. CPUE by Size Class of Blue and Flathead Catfish during RC Byrd Fall Trotline Surveys 2016-2018

UPSTREAM MIGRATION AND USE OF FISHWAYS BY AMERICAN EELS IN THE SHENANDOAH RIVER

Principal Investigator: Stuart A. Welsh

Years Ongoing: 2009 – 2019

Expected Completion: 2020

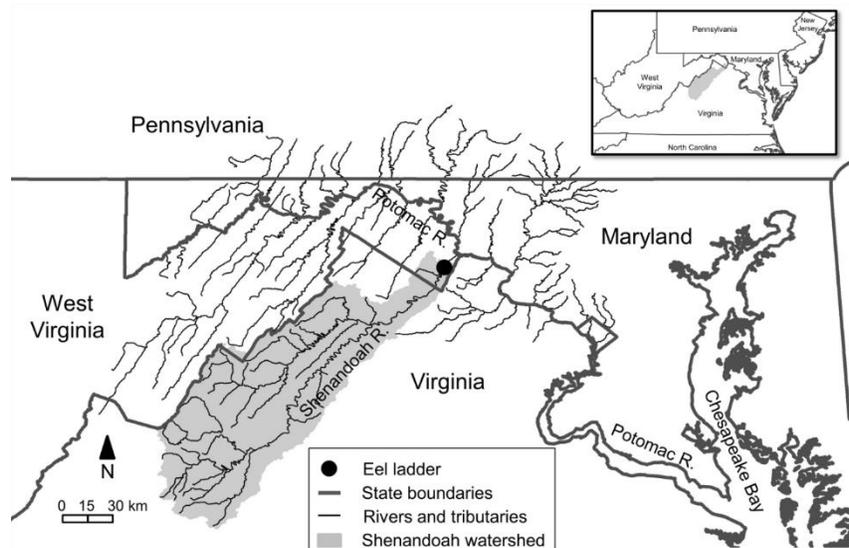
Funding Source: currently not funded

Objectives:

Examine upstream migration of American eels through monitoring of eel-specific fishways on hydroelectric dams of the lower Shenandoah River.

Progress:

The American eel is a migratory species with extensive upstream migrations in rivers during the yellow phase of its life cycle. Given recent concerns of population declines, studies have focused on obstructions to migration, specifically in relation to dams and associated influences on upstream migration. Improving technologies for upstream eel passage has been listed as a high research priority. Managers of fisheries and those of hydroelectric facilities have installed eel-specific fishways (commonly called eel ladders) on some rivers to assist eels in passing dams during upstream migration. However, little is known about eel movements and behaviors near dams and eel ladders, and few dams have been evaluated for ladder effectiveness.



There are five hydroelectric facilities on the Shenandoah River. All reservoirs are run-of-the-river. The dams and associated hydropower facilities are operated by PE Hydro Generation, LLC. An eel ladder was installed in 2003 on Millville Dam within the lower Shenandoah River (Figures 1, 2). Eel ladders have also been installed upstream at Warren and Luray dams, but these two ladders have passed relatively few eels.

Figure 1. Map of the Potomac River drainage and the location of the Millville Dam eel ladder on the Shenandoah River.

The Millville Dam eel ladder is currently monitored with a semi-automated eel ladder camera. This method allows for daily counts, estimation of lengths, and documentation of the time of passage of each eel. Based on data collected at the Millville ladder, upstream migrant eels range from 3 to 11 years in age and average 30 cm in length (primarily ranging from 19 cm to 50 cm). Eels often use the ladder during time periods near the new moon or periods of increasing river discharges. Eels are crepuscular and nocturnal and primarily use the ladder at night. The largest numbers of eels have passed the Millville ladder during high river discharges of spring, summer, and fall, and relatively few eels move upstream during periods of low river discharge. Since 2003, the eel ladder at Millville, WV, has passed over 30,000 eels. In 2018, the Millville ladder passed 1201 eels, but is currently under repair as it was damaged by debris on June 3 by flood flows. The eel ladder was repaired and working as of June 2019, where 366 eels have used the ladder between June and 24 September 2019.



Figure 2. Millville Dam eel ladder on the lower Shenandoah River.

INDIVIDUAL VARIATION OF GROWTH (C-MAX) AND METABOLISM IN BROOK TROUT A PRIMER OF RESILIENCY TO CLIMATE CHANGE

Student Investigator: Cory M. Hartman

Principal Investigators: Dr. Patricia Mazik

Collaborators: Dr. Quinton Phelps

Years Ongoing: 2016 - Present

Expected Completion: December 2019

Funding Source: West Virginia Division of Natural Resources

Objectives:

- Determine individual variation of growth and metabolism in Brook Trout from 4 different head water streams in West Virginia.
- Determine if life history (i.e. fluvial population of fish) affects performance at elevated temperatures
- Evaluate size dependencies on growth and metabolism

Progress:

All fish were collected from source streams in July of 2018, and acclimated to the laboratory setting between August 2018 and December 2018. Respiration chambers constructed summer 2018 and laboratory designs and tank set ups were conducted in fall of 2018. Experiments were initiated January 2019 and concluded August 2019, including an added component to tease out how size may affect the growth and metabolism of various sized fish. Initial analysis is indicating that one of the streams is out performing the other three streams at elevated temperatures. Thesis writing and data analysis has begun.

INFLUENCE OF SEASONAL COCCIDIOSIS, TISSUE CONTAMINANTS AND FISH AGE ON LIVER AND BILIARY PATHOLOGY IN WHITE PERCH *MORONE AMERICANA* FROM CHESAPEAKE BAY

Student Investigator: Mark Matsche

Principal Investigators: Dr. Patricia Mazik

Collaborators: Dr. Vicki Blazer

Years Ongoing: 2018 – Present

Degree Program: PhD

Expected Completion: May 2022

Funding Source: Maryland Department of Natural Resources Cooperative Oxford Laboratory (NCTC Student)

Purpose:

This research will involve the study of coccidia in white perch and collaboration on a survey of chemical contaminants in white perch, which will provide information on multiple, potentially important contributors to stress in fish. The goals of this proposed research are to describe and document this coccidian parasite, describe the histopathological and clinical plasma chemistry changes before, during and after coccidiosis events, determine the concentration of chemical contaminants in white perch tissues, and to assess the relative importance of chemical contaminants, parasitism and other factors in how they relate to hepatic and biliary disease.

Objectives:

1. Description of a seasonal coccidian parasite in white perch from Chesapeake Bay, Therefore the goals of this portion of proposed research are to:
 - Continue efforts to extract DNA from coccidia for genetic comparisons,
 - Name and describe this parasite as new species if the morphological and genetic data support such actions,
 - Fit these species within the current taxonomic structure of Apicomplexan species,
 - Describe the geographical distribution of these parasites in white perch populations around the Chesapeake bay region, and
 - Describe the annual cycle of parasite infections in white perch, and any important biological factors, such as temperature and fish sex and size, which may influence infections.

2. Histopathology and clinical pathology changes in white perch during biliary coccidiosis

Therefore, the goals of this component of research are to:

- Conduct a histopathological analysis of livers and gall bladder collected for the study of coccidia (2016-2018),
- Provide a monthly summary of morphological changes in the liver using a semi-quantitative index (Bernet et al. 1999) based on weight of importance and severity of lesions observed, and
- Analyze data using non-linear, non-parametric correlations, cluster analysis, and other tools to compare liver histopathology index data (LHI) with parasite spore intensity and prevalence data.

3a. Proposed method to evaluate macrophage aggregates in fish tissues, therefore the goals are:

- Develop a method to efficiently measure the total volume of MA in an organ using unbiased techniques,
- Compare total volume of MA to density of MA, and
- Use a general linear model and non-parametric correlation to compare sensitivity of different measurement techniques (total volume vs density) to detect differences in MA by disease and age of fish.

3b. Once this technique is validated, analysis of splenic MA will be evaluated in white perch, yellow perch and largemouth bass, as part of a collaborative USGS fish health study, the goals are:

- Measure spleen volume and total splenic volume of MA in fish collected during collaborative USGS health study, and
- Use multivariate models to assess volume of MA by important abiotic and biotic factors including contaminant concentrations, age, sex and species.

4. Study of tissue contaminants and relationship to disease, parasitism and morphological changes in the liver of white perch, therefore the goals of this research component are to collaborate with USGS on a fish tissue contaminant and health survey to:

- Measure tissue contaminant concentrations in white perch,
- Estimate concentrations of coccidian spores in the gall bladder,
- Assess fish for hepatic histopathology,
- Use stereological techniques to estimate a variety of tissue parameters in the liver (e.g. bile duct length, relative fraction of parenchyma and stroma, volume of coccidia, MA volume) and spleen (e.g. MA volume, lesion volume), and
- Use a variety of multivariate techniques to evaluate morphological changes in organs with chemical contaminant concentrations, disease, parasitism, and age.

Results:

Data collection is ongoing and 2 papers have been published:

Matsche, M.A., V.S. Blazer and P.M. Mazik. 2019. Comparisons of stereological and other approaches for quantifying macrophage aggregates in piscine spleens. Journal of Aquatic Animal Health. In Press. ISSN: 0899-7659 print / 1548-8667 online DOI: 10.1002/aaah.10086

Matsche, M.A., V.S. Blazer and P.M. Mazik. 2019. Seasonal development of the coccidian parasite *Goussia bayae* and hepatobiliary histopathology in white perch *Morone americana* from Chesapeake Bay. *Diseases of Aquatic Organisms*. 134:112-135.

Macrophage aggregates (MAs) are focal accumulations of pigmented macrophages in the spleen and other tissues of fish. A central role of MAs is the clearance and destruction of degenerating cells and recycling of some cellular components. Macrophage aggregates also respond to chemical contaminants and infectious agents and may play a role in the adaptive immune response. Tissue damage or physiological stress can result in increased MA accumulation. As a result, MAs may be sensitive biomarkers of environmental stress in fish. Abundance of MAs in tissues has been reported in a variety of ways—most commonly as density, mean size, and relative area—but the utility of these estimates has not been compared. In this study, four different types of splenic MA abundance estimates (abundance score, density, relative area, and total volume) were compared in two fish populations (Striped Bass *Morone saxatilis* and White Perch *M. americana*) with a wide range in ages. Stereological estimates of total volume indicated an increase in MA abundance with spleen volume, which generally corresponded to fish age, and with splenic infections (mycobacteria or trematode parasites). Abundance scores were generally limited in the ability to detect changes in MA abundance by these factors, whereas density estimates were greatly influenced by changes in spleen volume. In some instances, densities declined while the total volume of MAs and spleen volume increased. Experimentally induced acute stress resulted in a decrease in spleen volume and an increase in MA density, although the total volume of MAs remained unchanged. Relative area estimates accounted for the size and number of MAs but not for changes in organ volume. Total volume is an absolute measure of MA abundance irrespective of changes in organ volume or patterns of accumulation and may provide an improved means of quantifying MAs in the spleens of fish.

