



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

FY 2018

ANNUAL REPORT

1 October 2017 - 30 September 2018



COOPERATING AGENCIES:

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

TABLE OF CONTENTS

	<u>Page</u>
WVCFWRU HISTORY.....	1
COORDINATING COMMITTEE.....	2
UNIT STAFF.....	2
COOPERATORS.....	3
STUDENTS.....	4
UNIT STAFF COURSES TAUGHT.....	5
PROGRAM DIRECTION STATEMENT.....	5
COMPLETED PROJECTS	
Aquatic.....	6
Wildlife.....	24
CONTINUING PROJECTS	
Aquatic.....	38
Wildlife.....	58
PUBLICATIONS, REPORTS, THESES, DISSERTATIONS, PRESENTATIONS, AND HONORS, AWARDS, AND APPOINTMENTS	
Scientific Publications.....	79
Theses and Dissertations.....	80
Presentations.....	81
Awards.....	82



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, 2018, 118 students have completed their degree requirements: 90 Masters and 28 Ph. D. The Unit scientists are currently supervising 9 Master's students and 7 Ph.D. students.



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U.S. GEOLOGICAL SURVEY
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COORDINATING COMMITTEE

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UNIT STAFF

UNIT LEADER

Patricia M. Mazik, Adjunct Professor of Fisheries
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

Becky Nestor, Unit Secretary, Division of Forestry and Natural Resources
Lara Hedrick, Research Assistant, Division of Forestry and Natural Resources

PROJECT COOPERATORS

UNIVERSITY

Jim Anderson, Professor, Division of Forestry and Natural Resources
Donald Brown, Assistant Research Professor, Division of Forestry and Natural Resources
Kyle Hartman, Professor, Division of Forestry and Natural Resources
Chris Lituma, Assistant Professor, Division of Forestry and Natural Resources
Yong Lak Park, Associate Professor, Entomology
Todd Petty, Professor, Division of Forestry and Natural Resources
Quinton Phelps, Assistant Professor, Division of Forestry and Natural Resources
Chris Rota, Assistant Professor, Division of Forestry and Natural Resources
Michael Strager, Associate Professor, Division of Resource Management
Amy Welsh, Associate Professor, Division of Forestry and Natural Resources

STATE

Rich Bailey, Division of Natural Resources
Steve Brown, Division of Natural Resources
Dan Cincotta, Division of Natural Resource
Gary Foster, Division of Natural Resources
Jim Hedrick, Division of Natural Resources
Brandon Keplinger, Division of Natural Resources
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Mike Peters, Division of Natural Resources
Chris Ryan, Division of Natural Resources
Mark Scott, Division of Natural Resources
Scott Warner, Division of Natural Resources

FEDERAL

Vicki Blazer, U.S. Geological Survey
Doug Chambers, U.S. Geological Survey
Randy Dettmers, U.S. Fish & Wildlife Service
Nathaniel Hitt, U.S. Geological Survey
Cathy Johnson, U.S. Forest Service
Cyndy Loftin, U.S. Geological Survey
John Perez, National Park Service
Alan Temple, U.S. Fish & Wildlife Service
Dave Smith, U.S. Geological Survey
Scott Stoleson, U.S. Forest Service

OTHER

Margaret Brittingham, Pennsylvania State University
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>
Kyle Aldinger	Ph.D.	Completed Aug 2018	Petra Wood
Ryan Braham	Ph.D.	Completed May 2018	Pat Mazik
Hannah Clipp	Ph.D.	Expected May 2022	Petra Wood/Chris Rota
Sara Crayton	M.S.	Expected May 2019	Petra Wood/Donald Brown
Laura Farwell	Ph.D.	Completed Aug 2018	Petra Wood
Mack Frantz	Ph. D.	Expected Dec 2018	Petra Wood
Cory Hartman	M.S.	Expected Aug 2019	Pat Mazik
Brin Kessinger	M.S.	Expected May 2020	Stuart Welsh/Amy Welsh
Carlos Martinez (NCTC)	M.S.	Expected Dec 2019	Pat Mazik
Eric Margenau	Ph. D.	Expected May 2020	Petra Wood
Gretchen Nareff	Ph. D.	Expected Dec 2018	Petra Wood
Lincoln Oliver	M.S.	Expected Dec 2020	Petra Wood/Chris Lituma
Nathaniel Owens	M.S.	Expected May 2019	Stuart Welsh
Lenza Paul	M.S.	Expected May 2019	Donald Brown
Brian Rolek	Ph. D.	Expected Dec 2018	Petra Wood/Cyndy Loftin
Dustin Smith	Ph.D.	Expected Dec 2018	Stuart Welsh
Kristin Stockton	M.S.	Expected May 2020	Pat Mazik
Thomas Waldrop (NCTC)	M.S.	Expected Dec 2018	Pat Mazik
Emily Underwood	M.S.	Expected Dec 2018	Pat Mazik
Heather Walsh	Ph. D.	Expected Dec 2018	Pat Mazik
Kelsey Young	M.S.	Completed May 2018	Pat Mazik

UNIT STAFF COURSES TAUGHT

Patricia M. Mazik, Adjunct Professor of Fisheries
Fish Physiology Fall 2017 3 credits

Stuart A. Welsh, Adjunct Professor of Fisheries
Advanced Ichthyology Fall 2017 3 credits

Petra B. Wood, Adjunct Professor of Wildlife
Wildlife and Fisheries Graduate Seminar Spring 2018 1 credit 20 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

AN EXAMINATION OF CYANOTOXINS IN THE UPPER AND MIDDLE POTOMAC RIVER DRAINAGE, USA

Student Investigator: Ryan Braham, M. S.

Principal Investigators: Dr. Patricia Mazik

Collaborators: Dr. Vicki Blazer - USGS Leetown Science Center, Jim Hedrick - WVDNR

Years Ongoing: 2013 - 2018

Degree Program: PhD

Completed: May 2018

Funding Source: West Virginia Division of Natural Resources

Justification:

The Potomac River is a large tributary of the Chesapeake Bay. Large fish die-offs were reported in the Potomac watershed (specifically the South Branch of the Potomac) in 2002 and 2005, as well as smaller episodic events in recent years which have no clear correlation to any one pathogen or contaminant. Although a variety of pathogens have been identified (such as trematodes, myxozoans, *Aeromonas hydrophila*, *A. salmonicida*, *Flavobacterium columnare*, and largemouth bass virus) in recent die-offs of smallmouth bass *Micropterus dolomieu*, no clear cause has been derived. Additionally, reproductive endpoints were reported in smallmouth bass and likely associated with instream estrogenic compounds. There has been increasing support for estrogenic compounds associated with agricultural runoff as one of the causes. However, there are little data on the contribution from primary producers such as cyanobacteria. Harmful algal blooms are of increasing concern to human health worldwide and their effects on the aquatic community within the South Branch Potomac watershed is largely unknown. To address knowledge gaps associated with fish health in the South Branch Potomac watershed, an assessment of the temporal and spatial distribution of algal toxins and environmental estrogens was required.

Objectives:

The primary objective of this study is to take an investigative approach to quantifying the extent of cyanotoxins, as well as their possible effects on the aquatic community in the upper and middle Potomac River drainage. The specific research objectives are to:

1. Quantify cyanotoxins (specifically microcystins) in the pelagic and periphyton community at selected sites in West Virginia, Maryland, and Virginia. Investigate spatial and temporal distribution of microcystin toxins present in the water column, as well as the potential toxin available in both the water column and the periphyton communities. Quantify the total microcystins present in wild smallmouth bass (*Micropterus dolomieu*) and golden redbreast suckers (*Moxostoma erythrurum*) at selected sites in West Virginia,

Maryland. Investigate the potential spatial, temporal, and species differential presence of microcystin toxins within liver tissue.

2. Quantify transcriptional changes to hepatocytes challenged with MC-LR *in vivo*. Examine change in transcriptional abundance in smallmouth bass leukocytes resulting from co-exposure to microcystin-LR toxin and peptidoglycan.
3. Quantify total estrogenicity in the pelagic and periphyton community at selected sites in West Virginia, Maryland, and Virginia. Investigate spatial and temporal distribution of total estrogenicity present in the water column, as well as the estrogenicity available in both the water column and the periphyton communities. Investigate the possible correlation among total estrogenicity and microcystin toxins in the water column.
4. Assess the potential toxicity of water and periphyton extracts on the yeast strain *Saccharomyces cerevisiae* BLYR by quantifying cell viability. This analysis is done both on field samples and laboratory controls.

Results:

Objective 1:

Microcystin (MC) toxins are exclusively produced by cyanobacteria. Historically, blooms of pelagic-oriented cyanobacteria have been documented in the Potomac River watershed. We investigated the occurrence of MCs in the upper and middle Potomac River watershed. We sampled both the filtered and whole (unfiltered) water fractions to determine the relative contribution of pelagic-oriented MC-producing taxa; as well as the periphyton to determine the contribution from benthic-oriented MC-producing taxa. Samples were collected at sites throughout Maryland, Virginia, and West Virginia monthly (or bi-monthly) for 28 months. We compared these MC concentrations to both in-stream physical metrics, as well as nutrient parameters. We also sampled smallmouth bass (*Micropterus dolomieu*) and golden redhorse sucker (*Moxostoma erythrurum*) at 19 time points to quantify the accumulation of MCs in hepatic tissue. All MC data were quantified using an enzyme-linked immunosorbent assay (ELISA). We observed relatively low concentrations of MCs in water, periphyton, and livers at all of the sites sampled as compared to acutely toxic concentrations reported in literature and the World Health Organization guideline for microcystin-LR in drinking water (1.0 µg/L). We observed seasonal variability of MCs in the water column that positively correlated to stream temperature. We observed seasonal variability of MCs in hepatic tissue similar to those observed in the water column. Overall, although the concentrations of MCs are low, further investigation is warranted into the specific taxa present in each fraction, as well as overall biomass may yield a fuller picture to this initial research.

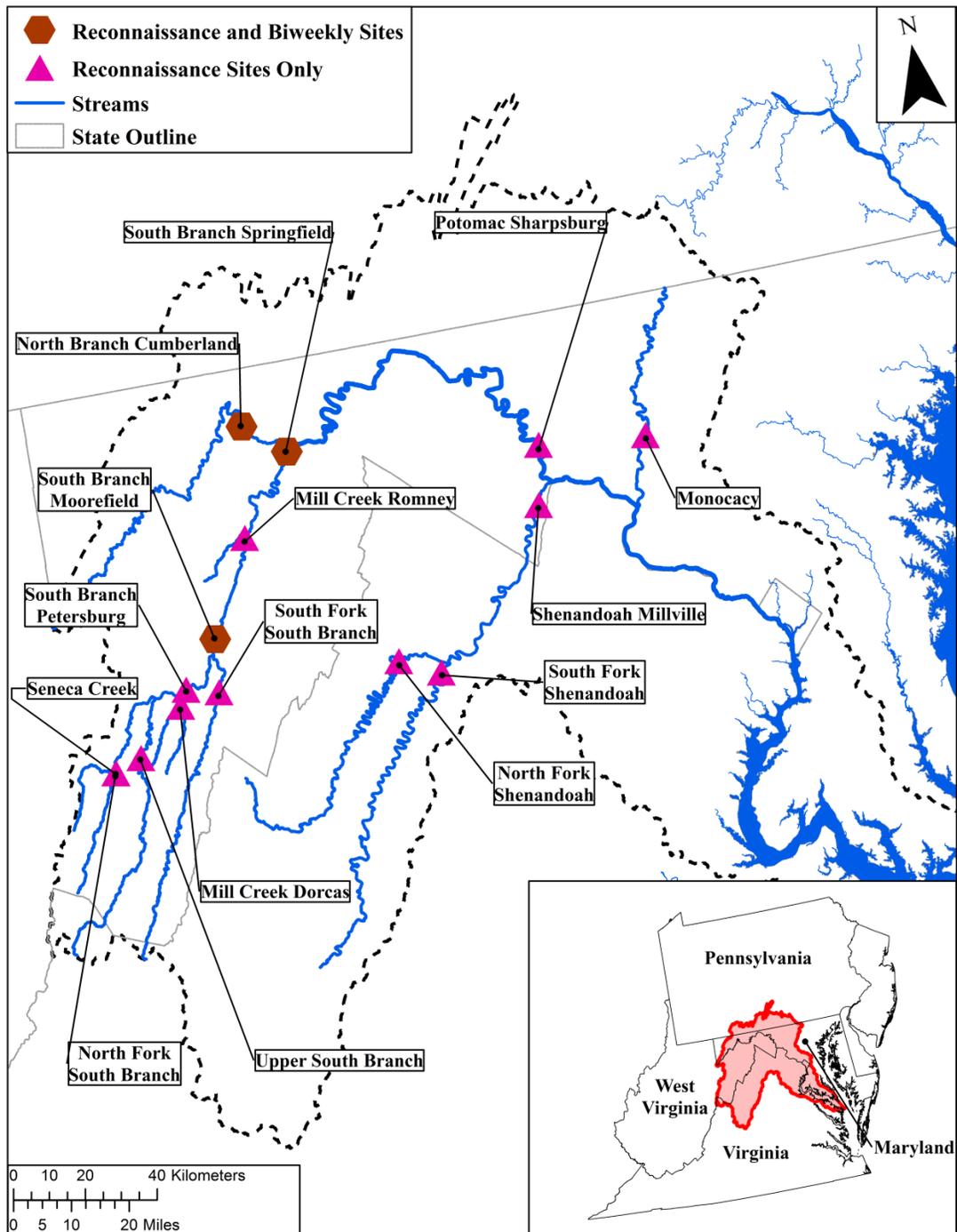


Figure 1. The location of all 15 sites included in this study. All sites were located in the states of West Virginia, Maryland, and Virginia within the Potomac River watershed.

Table 1. Summary of total microcystins/nodularins in each sample type grouped by season and year. Sample size is presented as *n* and standard error is presented as SE.