The coccidium *Goussia bayae* infects the gallbladder and bile ducts of white perch *Morone americana* from Chesapeake Bay, USA. Seasonal changes in coccidian infections were analyzed from bile specimens of 1588 fish from the Choptank River during 2016–2018 using wet mount preparations with a Sedgwick-Rafter counting chamber. Histopathology of the gallbladder and liver was analyzed from a subset (n = 480) of these fish. Maximum parasite prevalence (100%) and intensities in the gallbladder occurred during the fish spawning season in March and April. Asynchronous coccidian development and prevalence of infections in fish increased gradually during autumn and winter, but coccidian intensity increased sharply 2–4 wk prior to the onset of fish spawning activity and decreased after spawning activity concluded. Sporulation was internal, and the gallbladder was the primary reservoir for oocysts. Two previously undescribed species of coccidia were observed in the intestine. Lesions in the gallbladder were rare and included cholecystitis and epithelial necrosis. Intrahepatic bile duct lesions were more common and included distension, cholangitis, epithelial erosion and necrosis, cholestasis, hyperplasia, and neoplasia. Cholangitis and necrosis of intrahepatic bile ducts were significantly associated with coccidian infections, while plasmodia of a myxosporean (spore morphology consistent with the genera *Myxidium* and *Zschokella*) were significantly associated with bile duct hyperplasia. Biliary neoplasia included cholangiomas (5% prevalence) and cholangiocarcinomas (1% prevalence). No association was detected between *G. bayae* and biliary neoplasms, but an association may exist between these lesions and the myxosporean plasmodia.

ASSESSMENT OF IMMUNE STATUS IN SMALLMOUTH BASS

Student Investigator: Cheyenne R. Smith

Principal Investigators: Dr. Patricia Mazik

Collaborators: Dr. Vicki Blazer – LSC, Jim Hedrick/Brandon Keplinger – WVDNR

Years Ongoing: 2018 – Present

Degree Program: PhD

Expected Completion: May 2022

Funding Source: West Virginia Division of Natural Resources

Objectives:

1. *In vitro* exposures – Utilize anterior kidney cells from laboratory-reared smallmouth bass to assess the effects of exposure to environmentally-relevant concentrations of commonly measured contaminants. Expose cells to chemicals that have been documented during the past few years during analyses of water samples: pesticides (i.e. atrazine), estrone, phytoestrogens and microcystin. Functional analyses as well as gene expression will be assessed. Goal for gene expression is to finish transcriptome analysis using anterior kidney tissue from various sources as well as isolated anterior kidney cells exposed to various chemicals. This will help to assess immune function in relation to contaminant exposure and identify gene pathways affected by contaminants.
2. Wild fish sampling – Compare the health of wild smallmouth bass from two South Branch sites in the Potomac River drainage, two sites in the Greenbrier drainage and one site at Cheat Lake in the spring and fall of 2019 and 2020. Functional immune assays and gene expression will be combined with the established fish health assessment methods (documenting external and internal abnormalities, histopathology, plasma analyses, pathogen identification) to better understand the relationship between health, land-use and chemical contaminants. The South Branch site near Moorefield, WV is co-located with the USGS EDC and Priority Ecosystems projects for which monthly water chemical analyses and some tissue analyses is being conducted.
3. Depending on the findings in 2019, it will be determined by WVDNR together with USGS, as to whether additional sites should be assessed or further work should be done at a subset of the 2019 sites with some funding being used for chemical analyses of water and tissue of sites not part of the USGS projects. It will also be determined whether more laboratory fish are needed.

Progress:

Objective 1: Healthy stock of laboratory-reared smallmouth bass established at Leetown Science Center since spring of 2016. Methods finalized and sampling for *in vitro* exposures began August 2019. Cells yields from anterior kidney tissue initially too low for both functional and gene expression assays, so only functional immune tests were performed. Working on increasing cell yields to include gene expression testing and compiling genes of interest for codeset development. Preliminary results for atrazine and estrone effects on immune function (bactericidal activity, respiratory burst activity, and mitogenesis) are available. Analysis is

ongoing as we challenge leukocytes with more chemicals. Challenges limited to number of laboratory fish available, so chemical combinations may be looked at versus additional chemicals – pending full analysis from first two chemical treatments.

Objective 2: Fish collected from all five WV sites in spring 2019 and functional immune assays were completed. Data has been collected for spring 2019 samples and analysis is ongoing. Fall collection anticipated October 2019.

Objective 3: Pending analyses from first two objectives.

**WASTEWATER TREATMENT OUTFALL AS A LIMITING FACTOR TO FRESHWATER MUSSEL
REINTRODUCTION AND RECOLONIZATION**

Student Investigator: Kristin A. Stockton

Principal Investigator: Patricia Mazik

Collaborators: Janet Clayton

Years Ongoing: 2018-Present

Expected Completion: May 2020

Funding Source: West Virginia Division of Natural Resources

Objectives:

- Determine if proximity to wastewater treatment outfall can result in negative impacts to growth and survival in juvenile mussels
- Determine if Opequon Creek is a candidate for future mussel reintroduction
- Identify water quality factors contributing to mortality and health of juvenile freshwater mussels

Progress:

- Juvenile *Lampsilis cardium* mussels placed at 8 sites within Opequon Creek, above and below WWTP outfall and 4 sites on Back Creek as a control
- Mussels checked for mortality and measured once a month
- Water quality measurements taken 2-3 times a month with YSI meter
- Water samples collected and processed by Reliance Labs for Copper, Nitrite, Chloride, Mercury, and Ammonia Nitrogen once a month.
- Anticipated completion of field sampling – November 2019



Lampsilis cardium

CONSERVATION ASSESSMENT OF WEST VIRGINIA CRAYFISHES

Principle Investigators: Zac Loughman and Stuart Welsh

Years Ongoing: 2007-2019

Expected completion: 2021

Funding Source: WVDNR

Objectives:

- Identify species in need of conservation
- Document distribution ranges of invasive species
- Document range expansion and conservation standing of “common” species
- Conduct surveys for new state records and undescribed forms
- Publish a book on the Crayfishes of West Virginia

Progress:

Crayfishes have received moderate attention within the state of West Virginia. The first major work on decapods was performed by Faxon (1885), who listed only two taxa in WV. Since this initial research, several more species have been added to the crayfishes of West Virginia, with 22 known taxa residing within the state’s borders as of 2006. Several of these species additions were the result of crayfish surveys throughout various ecological regions within the state. Survey efforts within the state reached their peak during the 1980’s, with the last formal statewide survey of West Virginia’s crayfish fauna performed by Jezerinac during the summers of 1987 and 1988.

Efforts within the state to identify conservation threats, potential state records, and conservation concerns since the publication of Jezerinac et al. have been disjunct in nature. Key species (*Cambarus elkensis*, *Cambarus veteranus*), have received attention since Jezerinac’s effort, while the majority of taxa have remained understudied. Conservation concerns have arisen since the publication of Jezerinac et al. that were not of major consequence in the 1980s to crayfish populations. Land use issues, stream alteration, invasive species, and habitat loss have occurred throughout the state in areas with diverse crayfish populations, and the ultimate impact of these activities on crayfishes remains poorly understood.

This research will provide a manual or guide to the crayfishes of West Virginia. Given recent developments in electronic media, an interactive key to the crayfishes of West Virginia with multiple pictures of a species, list of key characters, and current range maps is a possibility that did not exist during the initial statewide crayfish survey. This product would be one output of a current statewide crayfish census, and would make it possible for field biologists across West Virginia to ID specimens in the field via an electronic and interactive identification key.

Using a probabilistic-random sampling of stream sites, we have sampled crayfishes from approximately 10 sites from each 10 digit sub-basin within the Cheat, Elk, Potomac, Youghiogheny, Greenbrier, Guyandotte, James, and Tug Fork river drainages, as well as drainages from the northern panhandle of WV. Analyses of these data are underway, and the

remainder of the state will be sampled in future efforts. Separate efforts will also target burrowing crayfishes.

In addition to the overall distributional information on the WV crayfish fauna, some interesting findings of this work include rediscovery of *Cambarus veteranus*, and the discovery and description of four new species of *Cambarus*: *Cambarus smilax*, *Cambarus theepiensis*, *Cambarus hatfieldi*, and *Cambarus appalachiensis*.

The first species description, resultant of specimens collected during this study, was published in the *Proceedings of the Biological Society of Washington*. The new species, *Cambarus smilax* (Greenbrier Crayfish; Figure 1A), is endemic to streams occurring in the Greenbrier River system, and reaches its highest population densities in the headwaters of the Greenbrier River, specifically the East and West Forks of the Greenbrier, Thorny Creek, and Deer Creek. Populations of this animal are stable but are limited to the Greenbrier River system.

Secondly, *Cambarus theepiensis* (Coalfields Crayfish) was described as a new species in the journal *Zootaxa* in 2013 (Figure 1B). This species is a stream-dwelling crayfish that appears to be endemic to the junction of the Cumberland Mountains with the Appalachian Plateau in West Virginia and Kentucky. Within this region, it is prevalent in the Guyandotte and Twelvepole basins of West Virginia, the Little Sandy River and Levisa Fork basins of Kentucky, and tributaries of the Big Sandy River shared by both states. The specific name is the latinized form of the Shawnee word for river, theepi. The Shawnee were among the first settlers of the Big Sandy, Lower Ohio and Guyandotte watersheds.

Cambarus hatfieldi (Tug Valley Crayfish) was described as a new species in the journal *Zootaxa* in 2013 (Figure 1C). This stream-dwelling crayfish appears to be endemic to the Tug Fork River system of West Virginia, Virginia, and Kentucky. Within this region, it is prevalent in all major tributaries in the basin as well as the Tug Fork River's mainstem. The specific name is the latinized form of Hatfield in honor of the Hatfield and McCoy feud which occurred in the Tug Fork River Valley of Kentucky and West Virginia in the late 1800s.

Cambarus appalachiensis (Conhaway Crayfish) was described as a new species in the journal *Zootaxa* in 2017 (Figure 1D). This stream-dwelling crayfish is endemic to the greater New River basins of Virginia and West Virginia. Within the New, Gauley, and lower portions of the Greenbrier basins *C. appalachiensis* is the dominant tertiary burrowing *Cambarus* species. The common name Conhaway

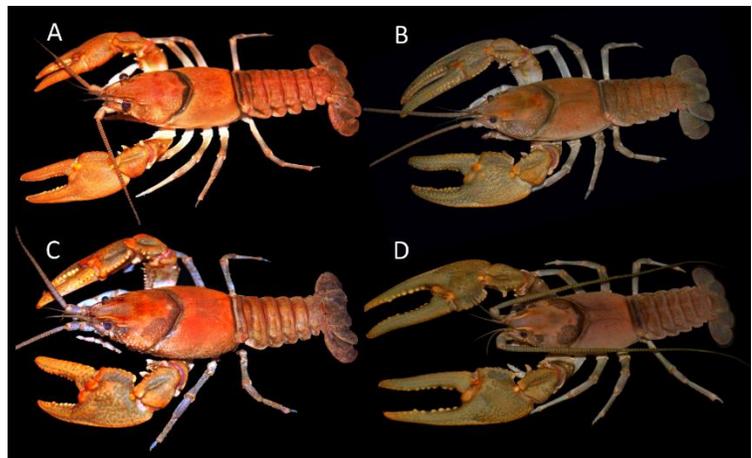


Figure 1. Four crayfish species described during this study: A. *Cambarus smilax*, B. *Cambarus theepiensis*, C. *Cambarus hatfieldi*, and D. *Cambarus appalachiensis*.

Crayfish is in reference to Conhaway River, which was the name of both New and Bluestone Rivers in the 1700's.

Cambarus fetzneri (Allegheny Mountain Mudbug) was described as a new species in the journal Zootaxa in 2019 (Figure 2). The distribution range of this primary borrowing crayfish includes the Allegheny Mountains and western Ridge and Valley physiographic provinces in Virginia and West Virginia at elevations above 1,800 feet (550 m).



Figure 2. Two crayfish described recently; *Cambarus fetzneri* (left) and *Cambarus Loughmani* (right; photo credit, Guenter Schuster)

Cambarus loughmani (Blue Teays Mudbug) was described by David Foltz et al. as a new species in the Journal of Natural History in 2018 (Figure 2). This primary borrowing crayfish species is endemic to the pre-glacial Teays River Valley of the Appalachian Plateau physiographic province in West Virginia, including Cabell, Kanawha, Lincoln, Mason, and Putnam counties.

EFFECTS OF CORRIDOR H HIGHWAY CONSTRUCTION ON BENTHIC MACROINVERTEBRATE COMMUNITIES

Principal Investigators: Stuart Welsh and Jim Anderson

Co-Investigator: Lara Hedrick

Years Ongoing: 2002-2016

Expected Completion: May 2018

Funding Sources: West Virginia Division of Highways

Objective:

To identify and compare changes in the benthic macroinvertebrate communities within watersheds impacted by construction of Corridor H, a four lane highway.

Progress:

This study was initiated in response to commitments made by the West Virginia Division of Highways established during the environmental impact assessment update for Corridor H. As part of the conditions for constructing the highway, the WVDOH must establish a long-term investigation focused on providing community level information on stream ecosystems. The

current list of streams that will be impacted by construction is as follows: Beaver Creek, Patterson Creek, tributaries of Elk Lick, Middle Fork of Patterson, Walnut Bottom Run, Waites Run, Smokey Hollow, Haddix Run, Baldlick Fork, Wilmoth Run, and Lazy Run. The sites located in the Beaver Creek watershed are in “during construction” phase. Sites located in the Patterson Creek and Walnut Bottom watersheds are in “post construction” phase. Sites located in Haddix Run, Baldlick Fork, Wilmoth Run, and Lazy Run are located on the alignment section from Parsons, WV to Kerens, WV.

Sampling has been completed in the Patterson Creek watershed. Twelve sites in the Patterson Creek watershed were sampled biannually for benthic macroinvertebrates beginning in Spring 2002. “Before Construction” samples were collected until construction began in 2007 and 2008. Sites PC-1, PC-2, PC-3, PC-4E, and PC-4W are located on tributaries of Elklick Run. Construction in this watershed began in 2008 and ended in November 2013. Samples have been collected “Before” (n=11), “During” (n=11), and “After” (n=8) construction.

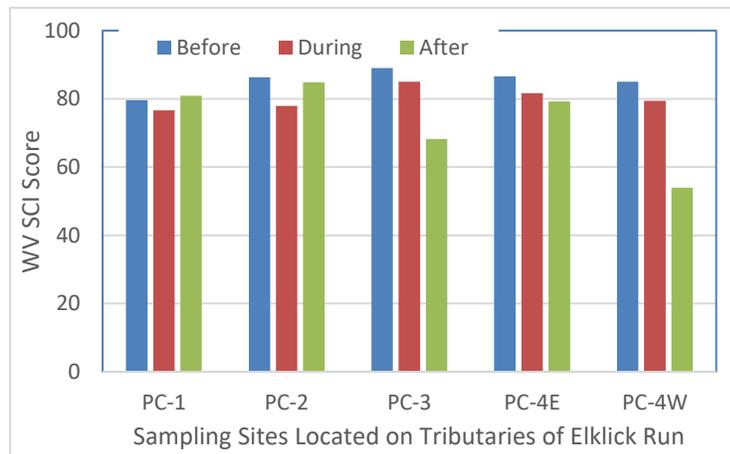


Figure 1. West Virginia Stream Condition Index Scores for sites located on tributaries of Elklick Run in the Patterson Creek watershed before (n=11), during (n=9), and after (n=6) construction.

PC-1, PC-2, PC-3, and PC-4E have maintained WV SCI scores in the “Good” to “Very Good” range throughout the sampling periods (Figure 1). All three sites have good riparian vegetation and good gravel / cobble habitats. Site PC-4W shows a decrease in WV SCI score in the post construction period. However, this site is located by the roadside, close to a culvert, and has potential to be annually influenced by other events such as water use in the stream and land use. Further samples will indicate if the benthic macroinvertebrate community has been impacted.

Sites PC-6, PC-7, and PC-8 are located on the Middle Fork of Patterson Creek. Site PC-6 is located at a secondary road crossing. It has marginal riparian habitat, however, the gravel substrate is optimal for benthic macroinvertebrates and it has maintained a “Good” WV SCI Score “Before” and “During” construction (Figure 2). Site PC-7 is located downstream of the highway alignment, some increase in sedimentation was noticed during construction. This site has maintained a “Good” WV SCI score during construction and post construction supports a healthy macroinvertebrate population. Site PC-5 is located on the North Fork of Patterson Creek in Greenland Gap. This site is in a relatively undisturbed area and sampling scores have always been “Good” (Figure 2).

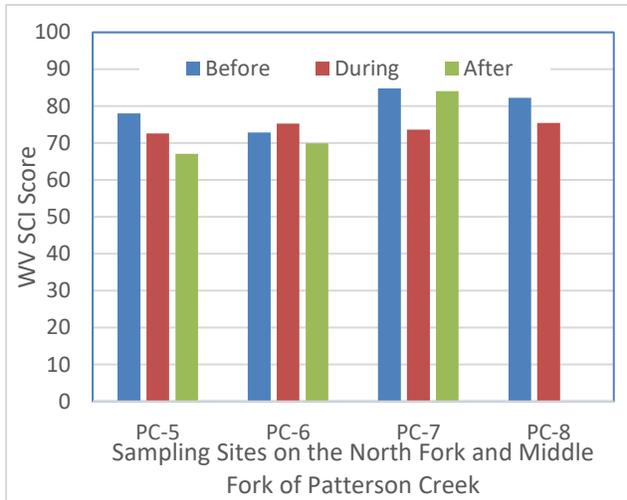


Figure 2. West Virginia Stream Condition Index Scores for sites located on the North Fork and Middle Fork of Patterson Creek watershed before (n=12), during (n=9), and after (n=6) construction.

Sites PC-9 and PC-10 are located on the main stem of Patterson Creek. Construction in this area began in 2007 and ended in November 2010. Benthic macroinvertebrate samples have been collected “Before” (n=10), “During” (n=7), and “After” (n=10) construction (Figure 3). Site PC-9 is located

upstream of the highway crossing, and site PC-10 is located in a farm field downstream of highway construction. Both sites had an average “Fair” WV SCI score “Before” and “During” construction, and average “Good” score post construction. Site PC-9 is located at a secondary road crossing and has marginal riparian habitat and fine gravel substrate. Site PC-10 is located at the edge of a farm field and has excellent riparian and good cobble/gravel substrate.

Two reference sites were sampled in conjunction with the Patterson Creek sites along the highway alignment (Figure 4). Sites PC-11 is located on the North Fork of Lunice Creek in Cosner Gap, and site PC-12 is located on Thorn Run, a tributary of Patterson Creek. Site PC-11 has a boulder and cobble substrate which may limit sensitive taxa that prefer cobble/gravel substrate. Site PC-12 is located upstream from a dam and has a ford crossing the stream. Construction would not have affected benthic macroinvertebrates at these sites, however, environmental factors such as high flows could have. Site PC-12 has consistently scored low, in the “Fair” WV SCI category, during spring sampling. Flushing of the stream and sediment inputs during high spring flows may contribute to this.

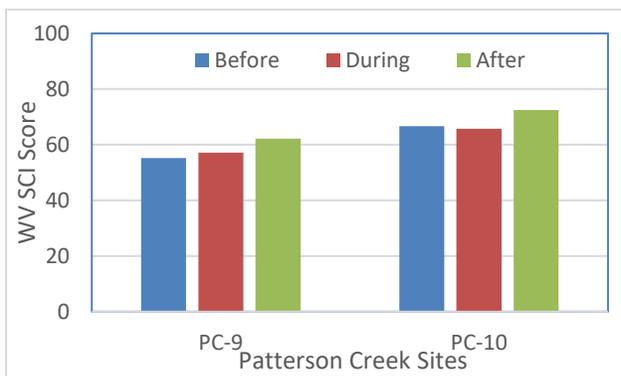


Figure 3. West Virginia Stream Condition Index Scores for sites located on Patterson Creek watershed before (n=10), during (n=7), and after (n=10) construction.