Site Name	Season and Year	<i>n</i>	Filtered Water		Unfiltered Water		Periphyton	
			Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/g)	SE
Monocacy River near Mouth near Frederick, MD	2013 Summer	2	5.39	2.26	16.66	0.29	0.05	0.02
	2013 Autumn	2	6.19	1.19	10.76	5.34	1.56	1.49
	2014 Winter	3	4.47	0.86	5.32	1.06	2.70	1.40
	2014 Spring	3	3.68	0.94	3.48	0.18	2.63	1.21
	2014 Summer	1	2.90	NA	4.72	NA	2.54	NA
Mainstem of the North Branch at Cumberland, MD	2013 Summer	3	9.18	2.86	27.33	16.83	1.60	0.61
	2013 Autumn	2	8.97	1.75	11.03	0.13	1.40	0.35
	2014 Winter	3	5.04	1.17	3.33	0.33	2.01	0.84
	2014 Spring	3	3.72	0.92	3.34	0.06	4.18	2.77
	2014 Summer	7	6.43	1.95	7.70	1.48	1.06	0.18
	2014 Autumn	6	6.25	1.59	7.91	3.30	10.37	4.06
	2015 Winter	7	3.76	0.25	3.41	0.53	2.78	0.89
	2015 Spring	6	2.93	0.47	3.10	0.90	1.63	0.78
	2015 Summer	7	6.22	1.36	7.47	1.92	1.81	0.80
	2015 Autumn	7	3.15	1.33	8.45	1.86	5.46	1.50
2016 Winter	1	2.05	NA	6.47	NA	2.38	NA	
Mainstem of the Potomac River near Sharpsburg, MD	2013 Summer	2	3.15	0.02	4.52	2.09	0.56	0.52
	2013 Autumn	2	7.89	2.89	4.86	1.54	1.26	0.96
	2014 Winter	3	5.09	0.24	3.29	0.74	1.99	1.31
	2014 Spring	3	4.32	0.85	6.00	1.75	3.50	1.22
	2014 Summer	1	4.73	NA	4.62	NA	0.07	NA

Site Name	Season and Year	n	Filtered Water		Unfiltered Water		Periphyton	
			Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/g)	SE
North Fork of the South Branch at Seneca Rocks, WV	2013 Summer	2	18.60	2.09	25.75	18.89	5.52	5.33
	2013 Autumn	2	9.17	3.53	9.88	4.99	0.46	0.41
	2014 Winter	3	5.41	0.68	4.35	0.27	2.43	1.93
	2014 Spring	3	4.47	0.71	4.30	0.53	5.68	2.64
	2014 Summer	1	11.21	NA	7.51	NA	3.76	NA
South Fork of the South Branch near Moorefield, WV	2013 Summer	2	7.15	4.02	22.85	12.44	4.23	4.18
	2013 Autumn	2	7.17	2.17	4.48	0.57	0.47	0.07
	2014 Winter	3	4.96	1.25	4.78	1.33	12.01	4.51
	2014 Spring	3	2.53	0.22	3.30	0.87	1.09	0.45
	2014 Summer	1	5.69	NA	9.12	NA	5.18	NA
Upper Mainstem of the South Branch	2013 Summer	2	8.74	2.60	15.26	5.83	0.61	0.55
	2013 Autumn	2	4.70	0.31	15.97	5.43	1.93	0.31
	2014 Winter	3	5.68	0.59	5.33	2.02	5.48	2.53
	2014 Spring	3	4.28	0.82	4.33	1.65	2.10	1.31
	2014 Summer	1	3.93	NA	11.61	NA	1.78	NA
Mainstem of the South Branch at Petersburg, WV	2013 Summer	2	8.42	5.38	29.32	3.72	5.97	1.84
	2013 Autumn	2	4.87	0.14	6.50	3.18	6.72	5.10
	2014 Winter	3	5.68	0.59	3.99	0.63	2.51	0.95
	2014 Spring	3	4.98	1.12	3.75	0.44	9.19	0.81
	2014 Summer	2	16.28	3.27	24.98	9.68	3.71	1.59

Site Name	Season and Year	n	Filtered Water		Unfiltered Water		Periphyton	
			Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/g)	SE
Mainstem of the South Branch near Moorefield, WV	2013 Summer	2	9.24	6.20	30.17	1.84	5.17	2.92
	2013 Autumn	2	11.45	4.69	11.10	6.98	111.62	111.00
	2014 Winter	3	5.21	1.01	3.65	0.22	2.98	1.30
	2014 Spring	4	3.94	0.48	4.81	0.19	0.97	0.79
	2014 Summer	8	23.15	5.05	39.35	11.09	2.25	0.73
	2014 Autumn	6	9.86	3.66	11.75	3.44	23.49	20.37
	2015 Winter	7	4.56	0.56	5.67	1.33	2.81	1.01
	2015 Spring	6	2.66	0.32	4.32	1.32	1.25	0.91
	2015 Summer	7	9.67	2.11	13.93	2.37	3.11	1.15
Mainstem of the South Branch near Moorefield, WV	2015 Autumn	7	4.05	1.28	9.32	2.74	3.61	1.23
	2016 Winter	1	8.06	NA	23.24	NA	1.64	NA
Mainstem of the South Branch near Mouth near Springfield, WV	2013 Summer	2	12.34	7.92	26.92	0.24	0.04	0.00
	2013 Autumn	2	10.45	5.27	19.80	4.12	1.29	0.69
	2014 Winter	3	5.97	0.63	4.30	1.63	6.56	2.61
	2014 Spring	3	4.65	1.32	6.42	3.20	4.42	2.18
	2014 Summer	6	28.04	5.52	39.70	7.38	2.63	1.43
	2014 Autumn	6	11.43	4.06	16.45	5.22	2.25	0.81
	2015 Winter	7	3.61	0.17	6.16	2.48	3.99	2.30
	2015 Spring	6	6.60	2.91	7.57	2.37	0.97	0.39
	2015 Summer	7	14.65	3.31	22.37	2.51	16.68	9.92
	2015 Autumn	7	4.30	0.70	8.00	1.57	5.48	3.43
	2016 Winter	1	12.19	NA	24.01	NA	1.97	NA

Site Name	Season and Year	n	Filtered Water		Unfiltered Water		Periphyton	
			Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/g)	SE
Mill Creek near Dorcas, WV	2013 Summer	2	5.62	2.49	10.95	8.52	0.34	0.27
	2013 Autumn	2	5.67	2.87	8.44	5.04	3.97	3.09
	2014 Winter	3	5.77	0.52	5.46	1.93	2.84	2.56
	2014 Spring	3	4.67	0.70	5.36	2.06	7.54	3.51
	2014 Summer	1	4.16	NA	4.48	NA	0.97	NA
Mill Creek near Romney, WV	2013 Summer	2	12.21	5.52	30.27	13.24	0.44	0.29
	2013 Autumn	2	3.62	0.58	8.89	3.84	1.78	1.13
	2014 Winter	3	5.77	0.52	5.87	2.34	2.89	1.03
	2014 Spring	3	3.72	0.92	3.34	0.06	1.03	0.48
	2014 Summer	1	39.46	NA	2.59	NA	6.51	NA
Seneca Creek near Seneca Rocks, WV	2013 Summer	2	10.77	1.30	26.52	0.19	0.57	0.49
	2013 Autumn	2	4.53	1.49	3.31	0.47	0.91	0.25
	2014 Winter	3	5.57	1.52	5.28	0.72	6.38	2.78
	2014 Spring	3	2.69	0.10	3.76	1.23	11.38	6.29
	2014 Summer	1	5.26	NA	9.88	NA	2.36	NA
North Fork of the Shenandoah River at Strasburg, VA	2013 Summer	2	6.06	2.93	3.96	1.53	0.17	0.11
	2013 Autumn	2	8.14	3.14	16.65	0.32	0.87	0.84
	2014 Winter	3	6.36	0.40	8.97	5.10	3.16	1.84
	2014 Spring	3	3.11	0.32	4.29	0.24	2.02	1.42
	2014 Summer	1	3.90	NA	4.39	NA	1.08	NA
South Fork of the Shenandoah River at Front Royal, VA	2013 Summer	2	3.28	1.75	7.32	1.98	0.03	0.00
	2013 Autumn	2	5.28	0.76	9.47	2.97	2.88	0.57
	2014 Winter	3	5.36	1.40	3.51	0.36	10.72	6.71
	2014 Spring	3	2.72	0.10	3.80	0.56	3.61	3.35
	2014 Summer	1	2.56	NA	2.37	NA	0.02	NA

Site Name	Season and Year	n	Filtered Water		Unfiltered Water		Periphyton	
			Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/L)	SE	Total MC/NOD (ng/g)	SE
Shenandoah River at Millville, WV	2013 Summer	2	7.74	0.94	13.10	3.72	0.04	0.02
	2013 Autumn	2	4.79	0.21	5.88	3.08	53.79	52.30
	2014 Winter	3	5.95	0.21	4.97	0.56	3.86	0.71
	2014 Spring	3	2.55	0.08	3.00	0.20	8.45	3.73
	2014 Summer	1	6.76	NA	2.81	NA	1.52	NA

Objective 2:

Cyanobacterial blooms are of increasing concern worldwide as both a nuisance and due to their production of toxins in select species. The cyanobacterial hepatotoxin microcystin-LR (MCLR) has been shown to disrupt a variety of genes in the liver and anterior kidneys of fishes. These toxins have been shown to occur within the Potomac River watershed in which the smallmouth bass (SMB; *Micropterus dolomieu*) is a popular sport fish. The temporal transcriptional response of SMB hepatocytes to environmentally relevant concentrations of MCLR (0.05, 0.5, and 5.0 ng/mL), as well as the effect of these concentrations of MCLR on peptidoglycan (PGN) stimulated SMB leukocytes was assessed. A reference leukocyte transcriptome was first developed using next-generation sequencing. Independent libraries of leukocyte and hepatic genes-of-interest were subsequently generated. Hepatic transcripts included those which are shown to be affected by MCLR, as well as those indicative of endocrine disruption. Leukocyte transcripts focused on the toll-like receptor 1/2 pathway (which is activated by PGN); as well as transcripts shown to be affected by MCLR. These were included in a customized CodeSet and analyzed using the NanoString® nCounter technologies. No significant differences among hepatocyte treatments or leukocyte treatments treated only with MCLR were reported. However differences in PGN-stimulated and PGN-MCLR coexposed leukocytes were observed when compared to unexposed. Although these data do not show statistically or biologically relevant transcriptional disruption by MCLR, it does show that MCLR acts concomitantly with peptidoglycan to up- or down-regulate select leukocyte transcripts.

Objective 3:

A suite of compounds identified to interfere with hormone pathways are termed endocrine disrupting compounds (EDCs). Considerable attention has been directed towards estrogenic chemicals in the environment within the Chesapeake Bay watershed. We quantified the net cumulative effects of these compounds in mixtures using a bioluminescent yeast estrogenicity screen (BLYES). Samples were collected at 15 sites through the middle and upper Potomac River basin and quantified the estrogenic equivalency potential in the water column. Data are presented as the estrogenic equivalency of strain *Saccharomyces cerevisiae* BLYES as compared to a 17 β -estradiol standard curve (EEQ_{BLYES}) and ranged from BD – 5.75 ng/L. We observed

considerable seasonal variability reporting data higher in the summer months. Estrogenicity was positively correlated with temperature; however, there were no significant correlations among discharge, pH, conductivity, total nitrogen or total phosphorous in the water column or benthos. The EEQ_{BLYES} was positively correlated with equol. Additionally, EEQ_{BLYES} was positively correlated with total microcystins/nodularins and we verified that purified microcystin variant – LR was not estrogenic by this assay. We assessed EEQ_{BLYES} in the context of land cover/land use and did not observe any significant correlations in any of our sampling watersheds. These data highlight temporal changes in estrogenicity at these sites. They also show a contribution from phytoestrogens to the instream estrogenicity. Both of these should be taken into account when quantifying environmental estrogenicity.

Table 2. Summary of EEQ_{BLYES} and total microcystins/nodularins in filtered water samples during the reconnaissance and biweekly sampling sites. r^2 is only represented in sites with significantly correlated association between MC/NOD and EEQ_{BLYES}. SE represents standard error.

Stream Name	Sampling Season/Year	Filtered EEQ _{BLYES} (ng/L)	Filtered EEQ _{BLYES} SE	Filtered Total Microcystins (ng/mL)	Filtered Total Microcystins SE	Association among Total Filtered MC/NOD and EEQ _{BLYES} (p-value, r^2)
Monocacy River near Mouth near Frederick, Maryland	2013 Summer	0.174	0.124	5.39	2.26	0.904
	2013 Autumn	0.359	0.024	6.19	1.19	
	2014 Winter	0.420	0.187	4.47	0.86	
	2014 Spring	0.479	0.080	3.68	0.94	
	2014 Summer	0.436	NA	2.90	NA	
Mainstem of the North Branch at Cumberland, Maryland	2013 Summer	0.352	0.054	9.18	2.86	>0.001*, 0.232
	2013 Autumn	0.309	0.259	8.97	1.75	
	2014 Winter	0.296	0.133	5.04	1.17	
	2014 Spring	0.318	0.180	3.72	0.92	
	2014 Summer	0.518	0.069	6.43	1.95	
	2014 Autumn	0.224	0.110	6.25	1.59	
	2015 Winter	0.218	0.090	3.76	0.25	
	2015 Spring	0.162	0.071	2.93	0.47	
	2015 Summer	0.501	0.147	6.22	1.36	
	2015 Autumn	0.382	0.072	3.15	1.33	
2016 Winter	0.274	NA	2.05	NA		
Mainstem of the Potomac River near Sharpsburg, Maryland	2013 Summer	0.289	0.239	3.15	0.02	0.075
	2013 Autumn	0.224	0.174	7.89	2.89	
	2014 Winter	0.440	0.040	5.09	0.24	
	2014 Spring	0.577	0.063	4.32	0.85	
	2014 Summer	0.050	NA	4.73	NA	
North Fork of the South Branch at Seneca Rocks, West Virginia	2013 Summer	0.296	0.246	18.60	2.09	0.620
	2013 Autumn	0.437	0.128	9.17	3.53	
	2014 Winter	0.397	0.209	5.41	0.68	
	2014 Spring	0.417	0.041	4.47	0.71	
	2014 Summer	0.448	NA	11.21	NA	