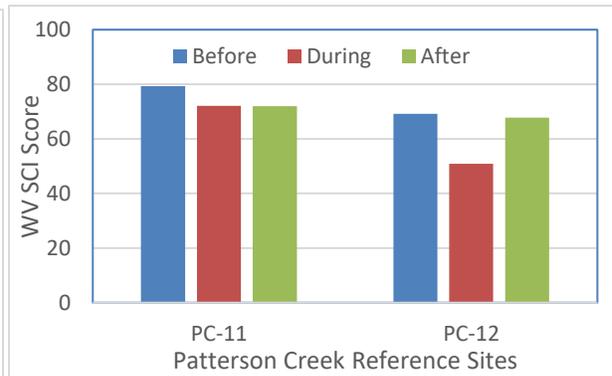


Figure 4. West Virginia Stream Condition Index Scores for Reference sites located on Lunice Creek and Thorn Run before (n=8), during (n=8), and after (n=6) construction.

WILDLIFE

THE EFFECTS OF IMIDACLOPRID ON TERRESTRIAL SALAMANDER COMMUNITIES WITHIN HEMLOCK FORESTS IN THREE SOUTHERN WEST VIRGINIA NATIONAL PARKS

Student Investigator: Lenza Paul

Principal Investigators: Yong-Lak Park, Donald Brown, Petra Wood

Cooperators: John Perez, Christine Arnott

Years Ongoing: 2016-Present

Degree Program: MS

Expected Completion: May 2020

Funding Source: National Park Service

Objectives:

- 1) Relate abundance and diversity of terrestrial salamanders with the frequency and extent of imidacloprid treatment and invertebrate abundance.
- 2) Compare body condition of salamanders at imidacloprid treatment sites to those at sites with no imidacloprid treatment by measuring morphological and physiological differences in individuals encountered.

Progress:

Hemlock forests make up a significant portion of the total forested area in New River Gorge National River (NERI), Gauley River National Recreation Area (GARI), and Bluestone National Scenic Area (BLUE) and play critical roles in the ecology, aesthetics, and recreational aspects of each park. Hemlock-dominated forests create unique micro-climates providing favorable habitats for certain wildlife species and affect understory plant species composition, biomass, and productivity (Fig. 1). Hemlocks are threatened by an exotic insect pest, the hemlock woolly adelgid (HWA). Although the effectiveness of imidacloprid on controlling HWA is well documented, the long-term effects of this insecticide on soil dwelling organisms and ecology of forest soils and water resources are poorly understood. This terrestrial salamander community research is one portion of a larger project that also is studying the effects and extent of imidacloprid on non-target meso- and macro-soil dwelling invertebrates.

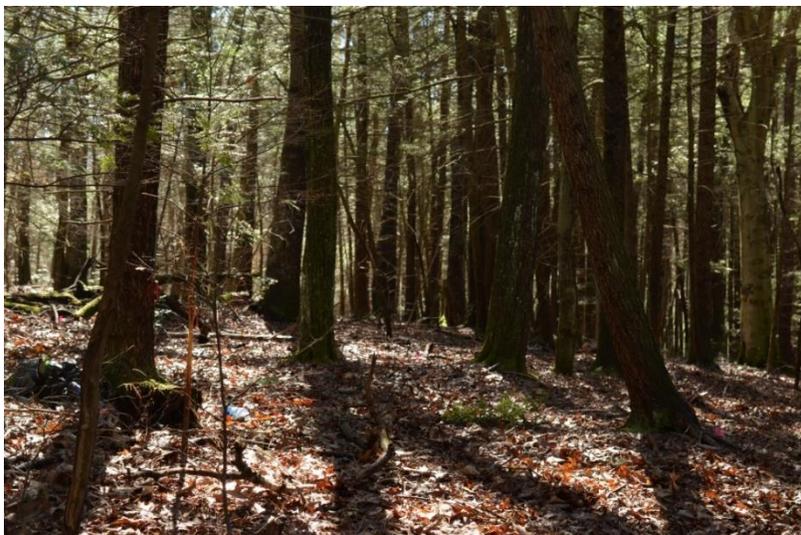


Figure. 1. Typical understory in an eastern hemlock dominated forest (photo by Lenza Paul, NPS)

To assure that the imidacloprid-treatment history in the parks reflected the current level of imidacloprid concentration in the soil, soil sampling was conducted at candidate sites by taking composite samples of 20 random soil cores (15 cm in depth) in each site. Concentration of imidacloprid in the sampled soil was determined using a competitive Enzyme-Linked Immunosorbent Assay (ELISA) technique. Eighteen study sites per year were chosen based on two levels of pesticide application (no and high imidacloprid inputs). In fall of 2016, coverboards for sampling salamander populations were placed in each plot (Fig. 2) and sampling began the spring of 2017. Eighteen new sites were chosen in late fall of 2017, and sampling began in the new plots in spring 2018. By the end of the fall 2018 sampling period, each site was sampled at least five times. In spring of 2019, all 36 plots were each sampled at least 5 times to make up for a lack of spring captures in previous sampling years. Habitat data was collected for all 36 plots in 2018. Soil pH, foliage cover, soil moisture, litter and duff depth, live basal area, and tree species inventories were completed for each plot for spring, summer, and fall.



Figure 2. Coverboard grid (Photo by Lenza Paul)

Captures in 2018 continued to be dominated by two species: eastern red-backed salamanders and northern slimy salamanders (Fig.3). Of 902 total captures across all sites and years (Table 1), eastern red-backed salamanders comprised 83% of overall captures, while northern slimy salamanders accounted for 8.5% of total captures. Control sites yielded 436 total captures of 10 species, while treatment sites had 466 captures of 11 species. Preliminary data analysis suggests no effect of insecticide treatment on body condition indices (calculated as individual mass/snout-to-vent length measurement) of either eastern red-backed or northern slimy salamanders ($p=0.76$ and $p=0.80$, respectively).



Fig. 3. The two most abundant species captured were the eastern red-backed salamander (left) and the northern slimy salamander (right)

Table 1. Total number of captures for each species for all years in control and treatment plots.

Species	Control	Treatment
Spotted salamander (<i>Ambystoma maculatum</i>)	0	1
Southern two-lined salamander (<i>Eurycea cirrigera</i>)	10	6
Four-toed salamander (<i>Hemidactylium scutatum</i>)	3	2
E. red-spotted newt (<i>Notophthalmus v. viridescens</i>)	12	8
E. red-backed salamander (<i>Plethodon cinereus</i>)	373	399
N. slimy salamander (<i>Plethodon glutinosus</i>)	35	42
Allegheny mountain dusky salamander (<i>Desmognathus ochrophaeus</i>)	1	2
Seal salamander (<i>Desmognathus monticola</i>)	0	2
N. dusky salamander (<i>Desmognathus fuscus</i>)	1	3
N. red salamander (<i>Pseudotriton r. ruber</i>)	1	1
Total captures	436	466

Prey availability sampling targeting ant species was completed 3 times for all 36 plots in summer 2019. Bait was placed in a small petri dish and deployed for 30 minute intervals. The sample was then taken back to the laboratory and placed in a freezer. Collected individuals were separated from the sampling material and air dried. Samples are currently being counted and identified to species, which will be completed by end of 2019. In early 2020, samples will be oven dried and weighed to add a mass variable to the analysis.

Synthesis of the information from this study could culminate in a reassessment of the HWA treatment program, guiding resource-based decisions. The results of this study will assist managers in prescribing and implementing effective, specific actions that preserve significant soil biodiversity and water quality, while still allowing appropriate treatments to control HWA. In addition, this research will document unacceptable impacts to soil fauna critically important to sustaining protected rare bird and amphibian species, enabling resource managers the opportunity to mitigate impacts by changing treatment protocols.

**EFFECTS OF CLIMATE CHANGE AND LANDSCAPE-SCALE FOREST MANAGEMENT ON AVIAN
COMMUNITIES, ABUNDANCE, AND NEST SUCCESS IN THE APPALACHIAN MOUNTAINS**

Student Investigator: Hannah Clipp

Principle Investigators: Petra B. Wood and Christopher T. Rota

Cooperators: Catherine Johnson, Kyle Crafts

Years Ongoing: 2018-Present

Degree Program: PhD

Expected Completion: May 2022

Funding Source: WVU Ruby Fellowship, NSF Graduate Research Fellowship Program, US Forest Service Monongahela National Forest

Objectives:

1. Review and explore potential effects of both climate and land cover change on forest songbirds of the Appalachian Mountains.
2. Understand if and why forest avian communities are changing over time and across elevational and latitudinal gradients.
3. Examine long-term avian responses to landscape composition and connectivity in both managed and unmanaged forested landscapes.
4. Assess the effectiveness of the recent habitat management guidelines for wood thrush (*Hylocichla mustelina*) and associated forest songbirds.
5. Identify the characteristics of wildlife openings that support a diversity of game birds, breeding songbirds, and post-breeding songbirds.

Progress:

Bird populations fluctuate over time from local to regional scales. Recent evidence from annual, nationwide breeding bird surveys indicates that numerous bird species, many of which are neotropical migrant bird species that breed in temperate forests, have declined throughout the eastern United States from 1966 to 2015. Major reasons for declining populations of forest songbirds include changes in the global climate, landscape (e.g., habitat loss and fragmentation), and local habitat structure. These different factors act both independently and in conjunction, and they affect individual bird species to varying degrees.

The Appalachian Mountains are a dominant land feature of the eastern United States, covering more than 190 million ha and stretching across a latitudinal range of 1,450 km. This extensive region is known for its diverse bird communities and is a focal area for avian research and conservation efforts. However, environmental conditions in the Appalachian Mountains region are changing, with likely consequences for regional bird populations and distributions, particularly along an elevational gradient. Gaining a better understanding of changes in forest songbird communities and abundance in the diverse habitats of the Appalachian Mountains is critical to evaluate the potential effects of a changing climate and landscape and to guide habitat management activities meant to minimize or mitigate impacts from these changes.

Beginning in 1992, a series of WV Coop Unit research studies have quantified avian communities on the Monongahela National Forest (MNF) and nearby private lands within the Central Appalachians. These efforts consist of both point count and nest success data. Additional avian monitoring in this area has been conducted by biologists from the US Forest Service, WV Division of Natural Resources, and West Virginia University. These existing historic datasets will be supplemented with datasets provided by collaborators from the Northern and Southern Appalachians and new additional survey efforts within the Central Appalachians. Collectively, these historic and new datasets will be used to evaluate our research objectives.

For objective 1, we will focus on the potential effects of both climate and land cover change on forest songbirds of the Appalachian Mountains. We will conduct a comprehensive review of published literature on this subject. We also will present a novel case study in which we look at the relative influence of climate vs. land cover change on forest songbird distributions and project those distributions based on future climate and land cover scenarios. For objective 2, we will use long-term historical point count data from the northern, central, and southern Appalachian Mountains to understand if and why forest avian communities are changing over time and across elevational and latitudinal gradients. Specifically, we will examine temperature and precipitation trends over time as possible explanations for any change in bird communities within the Hubbard Brook Experimental Forest (Northern Appalachians), MNF (Central Appalachians), and the Nantahela and Pisgah National Forests (Southern Appalachians).

To address objective 3, we shift our focus from climate factors to concentrate on the effects of landscape change. We will examine long-term avian responses to landscape composition and connectivity in the MNF, a predominantly unmanaged landscape, and in nearby industrial private forest lands, which represent a heavily managed landscape. We will also incorporate landscape-level factors in our analyses for both objectives 4 and 5, as well as local habitat features and management practices. As part of objective 4, we will assess the effectiveness of recent habitat management guidelines for wood thrush, a declining songbird species of conservation concern, and other forest songbirds. Although recommendations in the guidelines were developed based on best available science from peer-reviewed journal articles, they have not been explicitly tested with empirical field data. We will use point count data collected from a wide variety of habitat conditions to evaluate expected responses of wood thrush to the recommended management practices and to suggest revisions to the guidelines if warranted.



For objective 5, we aim to understand how and potentially why avian communities in wildlife openings (Fig. 1) vary within the MNF. In particular, we will identify the site- and landscape-level characteristics of wildlife openings that support a diversity of game birds, breeding songbirds, and post-breeding songbirds.



Figure 1. Examples of wildlife openings within the Monongahela National Forest in eastern West Virginia. Photos by Hannah Clipp.

New data collection for this dissertation project began in April 2019. Game bird surveys (including the simultaneous use of in-person surveys, game cameras, and autonomous recording units), breeding bird point count surveys, post-breeding mist-netting surveys, and vegetation surveys were conducted for 65 wildlife openings located through the MNF (Fig. 2). In addition, we completed point count surveys for nearly 40 historic transects located in the MNF, building upon the long-term dataset. New field data collection will continue in 2020 and 2021.



Figure 2. Images of an autonomous recording unit and game camera deployed for game bird surveys and an Indigo Bunting caught during a post-breeding mist-netting survey in a wildlife opening within the Monongahela National Forest in eastern West Virginia. Photos by Hannah Clipp.

WILDLIFE RESPONSE TO YOUNG FOREST HABITAT CREATION

Student Investigator: Eric Margenau
Principal Investigator: Petra Wood
Cooperators: Chris Ryan, Michael Peters, Gary Foster
Years Ongoing: 2015-Present
Degree Program: PhD
Expected Completion: August 2020
Funding Sources: West Virginia Division of Natural Resources

Objective:

Evaluate the response of bird (game and non-game) and salamander communities to young forest creation throughout West Virginia along gas pipelines, utility lines, and wildlife openings and within block cuts in mature forests.

Progress:

West Virginia is ~80% forest cover, which is predominantly comprised of mid- and late-successional age classes. Aging forest conditions in the northeastern United States have negatively affected over 70 wildlife species that require some form of disturbance mediated habitat. One of the main goals of this research is to better understand ways of managing for young forest dependent wildlife species in areas not often thought of as usable or optimal for many wildlife species (e.g., along gas pipelines and transmission powerlines) while also assessing the effect on mature forest dependent species.



Juvenile American woodcock observed in cut-back border at Beury Mountain

Young forest habitat was created by tree cutting and herbicide treatment in a variety of plot sizes, disturbance intensities, and surrounding matrices. Landscape matrices included rights-of-ways (ROW; gas pipeline or transmission powerline), wildlife openings, or 10-acre harvests within mature forests. Tree cutting along ROWs and wildlife openings was done at three different depths into the forest (50, 100, and 150 ft) and two different harvest intensities (20 ft²/ac and 60 ft²/ac basal area retention); these “cut-back borders” were compared to undisturbed control plots. Within forests, we implemented three different tree cutting-management techniques on 10-acre plots (clearcut-leave, clearcut-windrow, or hack-and-spray herbicide); these “regeneration stands” were also compared to undisturbed plots. We followed a before-after-control-impact (BACI) framework to study the effects of our various techniques, with one year of pre-treatment data collection and one or two years of post-treatment data collection.

In 2019, we finished data collection with assistance from WVDNR personnel. We sampled at 8 of the 11 total study sites (Fig. 1) in 2019. For species assessment, we conducted two rounds of ruffed grouse (*Bonasa umbellus*) drumming surveys from April 12 to May 15 between 530-1000 EST and one round of American woodcock (*Scolopax minor*) singing-ground surveys from April

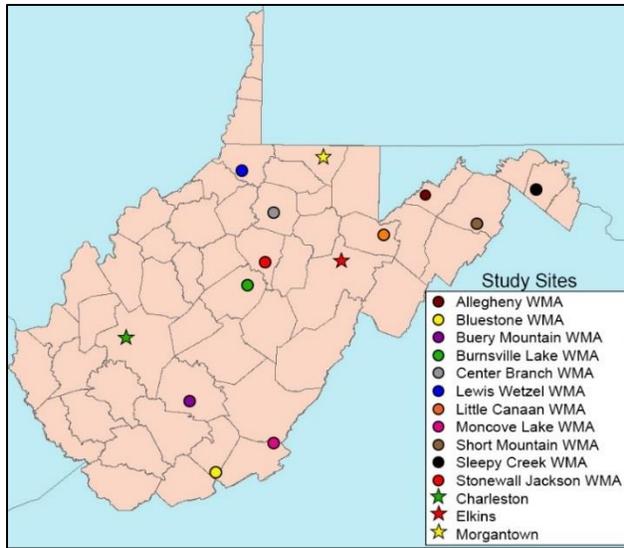


Figure 1. Map of study sites where sampling occurred during the entirety of the project.

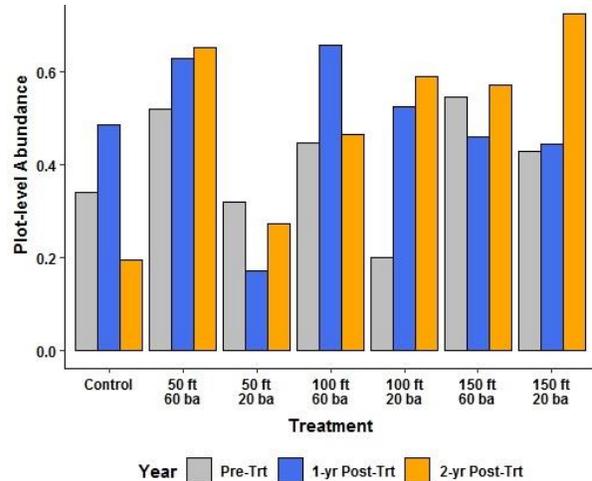


Figure 2. Estimated plot-level abundances of ruffed grouse in cut-back borders.

15 to May 5 between 1947–2100 EST. We calculated plot-level abundance estimates of ruffed grouse using a binomial N-mixture model for each year and estimated vegetation correlates. We detected a total of 28 drumming grouse in cut-back border plots during the study. No significant trends were observed among treatments or years (Fig. 2), but abundance was positively correlated with live understory vegetation cover 1–2 m above ground (slope coefficient = 0.592). We detected a total of 2 American woodcock in cut-back border plots during the study which was insufficient for statistical analysis.

We conducted two rounds of breeding songbird surveys from May 18 to June 27 between 0530-1000 EST. We calculated plot-level species richness of two habitat guilds (young forest and mature forest) for each year and estimated vegetation correlates. Young forest and mature forest guild richness increased from pre-treatment to two years post-treatment in all cut-back border treatments (Fig. 3). Young forest guild richness was also significantly higher in treatment plots

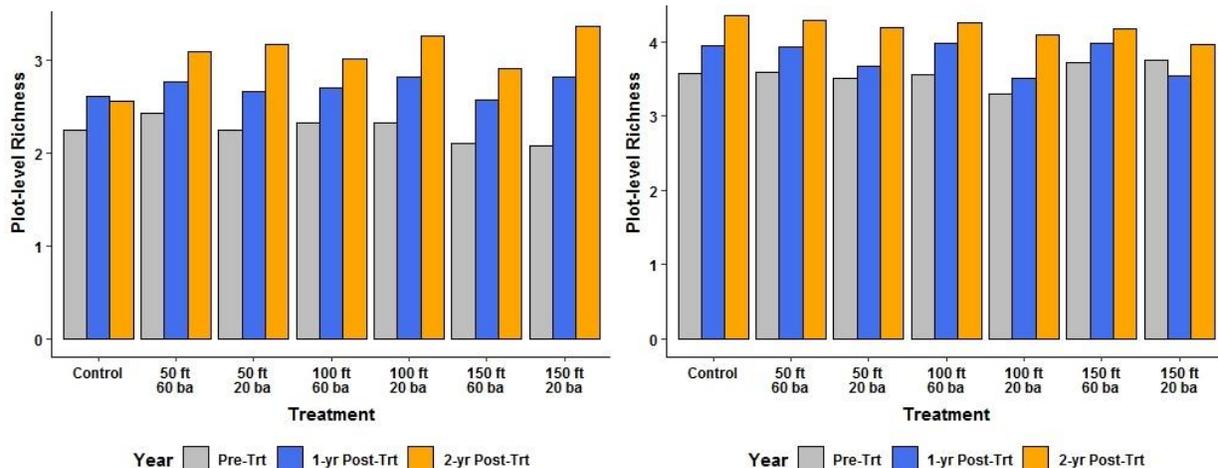


Figure 3. Plot-level richness of the Young Forest guild (left) and the Mature Forest guild (right) breeding songbird community in cut-back borders.

than the control plot at two-years post-treatment. Young forest guild richness was positively correlated with percent ground cover and live understory cover 1–2 m above ground (slope coefficients = 0.311 and 0.371, respectively). Mature forest guild richness was positively correlated with overstory cover and negatively correlated with number of woody stems 5–11.3 cm DBH (slope coefficients = 0.342 and -0.348, respectively).

We conducted three rounds of salamander sampling from April 15 to August 9 each year using passive sampling techniques (coverboards and natural cover objects). We captured a total of 243 individuals from the Genus *Plethodon* (eastern red-backed salamander [*Plethodon cinereus*], northern slimy salamander [*P. glutinosus*], and Wehrle’s salamander [*P. wehrlei*]) over the three-year sampling period. Total captures in cut-back border treatments remained relatively stable or slightly increased following tree cutting, while total captures in the control plots increased over the study period (Fig. 4). Statistical analyses that incorporate detection probability are underway for these data.