Stream Name	Sampling Season/Year	Filtered EEQ _{BLYES} (ng/L)	Filtered EEQ _{BLYES} SE	Filtered Total Microcystins (ng/mL)	Filtered Total Microcystins SE	Association among Total Filtered MC/NOD and EEQ _{BLYES} (p-value, r ²)
South Fork of the South Branch near Moorefield, West Virginia	2013 Summer	0.331	0.059	7.15	4.02	0.419
	2013 Autumn	0.651	0.601	7.17	2.17	
	2014 Winter	0.312	0.146	4.96	1.25	
	2014 Spring	0.251	0.108	2.53	0.22	
	2014 Summer	0.454	NA	5.69	NA	
Upper Mainstem of the South Branch	2013 Summer	1.003	0.953	8.74	2.60	0.647
	2013 Autumn	0.180	0.130	4.70	0.31	
	2014 Winter	0.163	0.113	5.68	0.59	
	2014 Spring	0.288	0.146	4.28	0.82	
	2014 Summer	0.399	NA	3.93	NA	
Mainstem of the South Branch at Petersburg, West Virginia	2013 Summer	0.474	0.096	8.42	5.38	0.074
	2013 Autumn	0.170	0.120	4.87	0.14	
	2014 Winter	0.342	0.052	5.68	0.59	
	2014 Spring	0.327	0.034	4.98	1.12	
	2014 Summer	0.475	0.037	16.28	3.27	
Mainstem of the South Branch near Moorefield, West Virginia	2013 Summer	0.627	0.063	9.24	6.20	0.161
	2013 Autumn	3.099	2.650	11.45	4.69	
	2014 Winter	0.353	0.077	5.21	1.01	
	2014 Spring	0.155	0.061	3.94	0.48	
	2014 Summer	0.417	0.057	23.15	5.05	
	2014 Autumn	0.319	0.023	9.86	3.66	
	2015 Winter	0.229	0.075	4.56	0.56	
	2015 Spring	0.381	0.331	2.66	0.32	
	2015 Summer	0.330	0.048	9.67	2.11	
	2015 Autumn	0.177	0.047	4.05	1.28	
	2016 Winter	0.050	NA	8.06	NA	
Mainstem of the South Branch near Mouth near Springfield, West Virginia	2013 Summer	0.505	0.082	12.34	7.92	0.035*, 0.070
	2013 Autumn	0.316	0.035	10.45	5.27	
	2014 Winter	0.453	0.046	5.97	0.63	
	2014 Spring	0.549	0.089	4.65	1.32	
	2014 Summer	0.382	0.071	28.04	5.52	
	2014 Autumn	0.199	0.067	11.43	4.06	
	2015 Winter	0.152	0.049	3.61	0.17	
	2015 Spring	0.157	0.068	6.60	2.91	
	2015 Summer	0.274	0.050	14.65	3.31	
	2015 Autumn	0.266	0.102	4.30	0.70	
	2016 Winter	0.050	NA	12.19	NA	
Mill Creek near Dorcas, West Virginia	2013 Summer	0.050	0.000	5.62	2.49	0.440
	2013 Autumn	0.317	0.031	5.67	2.87	
	2014 Winter	0.840	0.610	5.77	0.52	
	2014 Spring	0.532	0.057	4.67	0.70	
	2014 Summer	0.050	NA	4.16	NA	

Stream Name	Sampling Season/Year	Filtered EEQ _{BLYES} (ng/L)	Filtered EEQ _{BLYES} SE	Filtered Total Microcystins (ng/mL)	Filtered Total Microcystins SE	Association among Total Filtered MC/NOD and EEQ _{BLYES} (p-value, r ²)
Mill Creek near Romney, West Virginia	2013 Summer	3.088	2.476	12.21	5.52	0.312
	2013 Autumn	1.058	0.752	3.62	0.58	
	2014 Winter	0.359	0.155	5.77	0.52	
	2014 Spring	0.354	0.036	3.72	0.92	
	2014 Summer	0.696	NA	39.46	NA	
Seneca Creek near Seneca Rocks, West Virginia	2013 Summer	0.490	0.036	10.77	1.30	0.770
	2013 Autumn	0.227	0.177	4.53	1.49	
	2014 Winter	0.279	0.121	5.57	1.52	
	2014 Spring	0.423	0.038	2.69	0.10	
	2014 Summer	0.525	NA	5.26	NA	
North Fork of the Shenandoah River at Strasburg, Virginia	2013 Summer	0.398	0.080	6.06	2.93	0.907
	2013 Autumn	0.655	0.176	8.14	3.14	
	2014 Winter	0.265	0.131	6.36	0.40	
	2014 Spring	0.246	0.196	3.11	0.32	
	2014 Summer	0.438	NA	3.90	NA	
South Fork of the Shenandoah River at Front Royal, Virginia	2013 Summer	0.606	0.046	3.28	1.75	0.055
	2013 Autumn	0.736	0.032	5.28	0.76	
	2014 Winter	0.307	0.142	5.36	1.40	
	2014 Spring	0.452	0.093	2.72	0.10	
	2014 Summer	0.551	NA	2.56	NA	
Shenandoah River at Millville, West Virginia	2013 Summer	0.478	0.003	7.74	0.94	0.205
	2013 Autumn	0.696	0.133	4.79	0.21	
	2014 Winter	0.239	0.111	5.95	0.21	
	2014 Spring	0.519	0.038	2.55	0.08	
	2014 Summer	0.522	NA	6.76	NA	

Objective 4:

Estrogenic endocrine disrupting compounds (EEDCs) are a class of chemicals which have been shown to dysregulate reproductive pathways. The identification of these compounds is critical for understanding the biological effects potentially caused by EEDCs. The yeast strain *Saccharomyces cerevisiae* BLYR incorporates a constitutively expressed reporter and is designed to be a measure of toxicity; however, we reference output as interference. BLYR output was quantified among various concentrations of two solvents, methanol (MeOH) and dimethyl sulfoxide (DMSO), as well as 17 β -estradiol and extracts from both environmental water and periphyton samples. Challenged *S. cerevisiae* cells were also evaluated using flow cytometry to determine whether a decrease in BLYR output was a result of cell mortality. Temporal and spatial variability in BLYR output was assessed at select sites in the Potomac River watershed. Strain BLYR was run in conjunction with *S. cerevisiae* strain BLYES (bioluminescent yeast estrogen screen) to determine whether interference could be diluted out without a subsequent loss in the estrogenic signal. Low BLYR output was reported only at high concentrations of

MeOH (>10%), DMSO (>2.5%) and 17 β -estradiol (>0.25 ng/mL). There was only significant cell mortality at the highest concentrations of MeOH (50%) and DMSO (5%). Despite evaluating a range of BLYR output (3.21 – 98.61%) in environmental and 17 β -estradiol samples, we only observed \pm 5% mortality as compared to the control. Periphyton samples reported significantly higher interference as compared to water samples. There were minimal but site specific interference among water samples. Seasonally, summer and fall water samples have higher interference as compared to winter and spring. The dilution of individual samples resulted in a reduction of interference; however there was not a linear response in BLYES results suggesting diluting samples may be a viable option on a case-by-case basis. The comparison of strains BLYR and BLYES in diluted samples yielded variable results. Despite the minimal interference in water samples, it is recommended to report the BLYR interference along with estrogenicity values. This would aid in evaluating spatial and temporal trends; specifically outlier analysis. Due to the high reporter interference, it is recommended to only qualitatively assess estrogenicity in periphyton samples.

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

Student Investigator: Nathaniel Owens

Principal Investigator: Stuart Welsh

Years Ongoing: 2017

Degree Program: MS

Expected completion date: May 2019

Funding Sources: WVDNR

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

Progress:

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. The distribution of benthic fishes within the Kanawha River, particularly in relation to island habitat, is not well understood. This project will use a benthic trawl to sample for fishes, primarily in association with island habitats, but also in main channel and tributary mouth habitats.

During July of 2017, a pilot study was conducted to determine the effectiveness of the benthic trawl gear, as well as the potential for the gear to impact mussel populations. During the pilot study, 12 trawl hauls were conducted in an area of the Kanawha River with documented mussel densities (Figure 1). These hauls consisted of a total linear distance of 491.8 meters and a total area of 1,180.32 sq./meters. Fishes were captured in 11 of 12 hauls, including records of four fish species of interest: Slenderhead Darter (*Percina phoxocephala*), River Darter (*Percina shumardi*), Eastern Sand Darter (*Ammocrypta pellucida*), and Silver Chub (*Macrhybopsis*

storeriana). Mussel bycatch consisted of 3 dead mussel shells. The pilot study supports the method as effective at capturing benthic fishes, with little to no impact on mussel populations.

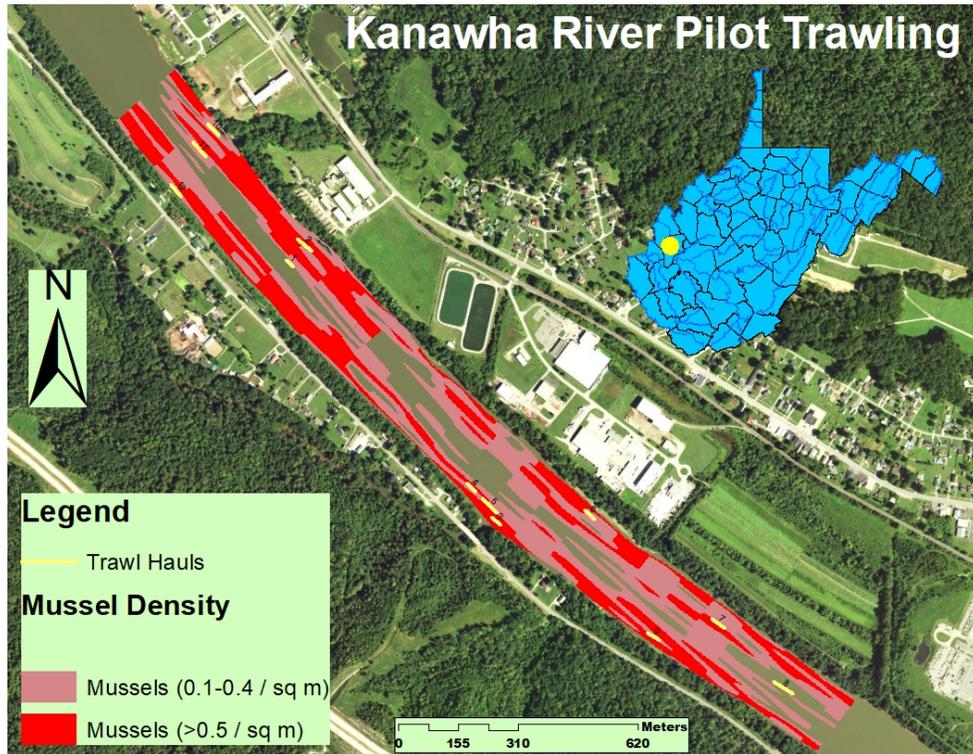


Figure 1. Transects of benthic trawl hauls in areas of relatively high mussel densities in the Kanawha River, WV.

Benthic trawling was conducted during September and October of 2017 and May to August in 2018. These efforts resulted in 270 total hauls to date, spanning 9 study sites, throughout all four pools in the Kanawha River (i.e., Robert C. Byrd, Winfield, Marmet, and London pools). Habitats that were sampled during these events consisted of main channel, main channel border, side channel, island head (upstream tip), island border main channel, island border side channel, island toe (downstream tip), and tributary mouths. Thus far 48 species in total have been captured via this technique several of which, designated as species of concern by the WVDNR, such as Shoal Chub (*Macrhybopsis hyostoma*), Silver Chub (*Macrhybopsis storeriana*; Figure 2), Popeye Shiner (*Notropis ariommus*), Bullhead Minnow (*Pimephales vigilax*), Northern Madtom (*Noturus stigmosus*), Eastern Sand Darter (*Ammocrypta pellucida*), Bluebreast Darter (*Etheostoma camurum*), Spotted Darter (*Etheostoma maculatum*), Tippecanoe Darter (*Etheostoma tippecanoe*), Channel Darter (*Etheostoma copelandi*), Gilt Darter (*Percina evides*), Slenderhead Darter (*Percina phoxocephala*), and River Darter (*Percina shumardi*). This updated the known distribution of some of these fishes in the Kanawha River by 10 – 30 years. In addition to these accounts, Young-of-the-year (YOY) and juvenile Blue Catfish (*Ictalurus furcatus*) were captured in considerable numbers in the Robert C. Byrd and Marmet pools suggesting that successful reproduction of this important gamefish species may be occurring within the Kanawha River proper (Figure 3).



Figure 2. A Silver Chub (Macrhybopsis storeriana) from the 2018 benthic trawl study on the Kanawha River, West Virginia.



Figure 3. Blue Catfish (Ictalurus furcatus) from the 2018 benthic trawl study on the Kanawha River, West Virginia.

ASSESSMENT OF MELANISTIC AREAS IN SMALLMOUTH BASS SKIN USING HISTOPATHOLOGY AND DIFFERENTIAL GENE EXPRESSION

Student Investigator: Kelsey Young
Principal Investigator: Dr. Patricia Mazik
Collaborators: Dr. Vicki Blazer, USGS Leetown Science Center
Years Ongoing: 2016 – 2018
Degree Program: MS
Completed: May 2018
Funding Source: U.S. Geological Survey (RWO 68)

Objectives:

The objective of this study is to determine potential causes or risk factors that may contribute to the development of melanistic areas (also known as “Blotchy bass” syndrome) in smallmouth bass (*Micropterus dolomieu*). Histopathological analysis will be used to determine morphological differences between normal skin and melanistic areas. RNASeq will also be employed for differential gene expression analysis to determine variations in genes between normal and melanistic skin. Depending on the findings, further analyses such as chemical contaminant concentrations in skin tissue or measuring various plasma hormone concentrations may be necessary.