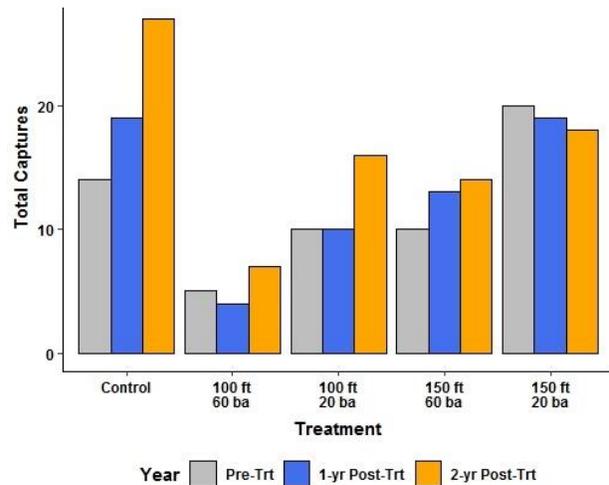


Figure 4. Total plot-level captures of *Plethodon* salamanders in cut-back borders.

With this project, we hope to better understand how species are using areas that are often not considered when developing management plans and goals. We are in the initial stages of analyzing the final data sets for this project. Future analyses will focus on assessing the songbird and salamander community and individual species response to cut-back border and regeneration treatments and the relations with vegetation characteristics.

Occupancy and Abundance of Cerulean Warbler and Golden-winged Warbler Following Private Land Implementation of Species-specific Habitat Management

Student Investigator: Lincoln Oliver

Principal Investigators: Petra B. Wood and Christopher Lituma

Cooperators: Rich Bailey, Kyle Aldinger

Years Ongoing: 2018-Present

Degree Program: MS

Expected Completion: Dec 2020

Funding: WV Division of Natural Resources, West Virginia University

Objectives:

1. Quantify the effects of Appalachian Forestland Enhancement Project management on Cerulean Warbler (*Setophaga cerulea*) occupancy and abundance in West Virginia
2. Quantify the effects of Working Lands for Wildlife management on Golden-winged Warbler (*Vermivora chrysoptera*) occupancy and abundance in West Virginia

3. Quantify the effects of Working Lands for Wildlife and Appalachian Forestland Enhancement Project management on Blue-winged Warbler (*Vermivora cyanoptera*) occupancy in West Virginia
4. Determine the longevity of effects of management for all species and provide guidelines for adaptive management on private lands
5. Evaluate management effects on the overall avian community

Progress:



Cerulean Warbler (*Setophaga cerulea*; CERW) and Golden-winged Warbler (*Vermivora chrysoptera*; GWWA) are two high-priority species for conservation that breed in West Virginia. Each was petitioned for listing under the Endangered Species Act (ESA) and both continue to experience population declines, however, efforts are underway to implement species-specific management on private lands. CERW is the focal species of the Appalachian Forestland Enhancement Project (AFEP) under the Regional Conservation Partnership Program (RCPP) (<http://www.nrcs.usda.gov/wps/portal/nrcs/detail/wv/newsroom/releases/?cid=STELPRDB1268019>) and GWWA is a focal species in the Working Lands for Wildlife (WLFW) program (<http://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/programs/initiatives/?cid=stelprdb1046975>). An extensive body of literature and recent research findings are informing on-the-ground management for these species but the effectiveness of these conservation practices for increasing CERW and GWWA populations on private lands has not been examined.

West Virginia is implementing both conservation programs which provides an opportunity to monitor the response of GWWA and CERW populations pre- and post-implementation. The Blue-winged Warbler (*Vermivora cyanoptera*, BWWA) is also a species of conservation concern and influences GWWA conservation in the Appalachians due to competition and hybridization between both species. The state falls in the core of all three-species' geographic range; thus, all are listed as species of conservation concern by the West Virginia Division of Natural Resources (WVDNR) State Wildlife Action Plan (SWAP). We are monitoring the effects of AFEP management on CERW and WLFW management on GWWA on sites throughout West Virginia to evaluate the effectiveness of each program at creating, improving, or maintaining CERW and GWWA habitat, as well as the longevity of management for both species. We are also monitoring BWWA to evaluate the effectiveness of each program in creating BWWA habitat and to document BWWA's influence on the success of WLFW.

Beginning in 2018, we partnered with NRCS and WVDNR biologists to identify enrolled private lands for both AFEP and WLFW and obtained landowner permission for access. We stratified sites based on their treatment status during the 2019 breeding season and surveyed points in 7

treatment categories (pre-treatment, during treatment, and 1-, 2-, 3-, 5-, and 6-years post-treatment) and points in untreated reference areas. Pre-treatment data was collected on 13 properties in Calhoun, Clay, Doddridge, Hampshire, Marion, Monroe, Hardy, Pocahontas, and Upshur Counties. Post-treatment data was collected on 10 properties in Hampshire, Monroe, Nicholas, Pendleton, Pocahontas, Randolph and Webster Counties. We followed a before-after-control-impact (BACI) framework to evaluate the effects of habitat management incorporated through AFEP and WLFW, prioritizing pre-treatment data collection in 2019 and post-treatment data collection in 2020.

Songbird and vegetation data were collected at 236-point count locations during 2019. We used multi-species, 100 m-radius, 10-minute avian point counts conducted during the peak breeding season (May 6 – June 28) at all sites. Counts were stratified by time and distance bands. To allow estimation of GWWA and CERW occupancy and abundance in Program R using package *unmarked*, we visited all points twice. Across all sites, we detected a total of 31 CERW, 3 GWWA, and 11 BWWA (Figure 1). We detected no CERW on post-treatment points, whereas all GWWA detections were on post-treatment points.

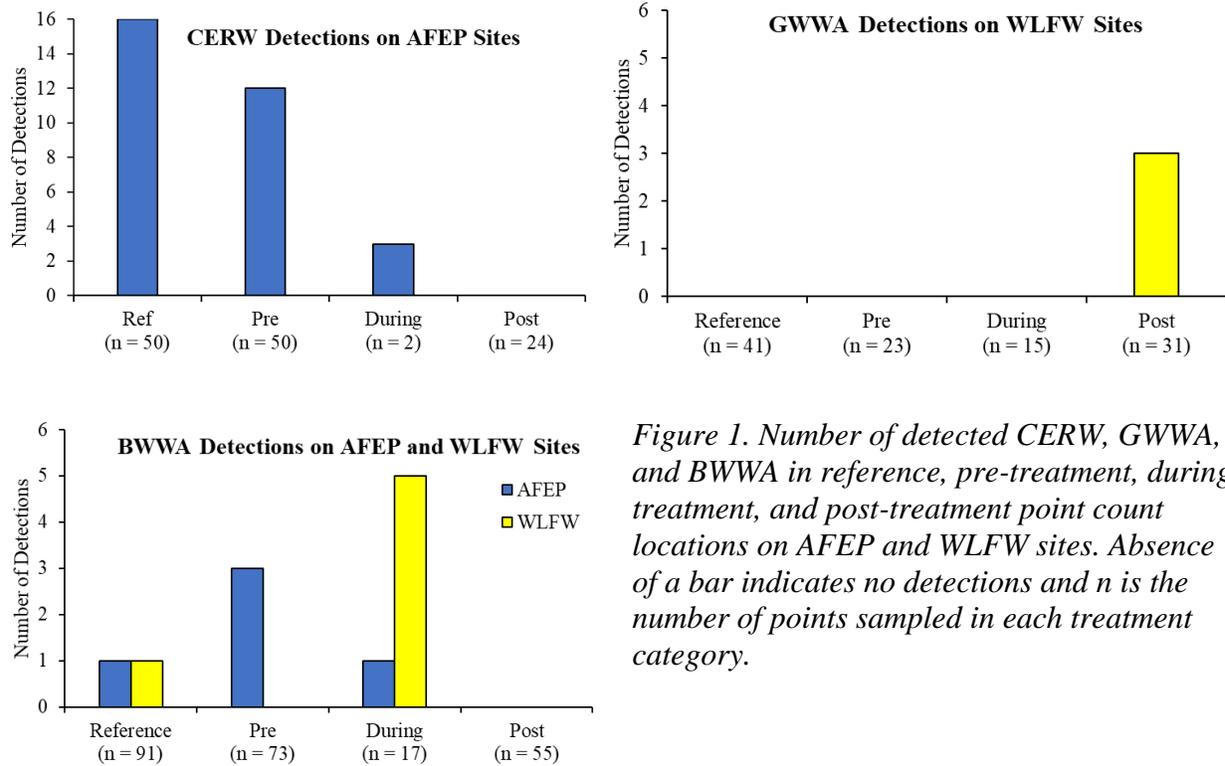


Figure 1. Number of detected CERW, GWWA, and BWWA in reference, pre-treatment, during treatment, and post-treatment point count locations on AFEP and WLFW sites. Absence of a bar indicates no detections and n is the number of points sampled in each treatment category.

We assessed avian community responses to habitat management incorporated through AFEP and WLFW using a guild approach which grouped species into one of three avian guilds (forest generalist, forest interior, and shrubland). Species richness of forest generalist and forest interior guilds was generally lower 1-year post-harvest on AFEP sites.