Results:

Over 100 smallmouth bass exhibiting melanistic areas were collected by boat electroshocking at various sites in Pennsylvania, Maryland, and West Virginia from spring of 2013 to spring of 2017 (Figure 1). Tissue samples of approximately 100 smallmouth bass with melanistic spots were processed for histological analysis. Slides were stained using the H&E stain and were examined by light microscopy for morphological differences such as morphology of melanophores and epithelial cells, presence of melanophores in different layers of the integument, inflammation, and presence of certain immune cells (Figures 2 and 3). Select samples of melanistic spots were also melanin bleach stained in order to determine the morphology of epithelial cells underlying melanin in the epidermis. Higher incidences of bass with melanistic lesions were documented in the Susquehanna River compared to the Potomac River. Typical melanistic lesions consisted of rounded to spindle shaped, melanin-containing cells (or melanophores) in a thickened epidermis whereas normal skin only contains melanophores in the dermal-epidermal interface.

Total RNA was extracted from six normal skin samples and six melanistic samples. These extracted samples were sent to Penn State Genomics Core Facility, University Park, PA for 150 nt single end read sequencing on the Illumina HiSeq 2500. Once the data was retrieved, the raw reads were processed by removing adaptors, poly-A tails, and low quality reads by Trimmomatic. Trinity was used to create a *de novo* transcriptome of smallmouth bass skin. False positives from the assembly were removed using Evgene. The transcriptome was then annotated using Diamond and count tables were created by Salmon. RNA-Seq data revealed the expression of a papillomavirus helicase exclusively in melanistic areas. Transcript abundance analyses of DCT (L-dopachrome tautomerase), MC5R (melanocortin receptor 5), MITF (microphthalmia-

associated transcription factor), PMEL (melanocyte protein), TYR (tyrosinase), TYRP-1 (tyrosinase-related protein 1), Rab38 (Ras-related protein Rab-38), Akt1 (RAC-alpha serine threonine-kinase), KRT8 (keratin type II cytoskeletal 8), and MT-1 (metallothionein) showed differential expression in melanistic areas. Further research will be needed to determine the roles of a potentially novel papilloma virus and chemical contaminants in induction of melanistic areas of smallmouth bass.

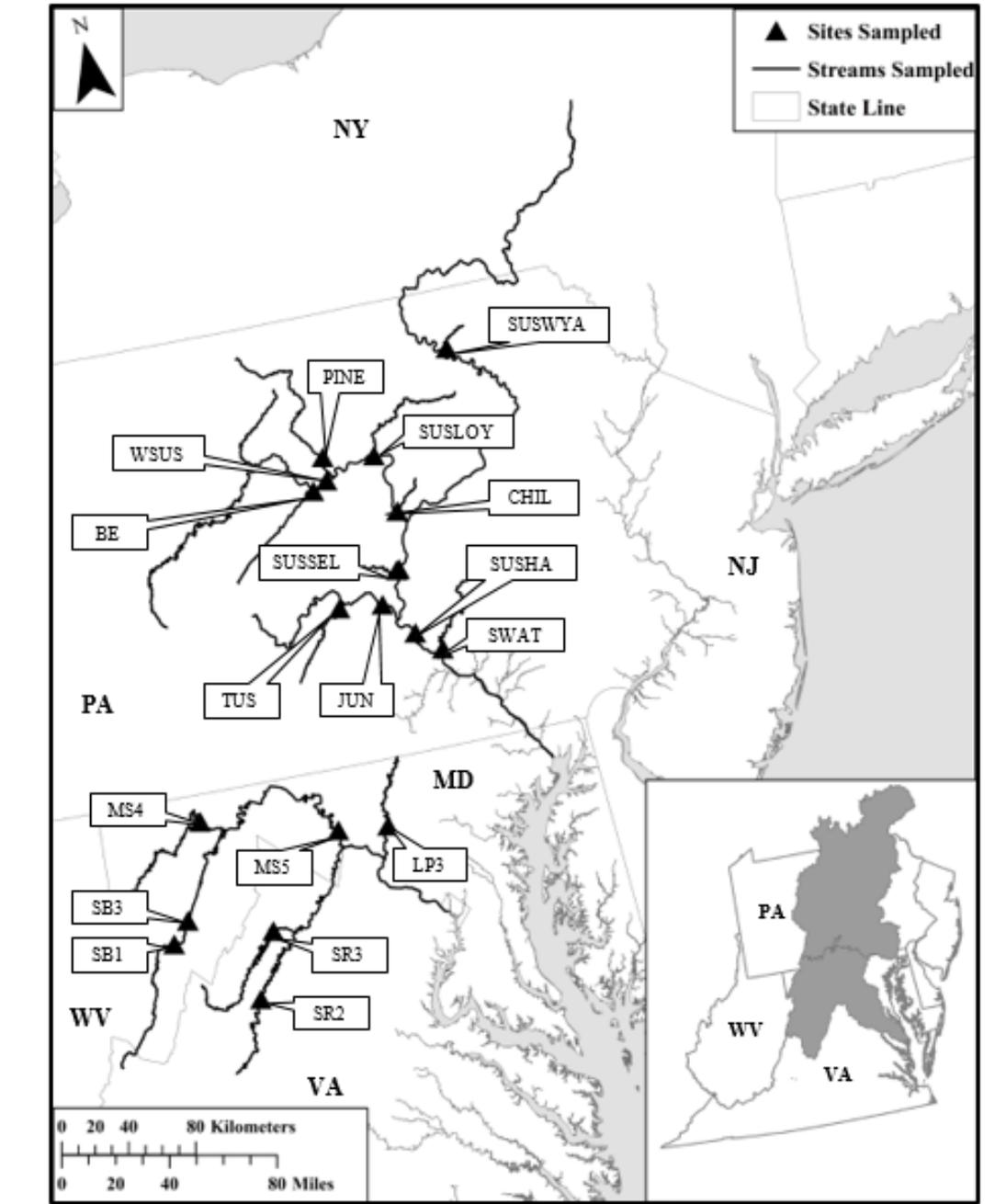


Figure 1. Map of sites sampled in Chesapeake Bay drainage in this study.

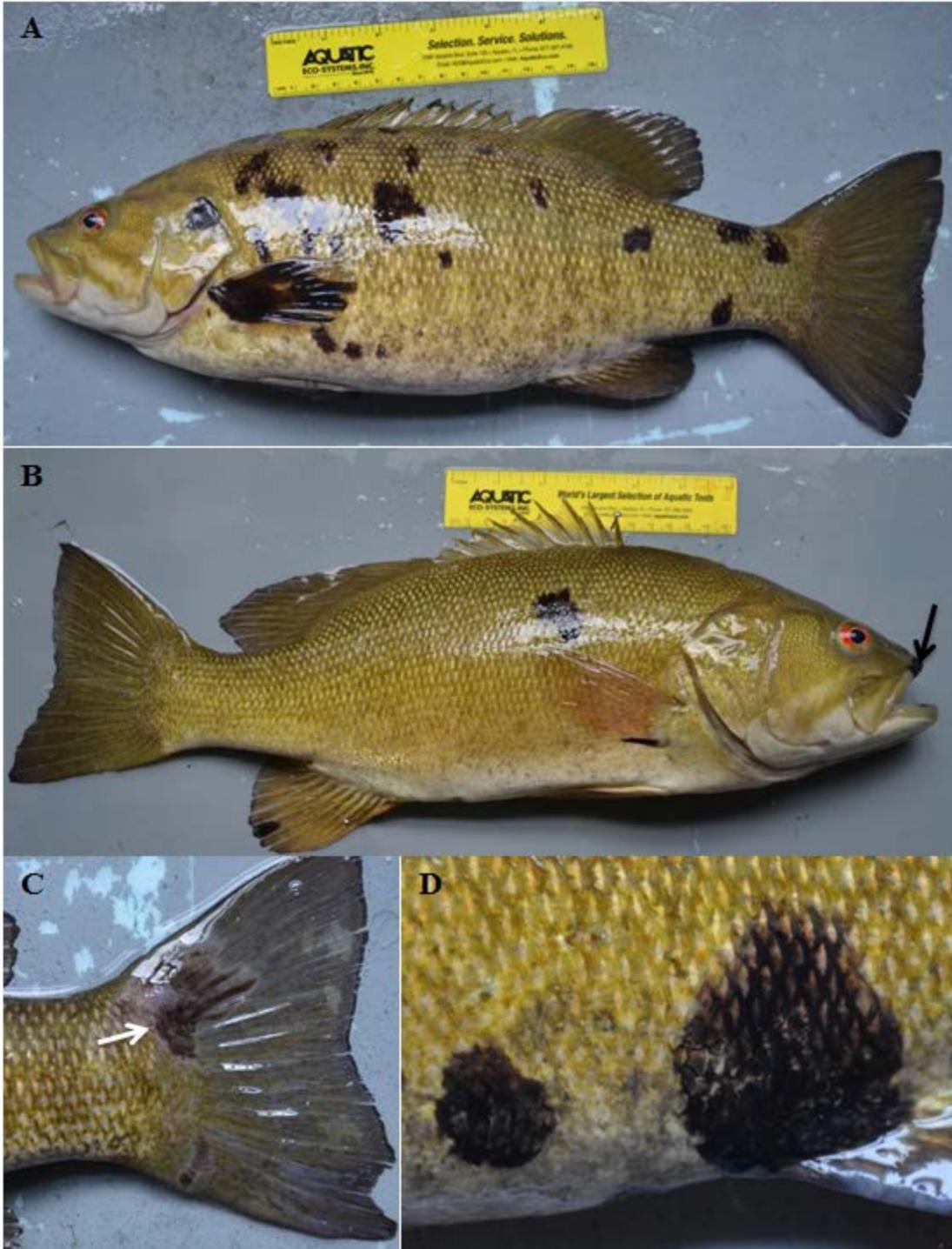


Figure 2. Grossly, melanistic areas of smallmouth bass are black to brown lesions on the body, fins, and mouth. A&B. Melanistic lesions that vary in size and number on each fish. C. Some melanistic lesions have depigmentation around the periphery. D. Melanistic lesions are unevenly pigmented.

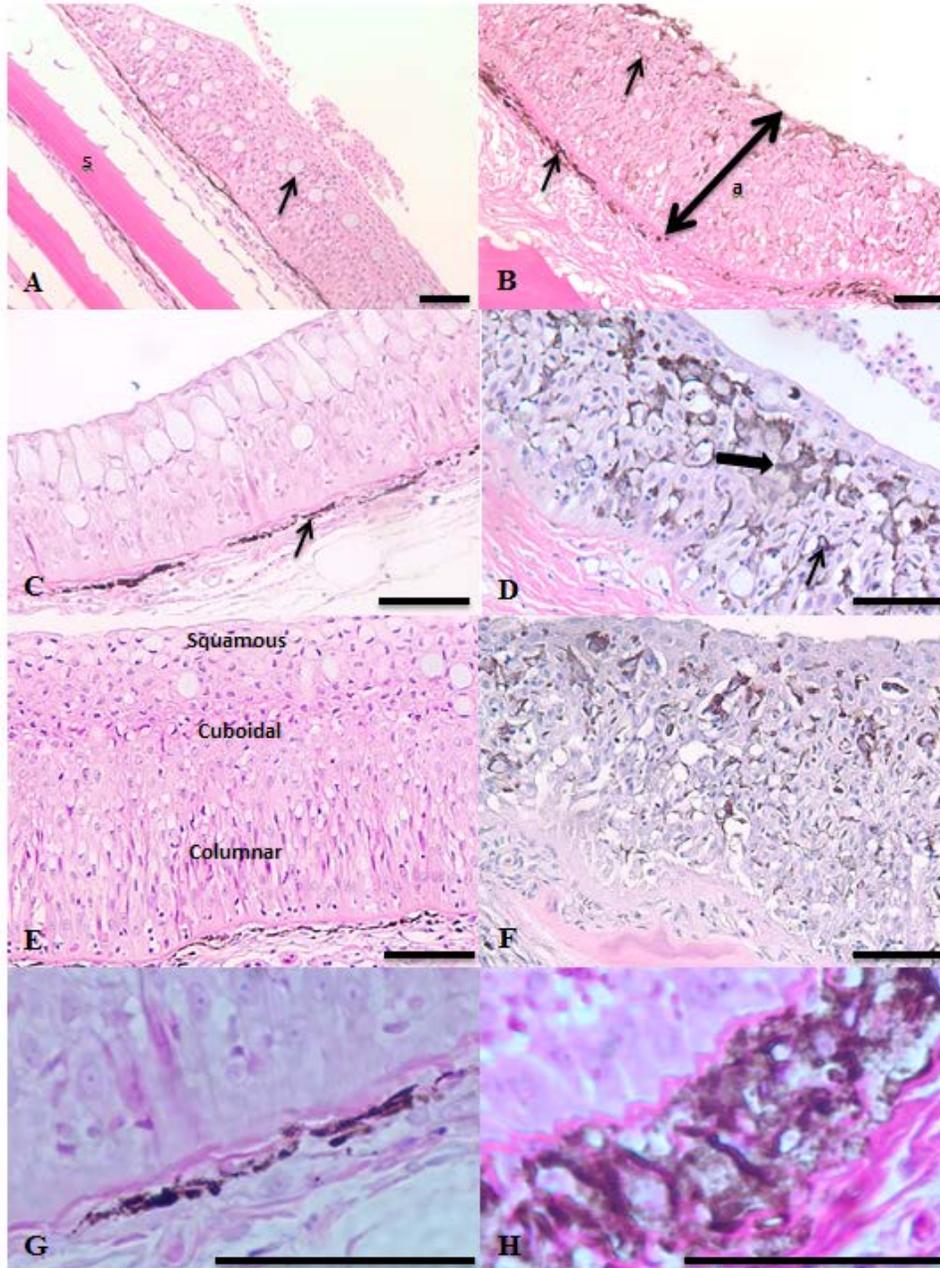


Figure 3. Comparison of normal and melanistic skin in smallmouth bass. A. Normal epidermis of smallmouth bass contains large mucous cells in the intermediate to superficial layers of the epidermis (small arrow). Melanophores are typically not present in the epidermis. B. Melanistic areas contain melanophores in the epidermis and dermal-epidermal interface (arrows) with a thickened epidermis (a). C. Normal skin contains small, somewhat elongated melanophores in the dermis (small arrow). D. Melanistic lesions often contain melanophores in the epidermis that appear less dense with melanin granules compared to melanophores found in the dermis of normal skin. Melanophores often appear rounded in the superficial layers of the epidermis (small arrow) and are spindle in shape in intermediate to basal layers of the epidermis (large arrow). E. Epithelial cell layering and structure of normal teleost integument with the apical layers containing squamous cells, cuboidal cells in the intermediate layers, and columnar cells in the basal layers. F. Loss of epithelial cell structure and layering in the intermediate layers of melanistic lesions. G. Higher magnification of normal melanophores in the dermis of normal skin. H. Higher magnification of melanophores in dermis of some melanistic areas. Melanophores are larger and pleomorphic in shape. Bar = 5 μ m. H & E stain.

WILDLIFE

ECOLOGY AND MANAGEMENT OF GOLDEN-WINGED WARBLERS AND ASSOCIATED AVIAN SPECIES IN THE ALLEGHENY MOUNTAINS OF WEST VIRGINIA

Student Investigator: Kyle Aldinger

Principal Investigator: Petra B. Wood

Cooperators: Rich Bailey, Cathy Johnson, Rob Tallman

Years Ongoing: 2008-2018

Degree Program: Ph.D.

Completed: August 2018

Funding: US Natural Resources Conservation Service, US Geological Survey, US Fish and Wildlife Service, US Forest Service, WV Division of Natural Resources, National Fish and Wildlife Foundation

Objectives:

1. Identify conditions supporting high Golden-winged Warblers (*Vermivora chrysoptera*) densities and evaluate the relationship between these conditions and overall avian community structure.
2. Quantify survival and dispersal of Golden-winged Warblers, Blue-winged Warblers (*V. cyanoptera*), and their hybrids.
3. Objectively quantify spatial configuration of shrub communities within Golden-winged Warbler territories on pastures to improve understanding of the spatially complex nature of nesting cover.
4. Evaluate annual persistence of shrubland-nesting birds on abandoned pastures.
5. Identify limiting factors for Golden-winged Warbler breeding populations.

Background:

Golden-winged Warblers (GWWA) breed in eastern North America and winter in Central and northern South America. They require dynamic forest landscapes for breeding, dominated by forest but with scattered patches of shrublands and young forests resulting from ecological and anthropogenic disturbance. In the Appalachian Mountains breeding population segment, they occur at elevations >500 m and defend breeding territories with a mix of herbaceous vegetation, shrubs, saplings, scattered canopy trees, and forest edges. GWWA fledge a single brood annually from a ground nest placed at the edge between dense shrub cover and a small herbaceous opening. Populations of GWWA have decreased for decades (West Virginia 1966–2015: -8.6% per year; range-wide 1966–2015: -2.3% per year). Forest succession, land-use change, lack of ecological and anthropogenic disturbances, patch- and landscape-level vegetation structure and composition, Brown-headed Cowbird (*Molothrus ater*) brood parasitism, topographic position, competition and hybridization with Blue-winged Warblers, and non-breeding season survival each are potential threats. No single threat or combination of these threats has definitively explained plummeting numbers of GWWA. As such, questions remain about proposed conservation strategies targeting single threats as opposed to a more comprehensive approach.

Results:

For objective 1, we evaluated variables at 4 spatial scales during 2008–2015 to identify conditions supporting high GWWA densities and associated avian species. Scales used for analyses represented annual dispersal (5-km radius), extra-territorial movement (1.5-km radius), intra-territorial movement (100-m radius), and local resource utilization (11.3-m radius). GWWA density peaked when minimum elevation was 804 m at the 1.5-km radius scale but was negatively associated with 100-m radius minimum elevation. Density was positively associated with 100-m radius shrubland cover. White-eyed Vireo (*Vireo griseus*), Blue-gray Gnatcatcher (*Poliophtila caerulea*), Gray Catbird (*Dumetella carolinensis*), Brown Thrasher (*Toxostoma rufum*), and Yellow Warbler (*Setophaga petechia*) were closely associated with these conditions. GWWA ordinated higher along a 100-m shrubland cover gradient than any other bird species, suggesting they were our most shrubland area-sensitive songbird. However, the species also requires heavily forested landscapes. Therefore, a species-specific conservation strategy that balances shrubland (patches 9–13 ha comprising 15% of the landscape) and contiguous forest area ($\geq 75\%$ of the landscape) could meet the needs of GWWA and many other avian species.

For objective 2, we used a spatial Cormack-Jolly-Seber (s-CJS) model to obtain minimally-biased estimates of annual survival and breeding and natal dispersal for GWWA, Blue-winged Warblers and hybrids during 2008–2015. Warbler annual survival did not vary by phenotype, sex, or study area, but adult annual survival (0.53, 95% CI = 0.46–0.60) was higher than juvenile annual survival (0.09, 95% CI = 0.05–0.13). Adjusting for mortality during the post-fledging period, juvenile annual survival may be about half of adult annual survival. Expected breeding dispersal (329 m, 95% CI = 316–344 m) was less than expected natal dispersal (544 m, 95% CI 500–592 m) based on our s-CJS model. We observed the longest distances for natal dispersal (mean = 1,587 m, median = 1,047 m, n = 18), intermediate distances for second year to after second year dispersal (mean = 492 m, median = 132 m, n = 46), and the shortest distances for after second year dispersal (mean = 290 m, median = 103 m, n = 103; Figure 2). Female (mean \pm SE = 716 \pm 162 m, n = 43) warblers dispersed farther than males (mean \pm SE = 404 \pm 64 m, n = 124; Figure 2). These are the first annual survival estimates that quantify and account for permanent emigration and have important implications for conservation network design.

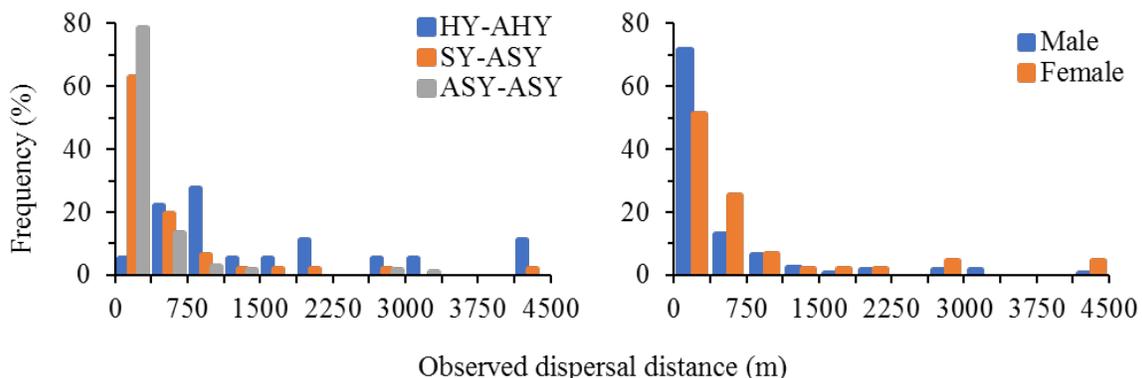


Figure 2. Observed *Vermivora* warbler dispersal distances by age (HY = hatch year, SY = second year, ASY = after second year) and sex in the Allegheny Mountains of West Virginia during 2008–2015.

For objective 3, we investigated shrub community spatial configuration within GWWA territories using field-measured and light detection and ranging (LIDAR) vegetation data during 2011–2014. GWWA selected nest sites with more shrub cover (mean \pm SE = $49.3 \pm 2.3\%$) than random locations (mean \pm SE = $42.9 \pm 2.9\%$) but did not select shrub edges or clumps. They selected territories with more pronounced edges ($\geq 60\%$ difference in shrub cover on either side of a given point) and a more clumped shrub configuration (shrub clumps 4.6–22.6 m wide) than would be expected given a random shrub configuration. Selection for pronounced edges and clumped shrub configuration at the territory scale rather than the nest scale, despite strong evidence that GWWA placed nests along edges, suggests that GWWA select territories to maximize potential nest sites for re-nesting or to reduce predation risk. GWWA nest sites had taller (mean height = 4.3 m) and more variable (mean standard deviation of height = 3.1 m) vegetation canopy height than random locations within the same territory. Across GWWA territories, 40–52% of the tallest vegetation canopy was ≤ 1 m tall consisting of grasses, forbs, blackberry, and seedlings, 29–33% of the vegetation canopy was >1 to ≤ 5 m tall consisting of shrubs and saplings, and 15–32% of the vegetation canopy was >5 to ≤ 20 m tall consisting of trees. We provide one of the first objective evaluations of the spatial configuration of GWWA nesting cover.

Objective 4 evaluated persistence of GWWA and other bird species on cattle pastures with varying amounts of time since abandonment and varying stages of vegetative succession representing a 62-year chronosequence during 2008–2014. Field Sparrow (*Spizella pusilla*) density peaked on active pastures, corresponding to the peak in herbaceous cover. GWWA density peaked 16–20 years after pasture abandonment and reached zero 33 years after abandonment, corresponding to the peak in shrubland cover. Shrubland-nesting bird species richness did not vary across the chronosequence. Forest cover increased linearly to a peak of 86% at 59 years since abandonment. Thus, abandoned pastures in our study area provided breeding habitat for a stable number but changing composition of shrubland-nesting bird species for approximately 60 years, though conservation value likely is highest 0–33 years after abandonment. The number and abandonment rate of farms in West Virginia and regionally are historically low, suggesting that managing for shrubland-nesting birds on existing or recently-abandoned pastures is important but alone may not support population persistence. Thus, increased forest management practices may be needed to supplement breeding habitat on pastures.

Finally, objective 5 focused on GWWA population trends in response to habitat management on pastures during 2008–2014. GWWA did not change nest placement behavior ($n = 109$ nests) because of mechanical vegetation management intended to maintain shrubland cover type on our pastures. Nest daily survival rate ($n = 123$ nests) was higher on pastures in the southern study area than the northern study area and was positively associated with proportion of territory-scale actively-managed shrubland cover type, shrubland patch size, and nest- and territory-scale elevation. We found that local GWWA population trends were associated with pasture-scale nest survival, with a nest daily survival rate of 0.978 presumably needed to offset other mortality and achieve a stable population in our study. A strength of these results was that we observed variation in population trends and identified potential limiting factors across a small geographic area during the breeding season, meaning birds were likely experiencing similar conditions during the non-breeding season.

Overall, our findings (Figure 2) help to justify, inform, and adapt state and regional GWWA conservation efforts during the breeding season. These findings also fill knowledge gaps and complement other novel research on GWWA throughout their breeding range.

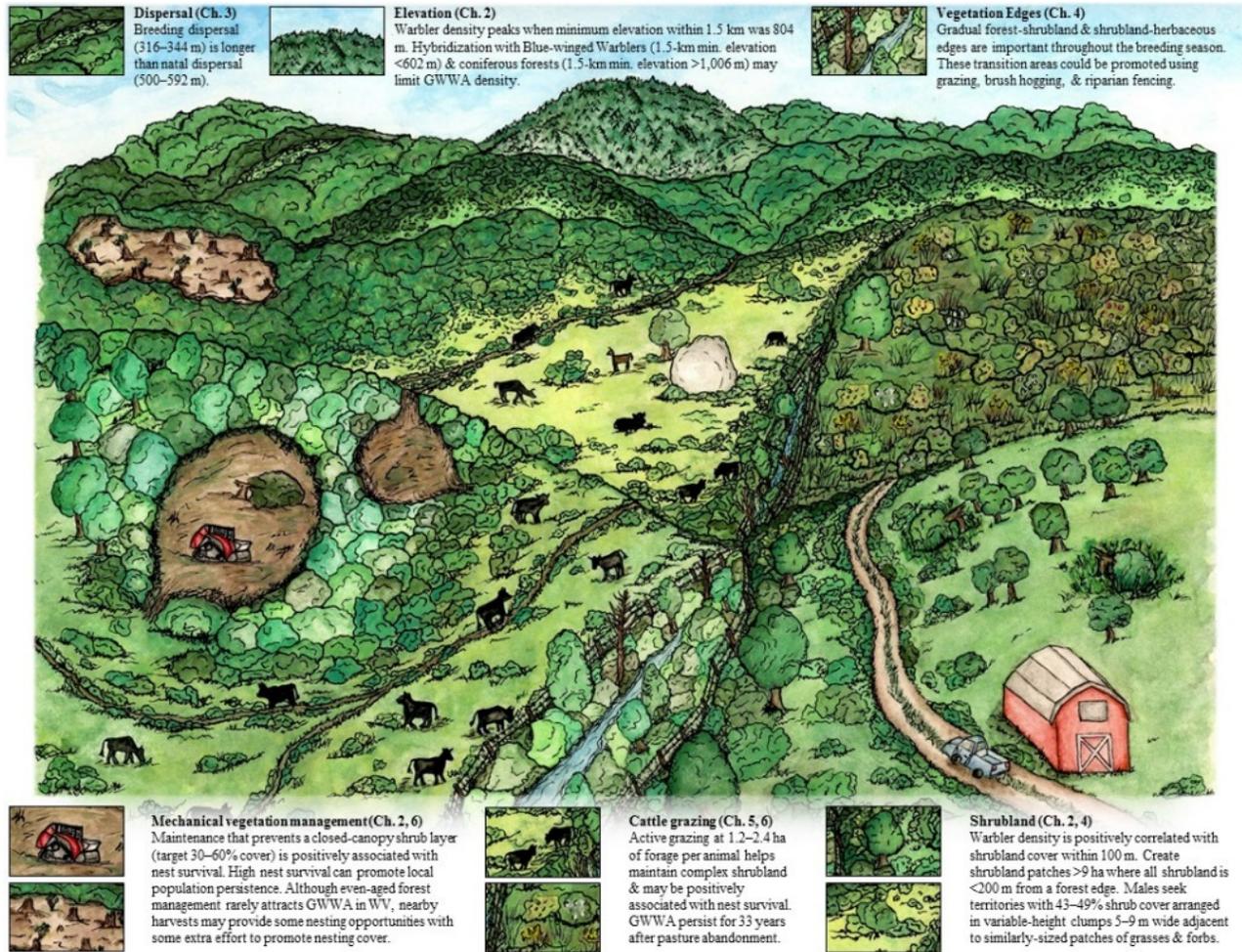


Figure 2. Visual abstract of study results.