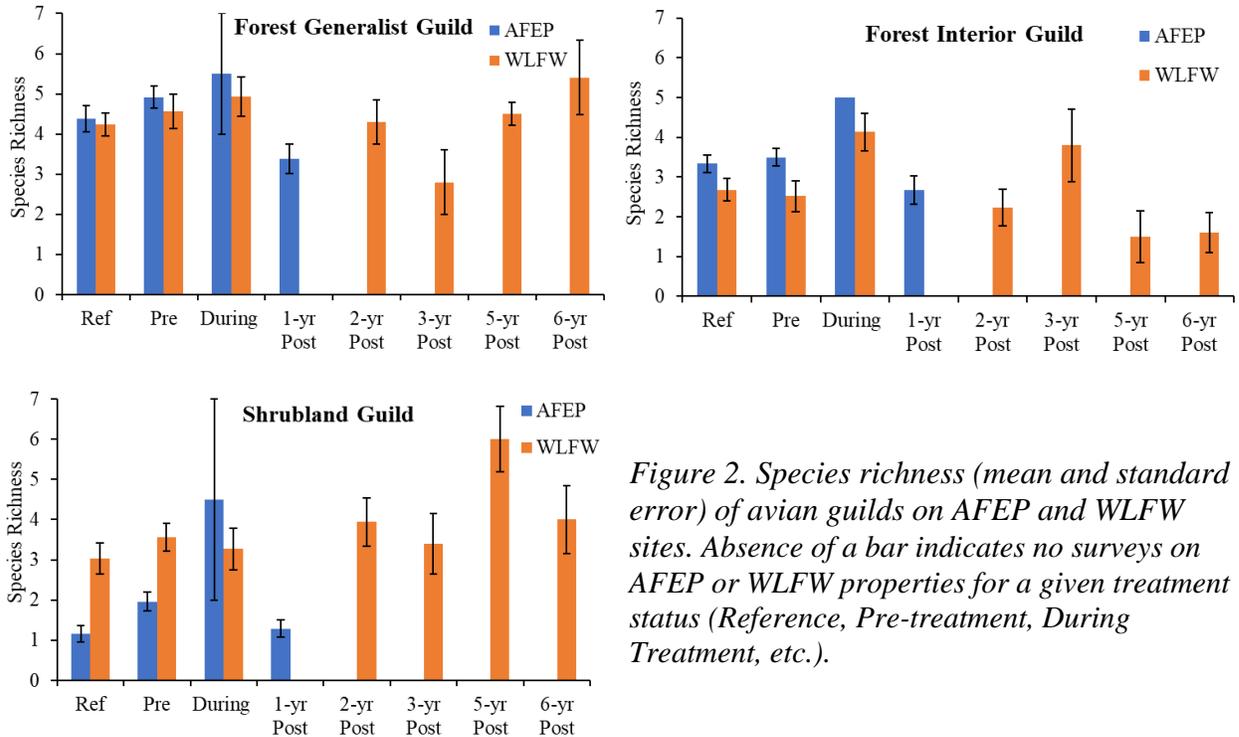


Figure 2. Species richness (mean and standard error) of avian guilds on AFEP and WLFW sites. Absence of a bar indicates no surveys on AFEP or WLFW properties for a given treatment status (Reference, Pre-treatment, During Treatment, etc.).

Songbird and vegetation data will be collected at the same 236-point count locations during 2020. Several of our pre-treatment sites surveyed in 2019 should have management implemented by the 2020 breeding season. Thus, we expect to have a larger sample size for evaluating post-treatment response after collecting the 2020 data.

LONG-TERM SONGBIRD POPULATION RESPONSE TO SHALE GAS DEVELOPMENT

Student Investigators: Eric Margeneau, Laura Farwell, Jim Sheehan, Greg George

Principal Investigator: Petra B. Wood

Years Ongoing: 2008-present

Expected completion: May 2020

Funding Sources: US Fish and Wildlife Service, West Virginia Division of Natural Resources, Department of Energy

Objectives:

1. Quantify the long-term effects of shale gas development on forest loss and fragmentation.
2. Determine if changes in avian guild species richness, focal species abundance, and spatial distributions reflect sensitivity to forest disturbance from shale gas development.

Progress:

Unconventional drilling for natural gas from the Marcellus-Utica shale has continued to expand in the central Appalachians, a heavily forested region that is a key conservation area for forest songbirds. The Lewis Wetzel Wildlife Management Area (LWWMA) in north-central WV had a substantial expansion of shale gas development beginning in 2008. During 2008-2019, we monitored annual breeding bird abundances at 142 survey points and annual land cover change across the site (Fig. 1).

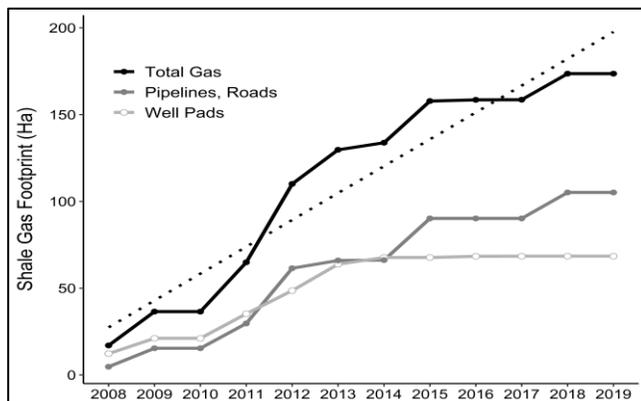


Figure 1. Site-wide increases in annual shale gas footprint (2008-2019), for all shale gas combined and separately for linear infrastructure (i.e. pipelines, access roads) vs. well pad areas (i.e. pads, buffers, fluid impoundments, storage areas). Dotted line represents linear trend obtained from ordinary least squares regression.

In Farwell et al. (2016), we documented the relationship between increasing shale gas development and avian species abundance and richness within three habitat guilds during 2008-2015. Construction of gas well pads and infrastructure (e.g., roads, pipelines) contributed to an overall 4.5% loss in forest cover at the site, a 12.4% loss in core forest, and a 51.7% increase in forest edge density by 2015. We evaluated the relationship between land-cover metrics and species richness within three avian guilds: forest interior, early-successional, and synanthropic, in addition to abundances of 21 focal species. Land-cover impacts were evaluated at a point-level within 100-m and 500-m buffers of each avian survey point and at a landscape-level across the study area (4326 ha). Although we observed variability in species-specific responses, we found distinct trends in long-term response among the three avian guilds. Forest-interior guild richness

declined at all points across the site and at points impacted within 100 m by shale gas but did not change at unimpacted points. Early-successional and synanthropic guild richness increased at all points and at impacted points. Our results suggest that shale gas development has the potential to fragment regional forests and alter avian communities, and that efforts to minimize new development in core forests will help to reduce negative impacts to forest dependent species.

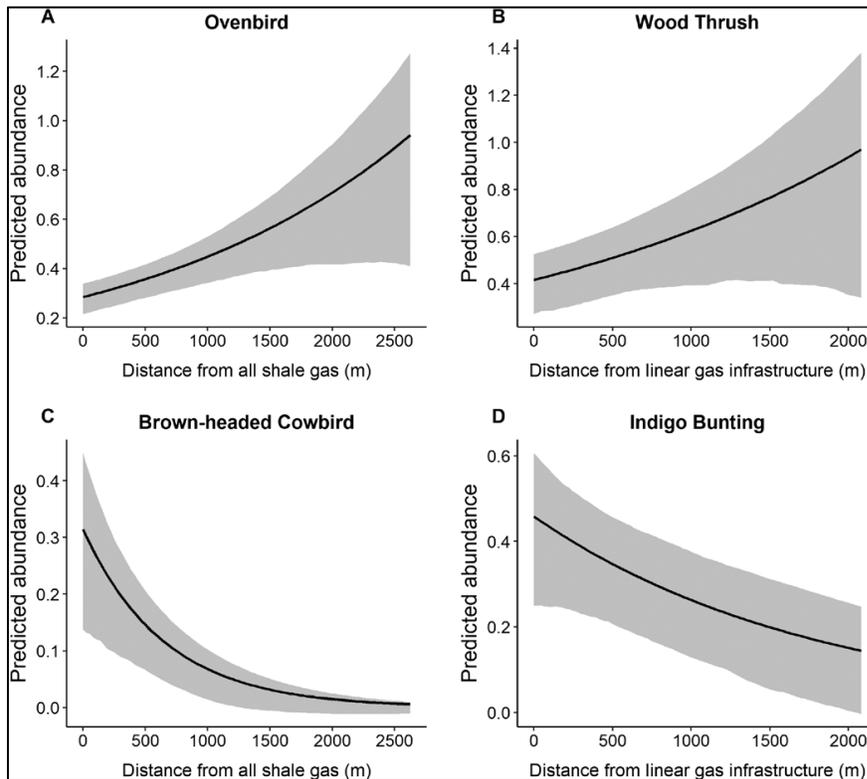


Figure 2: Examples of 2 species that increased in abundance with increasing distance (m) from unconventional shale gas development (A, B), and 2 species that decreased in abundance with increasing distance from gas development (C, D). Ovenbirds and Brown-headed Cowbirds showed significant responses to distance from all types of unconventional shale gas development (A, C), while Wood Thrush and Indigo Buntings responded to distance from linear shale gas infrastructure, in particular (B, D).

In Farwell et al. (2019), we evaluated the relationship between breeding passerine abundances and distance from shale gas development at LWWMA during 2008-2017. We found that more than half of the 27 focal species evaluated showed sensitivity to distance from shale gas infrastructure (e.g. well pads, access roads, pipelines). Five forest interior species occurred in higher abundances with increasing distance from shale gas (black-throated green warbler, hooded warbler, ovenbird, red-eyed vireo, and wood thrush; Fig. 2), while three forest interior gap specialists increased in abundance closer to shale gas (American redstart, cerulean warbler, eastern wood pewee). Early successional (common yellowthroat, indigo bunting) and synanthropic focal species (American robin, brown-headed cowbird, chipping sparrow) occurred in higher abundances closer to shale gas. We used interpolated distributions of 4 focal species to assess their spatial response to unconventional shale gas development over time. Our results indicate that breeding passerine distributions and community composition are changing with forest disturbance driven by unconventional shale gas energy development.

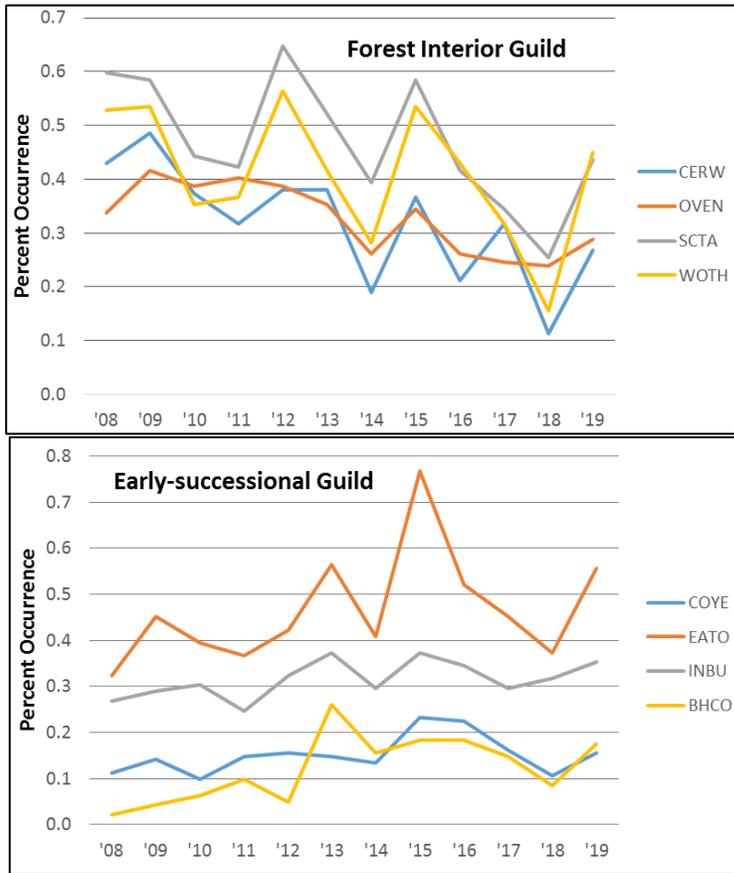


Figure 3. Site-wide occurrence (percent of points sampled) for 8 focal species from 2008 to 2019.

Across all years studied, 2008-2019, long-term trends show generally declining occurrence for forest interior species (Cerulean Warbler CERW, Ovenbird OVEN, Scarlet Tanager SCTA, and Wood Thrush WOTH) across the study area and increasing occurrence of early-successional species (Common Yellowthroat COYE, Eastern Towhee EATO, and Indigo Bunting INBU) and a nest parasite (Brown-headed Cowbird BHCO; Fig. 3). All species had some increase in 2019, likely in response to no additional disturbance from shale gas activities in 2019 (Fig. 1).

Through this long-term study, we have added to a growing body of research indicating that the rapid expansion of unconventional shale gas infrastructure in the Marcellus-Utica shale region is affecting forest ecosystems and altering native biological communities. Our results suggest that efforts to avoid shale gas infrastructure development in core forest areas of high conservation value will help mitigate negative impacts to native songbird communities, in a region with high value for global biodiversity.

Literature cited:

Farwell, L.S., P.B. Wood, J. Sheehan, and G.A. George. 2016. Shale gas development effects on the songbird community in a central Appalachian forest. *Biological Conservation* 201:78-91.

Farwell, L.S., P.B. Wood, D.J. Brown, and J. Sheehan. 2019. Proximity to unconventional shale gas infrastructure alters breeding bird abundance and distribution. *Condor* 2019:1-20. DOI: 10.1093/condor/duz020

PUBLICATIONS, THESES, DISSERTATIONS, PRESENTATIONS, AND HONORS, AWARDS, AND APPOINTMENTS

SCIENTIFIC PUBLICATIONS

- Farwell, L.S., P.B. Wood, D.J. Brown, and J. Sheehan. 2019. Proximity to unconventional shale gas infrastructure alters breeding bird abundance and distribution. *The Condor* 121(3) <https://doi.org/10.1093/condor/duz020>
- Frantz, M. W., P. B. Wood, and G. T. Merovich, Jr. 2018. Demographic characteristics of an avian predator, Louisiana Waterthrush (*Parkesia motacilla*), in response to its aquatic prey in a Central Appalachian USA watershed impacted by shale gas development. *PLOS ONE* 13(11): e0206077. Online at <https://doi.org/10.1371/journal.pone.0206077>. Published Nov 2018.
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- Margenau MTR paper Margenau, E. L., P. B. Wood, C. A. Weakland, and D. J. Brown. 2019. Trade-offs relating to grassland and forest mine reclamation approaches in the central Appalachian region and implications for the songbird community. *Avian Conservation and Ecology* 14(1):2. <https://doi.org/10.5751/ACE-01304-140102>
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- Matsche, M.A., V.S. Blazer and P.M. Mazik. 2019. Seasonal development of the coccidian parasite *Goussia bayae* and hepatobiliary histopathology in white perch *Morone americana* from Chesapeake Bay. *Diseases of Aquatic Organisms*. 134:112-135.
- Nareff, G.E., P.B. Wood, D.J. Brown, T. Fearer, J.L. Larkin, W.M. Ford. 2019. Cerulean Warbler (*Setophaga cerulea*) response to operational silviculture in the central Appalachian region. *Forest Ecology and Management* 448:409–423.
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THESES AND DISSERTATIONS

- Crayton, S.M. 2019. Stream Salamander and Benthic Macroinvertebrate Community Responses to Imidacloprid Exposure. MS Thesis, West Virginia University, Morgantown. Aug 2019.
- Frantz, M.W. 2019. Demographic, Spatial, and Epigenetic Response of the Louisiana Waterthrush (*Parkesia motacilla*) to shale gas development. PhD Dissertation, West Virginia University, Morgantown. May 2019.
- Nareff, G.E. 2019. Cerulean Warbler (*Setophaga cerulea*) and Associated Species Response to Operational Silviculture in the Central Appalachian Region. PhD Dissertation, West Virginia University, Morgantown. Sept 2019.
- Owens, Nathaniel. 2019. Distribution and Habitat Use of Benthic Fishes in the lower Kanawha River, West Virginia. MS Thesis, West Virginia University, Morgantown.
- Smith, Dustin. 2018. Evaluation of a re-established walleye population within a hydropower reservoir recovering from acidification. PhD Dissertation, West Virginia University, Morgantown.
- Underwood, Emily. 2018. An Interspecies Investigation of Thyroid Plasma Hormone Concentrations, Histology, and Gene Expression. MS Thesis, West Virginia University, Morgantown.

Walsh, Heather. 2019. Application of Molecular Pathology Techniques to Understand Mechanisms of Disease in Smallmouth Bass. PhD Dissertation, West Virginia University, Morgantown.

PRESENTATIONS

Brown, D. J., S. M. Crayton, T. J. Simpson, P. B. Wood, and Y-L. Park. Sept 2019. Bioaccumulation and sublethal effects on spotted salamanders from exposure to soil containing the pesticide imidacloprid. The Wildlife Society, 26th annual conference, Reno, Nevada (Oral Presentation).

Clipp, H. L., C. Johnson, C. T. Rota, and P. B. Wood. Mar 2019. Optimizing multi-species occupancy of game and non-game bird species in wildlife openings within Central Appalachian forests. Poster presented at The Wildlife Society Annual Conference, Reno, NV.

Clipp, H. L. Sept 2019. Multi-species avian occupancy of wildlife openings in the Monongahela National Forest. Poster presented at the West Virginia University Davis College Graduate Student Research Day, Morgantown, WV.

Crayton, S., P. Wood, D. J. Brown, K. Eliason, Y-L. Park, M. McKinney, T. Simpson, and K. M. Ku. July 2019. Impacts of imidacloprid exposure on spotted salamanders and benthic macroinvertebrates. Davis & Elkins College, Elkins, West Virginia (Oral Presentation).
Invited

Crayton, S., P. Wood, D. J. Brown, K. Eliason, Y-L. Park, M. McKinney, T. Simpson, and K. M. Ku. July 2019. Impacts of imidacloprid exposure on spotted salamanders and benthic macroinvertebrates. Washington & Jefferson College, Washington, Pennsylvania (Oral Presentation). **Invited**

Crayton, S., P. Wood, D. J. Brown, K. Eliason, Y-L. Park, and M. McKinney. Apr 2019. Benthic macroinvertebrate responses to imidacloprid exposure. West Virginia Chapter of The Wildlife Society, Morgantown, West Virginia (Oral Presentation).

Crayton, S., P. Wood, D. J. Brown, and Y-L. Park. Feb 2019. Effects of imidacloprid treatment on stream systems. West Virginia University, Women in Natural Resources Symposium (Poster Presentation).

Crayton, S., P. Wood, D. J. Brown, and Y-L. Park. Oct 2018. Effects of imidacloprid treatment on stream systems. The Wildlife Society, 25th annual conference, Cleveland, Ohio (Poster Presentation).

Farwell, L.S., P.B. Wood, R. Dettmers, M. Brittingham. Oct 2018. Region-wide Assessment of Marcellus-Utica Shale Gas Development Effects on Birds. Annual Conference of The Wildlife Society, Cleveland, OH, 10 Oct 2018. **Invited**

- Frantz, M. W., P. B. Wood, J. Sheehan, G. George, D. Becker, S. Latta, A. Welsh, and G. Merovich, Jr. Nov. 2018. Response of Louisiana Waterthrush and their benthic prey to shale gas development. Energy and the Environment Skype Lecture Series. Youngstown State University, Youngstown, OH, 14 November 2018. **Invited**
- Frantz, M. W., P. B. Wood, J. Sheehan, and G. George. Oct 2018. Louisiana Waterthrush demography in response to shale gas development. The Wildlife Society (TWS) 25th Annual Conference. Wildlife Responses to Marcellus-Utica Shale Gas Development and the Next Steps for Wildlife Conservation Symposium. Cleveland, OH, 10 October 2018. **Invited**
- Kramer, G.R., D.E. Andersen, D.A. Buehler, P.B. Wood, S.M. Peterson, J.A. Lehman, K.R. Aldinger, L.P. Bulluck, S. Harding, J.A. Jones, J.P. Loegering, C. Smalling, R. Vallender, and H.M. Streby. Oct 2018. Population trends of Vermivora warblers are linked to strong migratory connectivity. 2018 The Wildlife Society Annual Conference, Cleveland, Ohio USA.
- Margenau, E.L. and P.B. Wood. Apr 2019. Songbird response to young forest management using cut-back borders. West Virginia Chapter of The Wildlife Society Annual Conference.
- Margenau, E.L., P.B. Wood, and D.J. Brown. Oct 2018. Managing for birds and salamanders along forested edges of energy corridors. In Wildlife Responses to Marcellus-Utica Shale Gas Development and the Next Steps for Wildlife Conservation (Symposium). The Wildlife Society 25th Annual Conference. **Invited**
- Margenau, E.L. and P.B. Wood. 28 Aug 2019. Game and non-game bird response to young forest management. West Virginia Division of Natural Resources: Wildlife Resources Section Annual Meeting. Pipestem, WV. **Invited**
- Margenau, E.L. 11 Jul 2019. Reptiles and Amphibians: Terrestrial Salamanders. West Virginia Master Naturalist Program. Morgantown, WV. **Invited**
- Oliver, L.R., R. Bailey, K.R. Aldinger, C.M. Lituma, P.B. Wood. Sept 2019. Cerulean and Golden-winged Warbler Response to Private-Land Habitat Management. Poster Presentation at the The Wildlife Society Annual Conference, Reno, NV.
- Wood, P.B. Apr 2019. Forest Management for Cerulean Warblers and Associated Avian Species. Seminar at Frostburg State University Appalachian Ecology Lab. **Invited**

AWARDS

Hannah Clipp placed 1st in the category of PhD (Poster) in the Davis College Graduate Student Research and Creative Scholarship Day in March 2019. She is working towards her PhD degree through a NSF Graduate Research Fellowship.

Sara Crayton was awarded a WVU Swiger Doctoral Fellowship starting in August 2019 to support her PhD program.

Mack Frantz was a recipient of the 2019 BioOne Ambassador Award in April 2019. This prestigious award honors early-career researchers who have best communicated the importance and impact of their specialized research to the public at large.

Eric Margenau was awarded a Student Travel Award in July 2019 to attend and present at the Association of Field Ornithologist and Wilson Ornithological Society Joint Annual Meeting in October.