**SONGBIRD RESPONSE TO GAS WELL AND INFRASTRUCTURE DEVELOPMENT IN THE
MARCELLUS-UTICA REGION**

Student Investigator: Laura S. Farwell

Principal Investigator: Petra B. Wood

Cooperators: Randy Dettmers, Todd Fearer, Margaret Brittingham

Years Ongoing: 2013–2018

Degree Program: PhD

Completed: August 2018

Funding Source: US Fish and Wildlife Service, Southern Regional Education Board Dissertation Fellowship

Objectives:

1. Assess breeding songbird response to unconventional shale gas well and infrastructure development by comparing avian guild species richness and focal species abundance at sites containing shale gas development and at control sites with no shale gas development.
2. Evaluate potential threshold responses in birds to distance from human forest disturbance and percent forest cover, in impacted areas across the Marcellus-Utica shale region.
3. Develop recommendations regarding well pad location, size, and density to minimize negative impacts to birds.

Background: Since 2005, extraction of natural gas from Marcellus-Utica shale has increased exponentially in the central Appalachians, yet much is still known about potential biological impacts of this industry in forest ecosystems. We seek to identify the response of breeding songbirds to forest disturbance due to the development of shale gas well pads and infrastructure.

We sampled 190 study sites across the Marcellus-Utica region during 2014–2015, including 120 sites affected by shale gas development and 70 non-gas control sites (Fig. 1). Sites represented a gradient of forest cover (range = 25–99% forest; Fig. 2) as well as a range of well pad sizes, ages, densities, and placements on the landscape. Gas sites were centered on a focal shale gas well pad, a cluster of pads, or a pipeline. Non-gas control sites had no shale gas development within a minimum 1-km radius of any point sampled. Among gas sites with well pads ($n = 110$ sites), 45% had well pads located on public lands, 51% had well pads on private lands, and 4% had well pads on a mix of both public and private lands.

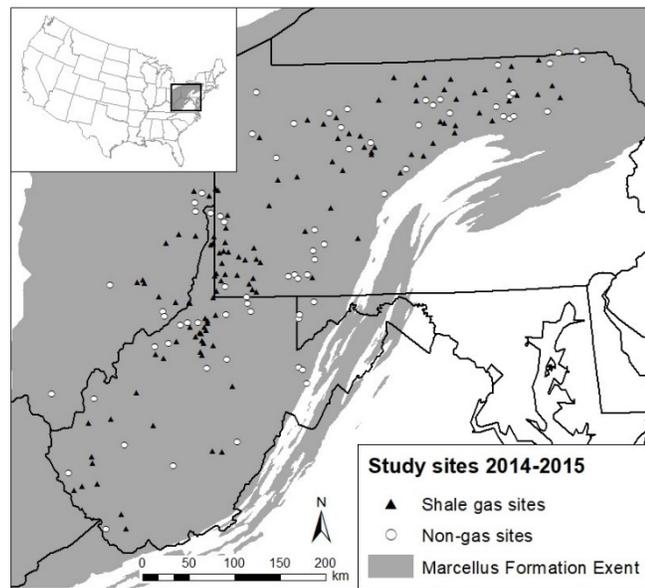


Figure 1. Field site locations from 2014-2015, in WV, OH, PA, MD, NY: black triangles represent shale gas impacted sites ($n = 120$), white circles are reference sites ($n = 70$).

We conducted avian point count surveys at 2,590 sampling points within the 190 study sites ($\bar{x} = 13.5$ points per site). At least 2 points per site were placed adjacent to forest edges in habitat directly disturbed by shale gas development (or other types of human development at non-gas control sites), and remaining points were distributed at varying distances from human development in surrounding forests (Fig. 2). We digitized land cover within a 1-km radius (314 ha) of each individual sampling point, to evaluate the influence of shale gas development and surrounding landscape matrices on breeding birds (Fig. 2). Because we were primarily interested in the response of birds to relatively fine-scale land cover impacts, we hand-digitized land cover at each site (a total area of approximately 140,000 ha) using 1-m resolution aerial imagery from the USDA National Agriculture Imagery Program. We classified land cover within broad categories of land use, including forest, timber harvest, agriculture (predominantly hayland and pasture), unconventional shale gas development, and other forms of human development unrelated to shale gas. Within classes of gas and non-gas development, we specifically categorized linear infrastructure separately from non-linear development.

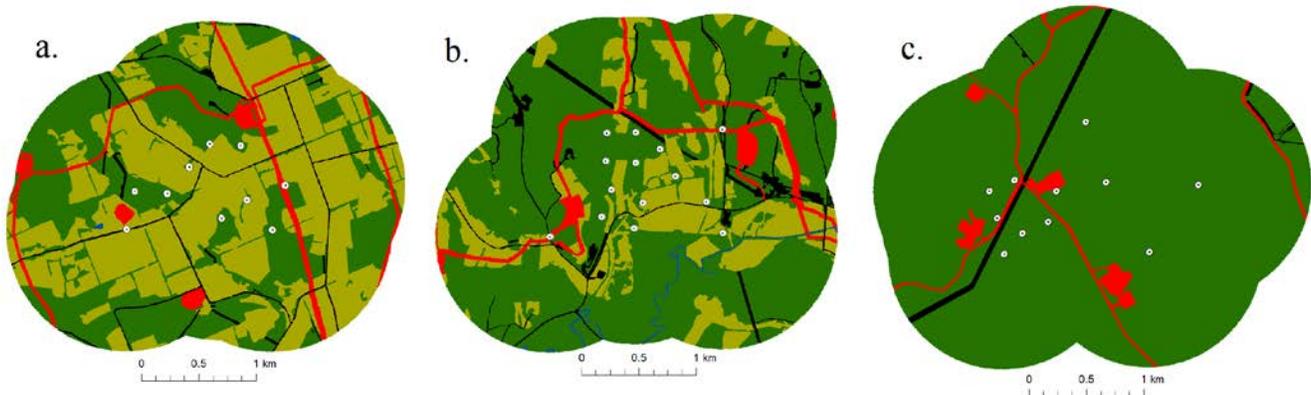


Figure 2. Hand-digitized, rasterized land cover maps, at three sites representing a gradient of forest cover: (a) low - 37%, (b) moderate - 69%, and (c) high - 94% forest. Forest cover is shown in green, shale gas infrastructure in red, agriculture in gold, non-gas development in gray. White dots represent locations of avian sampling points.

Results: A broad comparison of breeding songbird communities at gas sites versus non-gas sites showed significant overlap, suggesting avian communities generally responded similarly to shale gas development and other types of anthropogenic forest disturbance. However, finer-resolution comparisons of bird counts and land cover disturbance metrics revealed some distinct effects of unconventional shale gas development on birds. We compared species richness within 3 habitat guilds (forest interior, early successional, and synanthropic) and abundance of 24 focal species with land cover metrics calculated at 2 spatial extents: a point level within 500-m (79 ha) and 1-km (314 ha) buffers of each avian survey location, and a site level across each study area ($\bar{x} = 746$ ha). At the site level, pipelines and access roads in particular had a positive influence on synanthropic guild richness. At the point level, shale gas development within 500 m were negatively related to forest interior guild richness and positively related to successional and synanthropic guild richness. Although we saw variability in species-specific responses, effects of shale gas development on focal species abundance were generally consistent with guild-level effects. These region-wide results suggest that forest fragmentation from shale gas development is impacting avian communities across the Marcellus-Utica region, with higher numbers of early

successional and synanthropic species and lower numbers of forest interior species relative to increasing levels of unconventional shale gas development.

Lastly, we evaluated potential threshold responses in species abundance to distance from human development and percent forest/core forest cover, across the Marcellus-Utica shale region. We used Threshold Indicator Taxa Analysis (TITAN; Baker and King 2010) to identify change points in bird abundances at varying distances from anthropogenic forest edges, and across gradients of forest cover within 1-km radius landscapes surrounding avian sampling points. We calculated threshold responses for the overall avian community, within habitat guilds, and for individual species within each guild. Our results support the occurrence of some nonlinear avian responses to distance from anthropogenic edges among indicator species, although change point quantile intervals for distance thresholds were generally quite broad – for the overall avian community, within habitat guilds, and among indicator species. This indicates that distance threshold responses were typically gradual rather than sharply nonlinear. In general, species that increased along a gradient of distance from human development showed change points at greater distances than species that declined at greater distances from human development. This suggests that the benefits of anthropogenic disturbance for edge-associated species do not extend as far into affected forests as negative edge effects extend for forest interior species. For certain area-sensitive species (e.g., black-and-white warbler, blue-headed vireo, blackburnian warbler), negative effects of anthropogenic disturbance extended > 600 m into affected forests. We also identified threshold responses to the proportion of forest and core forest cover in landscapes altered by shale gas development. Some of these threshold responses were abrupt with narrow quantile intervals, particularly among positive threshold responses within the forest interior guild at 81.6%. This indicates that forest interior birds increased abruptly in abundance and frequency of occurrence at relatively high levels of overall forest cover, within the landscapes we evaluated.

Our region-wide findings are consistent with other localized studies from the region, which document decreasing abundance and diversity of forest interior birds and increasing numbers and diversity of early successional and human adapted species, in landscapes altered by unconventional shale gas development in the Marcellus-Utica region. Our results suggest that efforts to reduce forest fragmentation from shale gas development could reduce negative impacts on native biological communities in the central Appalachian region, particularly on area-sensitive, forest interior species.



Photo credit: WV Host Farms

Literature cited:

Baker, M.E., and R.S. King. 2010. A new method for detecting and interpreting biodiversity and ecological community thresholds. *Methods in Ecology and Evolution* 1:25–37.

**EVALUATION OF REPRESENTATIVE BIRD SPECIES' LANDSCAPE CAPABILITY MODELS
DEVELOPED BY THE DESIGNING SUSTAINABLE LANDSCAPES PROJECTS ON THE 13-STATE,
NORTHEASTERN REGION OF THE UNITED STATES**

Postdoctoral Investigator: Zachary Loman

Principal Investigators: Petra Wood, Daniel Harrison, Cynthia Loftin

Years Ongoing: 2015-2018

Completed: September 2018

Funding Sources: USGS Science Support Partnership Program (to Maine Coop Unit)

Objectives:

1. Test for relationships between abundance of representative species and DSL Landscape Capability (LC) products to enable practitioners to set concrete conservation goals.
2. Evaluate relationships between predictions of American Woodcock and Ruffed Grouse representative species LC models and occurrence predictions from singing ground surveys and drumming routes conducted in the Northeast.
3. Evaluate relationships between predictions of representative species Landscape Capability models evaluated in Objectives 1-2 and the species they represent.
4. Provide information to managers regarding priority, forest-associated avian species populations and forest structure and landscape conditions to inform conservation and management planning.

Summary:

The University of Massachusetts Designing Sustainable Landscapes (DSL) project (<http://www.umass.edu/landeco/research/dsl/dsl.html>) assesses the capability of the landscape to sustain wildlife populations under alternative climate change and urban growth scenarios. The project developed Landscape Capability models (LC) for representative species for current and future landscape conditions in the 13 state Northeastern Region of the USFWS. Species modeled by the DSL project are representative of habitat needs and ecosystem functions of groups of species so that conservation actions for representative species benefit the larger group. Several LC models are for USFWS priority species of high conservation concern in coniferous, hardwood-dominated or mixed coniferous-deciduous forest, or are expected to serve as surrogate models for other species with conservation priority designations. Before this project began LC models had not been validated with data collected in independent, systematic, repeated surveys, nor has the transferability of the representative species models been evaluated for the species they are assumed to represent.

We used bird point count survey data from ongoing and recently completed studies to evaluate relationships between occurrence and abundance of representative songbird species and LC predictions. Additionally, we evaluated transferability of the representative species' LC models to the species they were designed to represent. We evaluated LC models for two upland gamebird species Ruffed Grouse (*Bonasa umbellus*) and American Woodcock (*Scolopax minor*),

and published a paper on this topic (Loman et al. 2017). From the projects' initiation in September 2015, we have completed soliciting and compiling the necessary data to perform the validation and have completed an assessment and achieved the project objectives. We completed objective 1, assessing songbird models and published a paper on this topic (Loman et al. 2018); objective 2, assessing representativeness of LC models for surrogate species they are designed to represent (journal manuscript in prep); objective 3, assessing LC predictions of game bird occurrence and abundance (Loman et al. 2017; as mentioned above); and objective 4) assessing models relative to forest structure (journal manuscript in prep).

At a point-level for songbirds, LC is a moderate to excellent predictor of songbird occupancy for the seven songbird species modeled that we validated: Blackburnian Warbler (*Setophaga fusca*), Blackpoll Warbler (*Setophaga striata*), Cerulean Warbler (*Setophaga cerulea*), Northern Waterthrush (*Parkesia noveboracensis*), Louisiana Waterthrush (*P. motacilla*), Ovenbird (*Seiurus aurocapilla*) and Wood Thrush (*Hylocichla mustelina*). All songbird LC models used as a predictor of occupancy had high sensitivity, while the worst performing models had low specificity. For gamebirds, American woodcock (*Scolopax minor*) and ruffed grouse (*Bonasa umbellus*), LC models were reliable predictors of occurrence but were less indicative of relative abundance at route (11.5–14.6 km) and point scales (0.5–1 km). As predictors of occurrence, LC models had high sensitivity (0.71–0.93) and were accurate (0.71–0.88) and precise (0.88 and 0.92 for woodcock and grouse, respectively). Models did not predict point-scale abundance independent of the ability to predict occurrence of either species.



In evaluating representativeness of LC models as proxies for other species, we found that as predictors of represented species occurrence, LC models performed variably, but never were as good predictors as they were of the representative species themselves. LC models were an improvement on using macrohabitat classifications alone. In some instances, using multiple representatives in conjunction worked better than using one. Considerable range overlap was needed for models to provide meaningful predictive ability, but alone was no guarantee of accuracy or precision. Both ecological and taxonomic similarity between representatives and represented species had no effect on predictive ability. LC models worked similarly to using empirical observations, suggesting shortcomings in predictive ability were a function of imperfect surrogacy of representatives. Our analyses illustrate the usefulness of conservation proxies as representatives of species groups associated with macrohabitats, but underscore the necessity of using empirical data to evaluate their effectiveness.

LC models provide a continuous surface correlated to animal use across landscapes. How best to apply models depends on the relevant ecological scales at which animals choose habitat. Forest structure influences forest bird habitat selection, and measuring forest structure at fine scales may limit capability of models to predict patterns of animal occurrence. We sought to test how prediction errors from LC models used to predict three songbird species' abundances were influenced by point-scale forest structure. We used a subset of our larger data set, and developed a two-tier hierarchical model of LC as a predictor of point-level abundance with scale-dependent covariates. In the first tier, we used a generalized distance sampling approach and used latent indicator selection to determine form of LC data best used for prediction. In the second tier, we compared residuals between corrected point abundance estimates and the LC point predictions with point-level forest structure metrics. We used Bayesian model selection to derive inclusion probabilities for each variable. We repeated this process for three species. We found point level structural information did not influence model predictions of Blackburnian Warbler abundance, whereas with other species, such as Northern Waterthrush, prediction error resulted from unaccounted for point-level forest structure. Even within taxonomic family, an understanding of species ecology with finer scale information improves application of predictive models.

**INFLUENCE OF HARVEST TREATMENTS, VEGETATION, AND EDGES ON ABUNDANCE OF
BREEDING SPRUCE-FIR AVIAN SPECIES IN REGENERATING FORESTED STANDS**

Student Investigator: Brian Rolek

Principal Investigators: Petra Wood, Cynthia Loftin, Daniel Harrison

Years Ongoing: 2012–present

Degree Program: PhD

Completed: August 2018

Funding Sources: USFWS Migratory Bird Division, USFWS National Wildlife Refuge System, USGS Science Support Partnership Program, Maine Cooperative Forestry Research Unit, University of Maine Department of Wildlife, Fisheries, and Conservation Biology

Objectives:

We evaluated anthropogenic influences on avian communities in regenerating conifer forests, with a focus on the spruce-fir avian assemblage. To accomplish this we used multi-species point count surveys to survey birds. We examined:

1. associations between avian abundance and richness for spruce-fir avian species and seven ubiquitous forestry treatments,
2. focal species associations with harvest treatments, years-since-harvest, and vegetation, and
3. edge effects from roads and forest management on abundance and richness of avian species.

Background:

Several birds associated with the spruce-fir forest type (hereafter spruce-fir birds) have declining populations in the Atlantic Northern Forest (Fig. 1). The extent of coniferous forest (including spruce-fir forest) has declined, while the extent of deciduous forest has increased. This forested region is heavily managed for extraction of timber products and anthropogenic effects on bird communities and habitat are poorly understood. Numerous studies have focused on the immediate effects from forest management soon after harvesting (e.g., 0–5 years), but few examined abundance over longer durations of time as forest regenerates after disturbance.

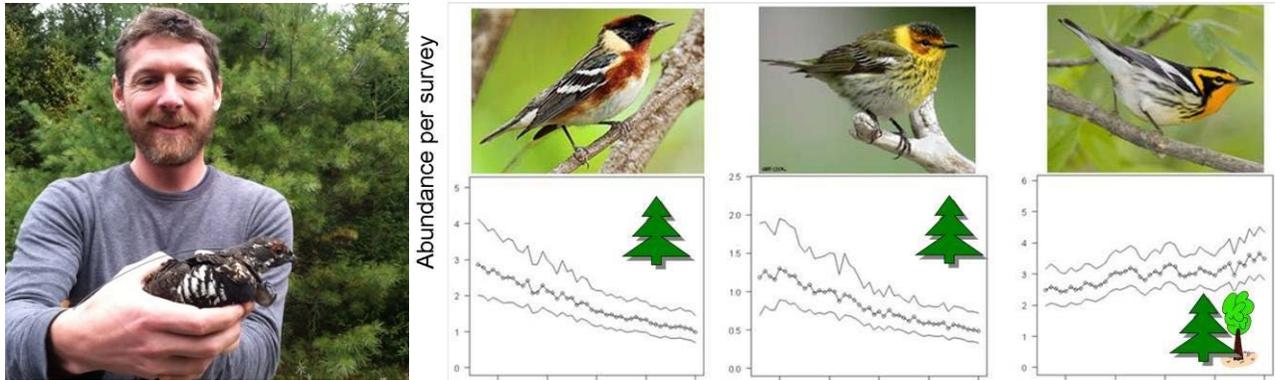


Figure 1. Left: Brian Rolek holds a Spruce Grouse (*Falcipennis canadensis*). Right: Focal species Bay-breasted (*Setophaga castanea*), Cape May (*Setophaga tigrina*), and Blackburnian (*Setophaga fusca*) warblers and their population trends from Breeding Bird Survey data in the North Atlantic Forest. Bay-breasted and Cape May warblers are spruce-fir species, while Blackburnian Warbler uses mixed-woods.

Results:

To understand how harvests influence conifer-associated birds during the breeding and post-breeding period over the forest regeneration period in the Atlantic Northern Forest (Bird Conservation Region 14) in lowland conifer (<500 m elevation) and mixed-wood forests, we used avian point count detection data and nonmetric multidimensional scaling to test for effects from seven common harvest treatments on 49 avian species. Harvest treatments had significant effects on avian assemblages (Fig. 2) and the spruce-fir avian assemblage reached greatest abundance in regenerating clearcut harvests combined with postharvest treatments (herbicide and precommercial thinning), and spruce-fir birds had greatest abundance in stands with $\geq 60\%$ spruce-fir tree composition and these stands had greatest spruce-fir tree composition.

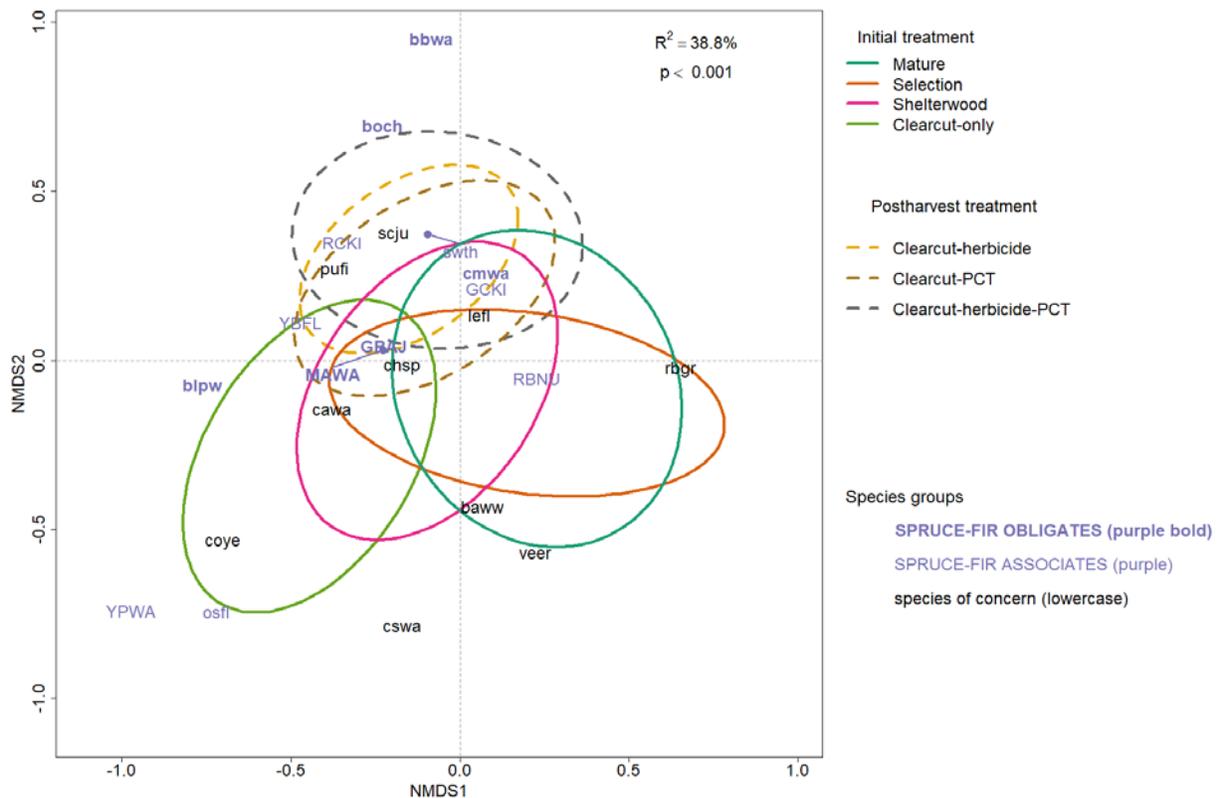


Figure 2. A linear regression of axes from nonmetric multidimensional scaling (NMDS, axes one and two) using abundance of 49 bird species modeled with treatment as an explanatory variable. Ellipses 67% confidence intervals are shown for each harvest treatment for visual aid. Spruce-fir species (obligates and associates), and species of concern are displayed here. All other species are omitted for visual clarity. R^2 and p value are shown for treatment effects. Text represents avian species as USGS alpha codes.

To test for effects from management, years-since-harvest, and vegetation on 19 conifer associated avian species, we examined associations with focal species abundance and used hierarchical Bayesian distance-removal models to estimate abundance. Abundance of only six species differed significantly among harvest treatments, indicating that treatments alone provided weak information regarding the abundance of individual species; however, 14 species had significant associations between abundance and vegetation variables (Fig. 3), suggesting that managers could target vegetative outcomes when directing management toward focal species. All vegetation variables were important for some species including basal area (nine species), midstory cover (five species), spruce-fir composition (four species), live crown ratio (three species), tree diameter at breast height (three species), shrub cover (three species), and shrub composition (one species). Only one species, the Blackpoll Warbler (*Setophaga striata*), was associated with years-since-harvest and abundance decreased as years-since-harvest increased.

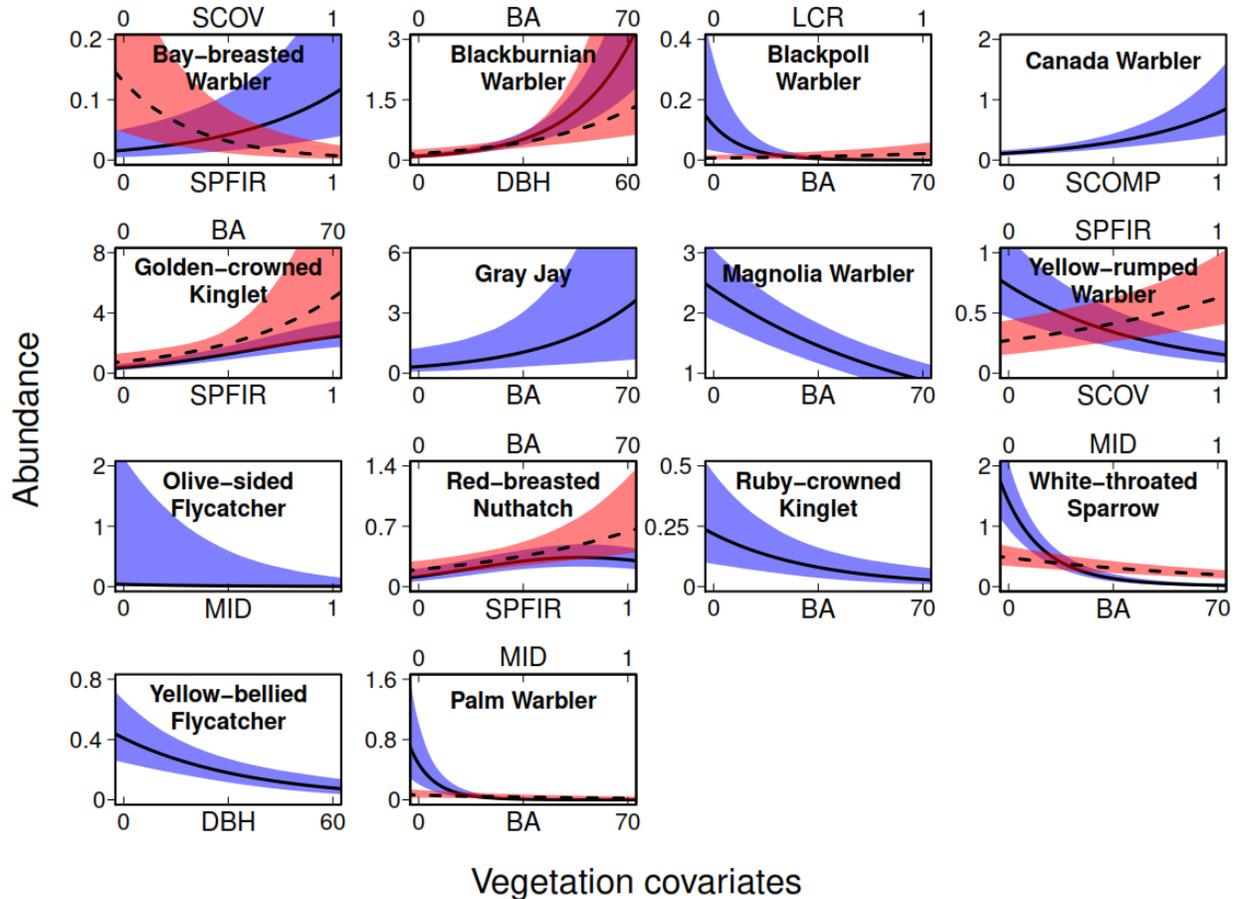


Figure 3. Vegetation associations with modeled abundance of 14 conifer-associated birds during the breeding and post-breeding periods. Each panel displays abundance for one species with the explanatory variable on the bottom x-axis (solid lines, blue CIs) having the greatest effect size and the top x-axis (dashed lines, red CIs) having the second greatest effect size. Vegetation variables include basal area (BA), diameter at breast height (DBH), spruce-fir tree composition (SPFIR), midstory (MID), shrub cover (SCOV), and live crown ratio (LCR).

To test for anthropogenically-induced edge effects on the avian community (72 species), we developed a novel Bayesian multi-species abundance model that accounts for detection probability and allows species to share information about abundance and detection probability to improve estimates. We conducted point count surveys at interior forest (≥ 130 m from edges), low-contrast edges where two regenerating managed stands meet, and high-contrast road edges with managed buffers. Spruce-fir birds had significantly greater richness at interior forest compared to high-contrast edge, and consistently trended toward greater median abundance for all fourteen species at interior forest (Fig. 4), although differences in abundance were not statistically significant. Interior forest had greater spruce-fir tree composition compared to high-contrast edge. Low-contrast edge was intermediate between interior forest and high-contrast edges in richness, abundance, and vegetation. We conclude that road edges contribute to habitat loss for spruce-fir birds, because high-contrast edges had less spruce-fir (51%) than the percentage needed to support the spruce-fir avian assemblage ($\geq 60\%$) while forest interior had much greater spruce-fir tree composition (73%).

Combined, our results provide essential information about the relationships between spruce-fir birds, forest management, vegetation, and edge effects that can guide practitioners toward management that will benefit the declining spruce-fir avian assemblage, and provides critical information for the conservation of biodiversity.

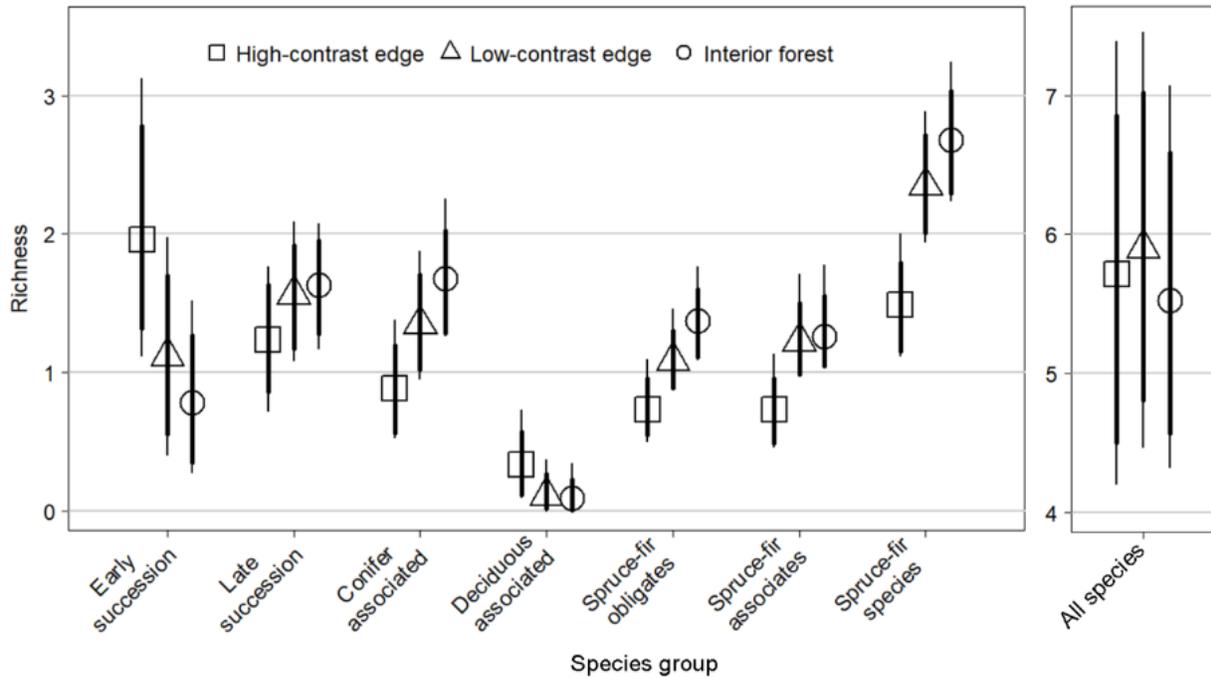


Fig 4. Richness estimates (y-axis) for species assemblages (x-axis) from a multi-species distance-removal model with a zero-inflated distribution for abundance that included 72 avian species. Points depict median abundance with 85% highest posterior density intervals (thick vertical lines) and 95% highest posterior density intervals (thin lines).

CONTINUING PROJECTS

AQUATIC

FISH USE OF MISSISSIPPI RIVER DREDGE AND DISPOSAL SITES

Student Investigator: Hae Kim

Principal Investigator: Quinton Phelps

Years Ongoing: 2018-2021

Expected Completion Date: December 31, 2021

Funding Source: USGS (RWO 75)

Objectives:

To evaluate patterns in vital rates and improve our understanding of the underlying factors that influence population dynamics, our primary research objectives are as follows: 1) Are there patterns of vital rates within and among species across time or space in the UMRs? and 2) How are vital rates within and across species associated with differences in abiotic and biotic drivers in LTRM reaches? With respect to UMRs, improved understanding of the role of hydrogeomorphic conditions (i.e., flow, temperature, habitat availability) on vital rates can provide insight into how river restoration can more effectively influence fish populations and communities.

Progress:

The LTRM fish specialists (state agency biologists) have commenced data collection from their respective pools. For each of the UMR fish species, the target goal is to collect 10 individuals of each centimeter length group from each LTRM pool annually. At this stage of the project, these targets have not yet been attained. That being said, a subset of samples have been collected from pool 4 (Lake City, Minnesota, RKM 1210-1283), pool 8 (La Crosse, Wisconsin, RKM 1092-1131), pool 13 (Bellevue, Iowa, RKM 841-896), pool 26 (Alton, Illinois, RKM 325-389), La Grange Pool (Illinois River, RKM 80-158) and the open river (Cape Girardeau, Missouri, RKM 47-129).

The number of samples collected varies by field station. Pool 4 field station staff have collected Emerald Shiner; ERSN (N=71), Bullhead Minnow; BHMW (N= 22), Channel Catfish; CNCF (N= 26), Freshwater Drum; FWDM (N=19), Bluegill; BLGL (N=14), and Gizzard Shad; GZSD (N= 45). Pool 8 field station staff have collected ERSN (N=41, BHMW (N=21), BLGL (N=97), FWDM (N=21), GZSD (N=23), and CNCF (N=89). Pool 13 staff have collected ERSN (N=44), BHMW (N=22), CNCF (N=33), FWDM (N=6), BLGL (N=15), and GZSD (N=48). Pool 26 staff to date have collected ERSN (N=100), BHMW (N=0), CNCF (N=50), FWDM (N=40), BLGL (N=40), and GZSD (N=50). Big Rivers and Wetlands Field Station staff have collected ERSN (N=14), BHMW (N=1), CNCF (N=17), FWDM (N=38), BLGL (N=2), and GZSD (N=2). LaGrange field station staff have collected ERSN (N= 80), BHMW (N=41), CNCF (N= 164), FWDM (N=46), BLGL (N=36), and GZSD (N=149). Standardized LTRM protocols have been followed in the collection of all species, whether collected during regular LTRM sampling or in

targeted sampling (Ratcliff et al. 2014). Upon collection, total length was recorded from each fish. Individual fish have been bagged with a unique individual fish barcode affixed, and frozen for storage until dissection. A trip is planned to collect all frozen specimens for the week beginning August 6, 2018. Fish hard parts (e.g., otoliths) will be removed from the fishes, sectioned and aged to determine population age structure. Age estimates will be determined by two independent readers. In the event of a disagreement, a third reader will be used to resolve discrepancies.

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

Principal Investigator: Stuart A. Welsh

Years Ongoing: 2009 – 2018

Expected Completion: 2019

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.

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.

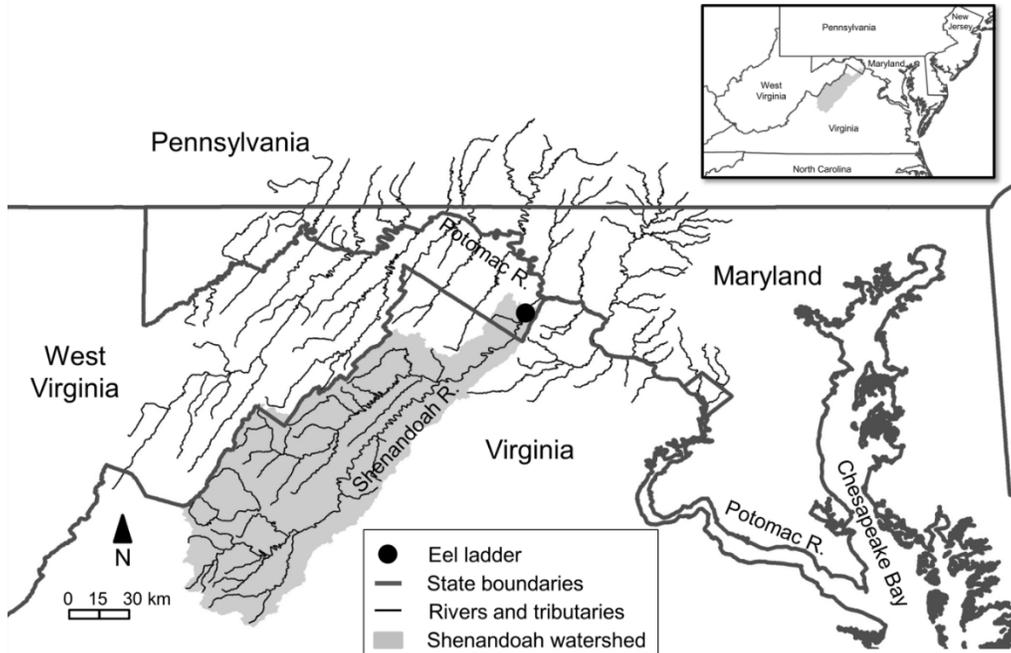


Figure 1. Map of the Potomac River drainage and the location of the Millville Dam eel ladder on the Shenandoah River.



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:

Fish collected from source streams, marked, and acclimating in the laboratory setting. Respiration chambers constructed. Initial data collection anticipated late August 2018.



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 (atrazine, simazine, metalochlor, certain neonicotinoids), estrone, phytoestrogens and microcystin. Functional analyses as well as gene expression will be assessed. This will help to assess immune function in relation to contaminant exposure.
2. Compare the health of wild smallmouth bass from 3 sites in the Potomac River drainage, two sites in the Greenbrier drainage and two sites in the Susquehanna drainage in the spring and fall of 2018-2020. The Susquehanna sites will be funded from a current USGS grant. 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. Two sites in the Potomac and two sites in the Susquehanna are 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 2018, it will be determined by WVDNR and DEP together with USGS, as to whether additional sites should be assessed or further work should be done at a subset of the 2018 sites with some funding being used for chemical analyses of water and tissue of sites not part of the USGS projects.
4. Based on the findings of the *in vitro* experiments, conduct short term exposures to YOY laboratory-held smallmouth bass and evaluate immune status as well as other responses as they reach age 1.

Progress:

Objective 1: We have established a healthy stock of laboratory-reared smallmouth bass at Leetown Science Center. Methods are being finalized for *in vitro* exposures. Sampling is tentatively scheduled fall/winter 2018 to avoid overlap with field season.

Objective 2: Site selection for source populations of fish has been identified. Immune function assays have been determined and validated for wild fish. Both Susquehanna sites and two Potomac sites were sampled in spring 2018. Greenbrier and the additional Potomac site will be added in fall 2018. Data has been collected for spring 2018 samples. Analysis is ongoing.

Objectives 3 & 4: Pending analysis from first two objectives.

**DETECTION OF FISH THYROID HORMONES BY MULTIPLEX ARRAY: AN INTERSPECIES
INVESTIGATION OF PLASMA HORMONE LEVELS, THYROIDAL HISTOLOGY, AND
TOXICOLOGICAL DATA**

Student Investigator: Emily Underwood
Principal Investigators: Dr. Patricia Mazik
Collaborator: Dr. Vicki Blazer
Years Ongoing: 2017-present
Degree Program: MS
Expected Completion: December 2018
Funding Source: U.S. Geological Survey (RWO 68)

Objectives:

Objective 1. Thyroidal endpoints in fish, such as plasma hormone levels (plasma thyroid stimulating hormone—TSH, thyroxine—T4, and triiodothyronine—T3), thyroid histology (thyroid epithelial cell height, colloid depletion), and deiodinase activity (deiodinase type I and deiodinase type II), are commonly used biomarkers of exposure to environmental contaminants. This thesis aims to compare these endpoints to gene expression data for four ecologically relevant species of fish (smallmouth bass, largemouth bass, brown bullhead, white sucker) from 7 Areas of Concern (AOCs) within the Great Lakes Basin. Each of these biomarkers have been used extensively in previous biomonitoring field studies, but no study has assessed them altogether.

Objective 2 & 3. Thyroid responses in fish are modulated by changes in circulating thyroid hormone (TH) concentrations. The current “gold standard” for measuring circulating levels of THs in vertebrates is Radioimmunoassay (RIA). While sensitive, RIA can lack concordance across platforms and laboratories, and can be prone to analytical interferences especially when using fish plasma. Perhaps the biggest weakness of this method is that a separate test must be employed for each TH. My second objective is to validate a new method, multiplex array, that allows for the simultaneous measurement of total T4 and total T3 in low volumes of fish plasma. Specifically, I aim to validate the commercially available Bio-Rad magnetic bead kit to measure T3 and T4 in smallmouth bass, largemouth bass, white sucker and brown bullhead. Additionally, I aim to validate a commercially available fish TSH ELISA for these species. Validation of this method will offer significant advantages relative to the current methods used for the detection of THs for these species.

Progress:

Objective 1.

Histology—Thyroid histology samples of 93 fish were assessed morphologically. At least ten thyroid follicles per fish were selected and digitally measured to quantify potential indicators of thyroid activity: thyroid epithelial cell height (TECH), ratio of thyroid epithelial cell area to colloid area (TECA:colloid), total colloid, and percentage colloid depletion. Light microscopy was performed at the 20x, 40x, or 63x magnification level with an Axio Observer inverted microscope (ZEISS, Jena, Germany) equipped with an AxioCam 503 color imaging camera (ZEISS, Jena, Germany). The measurements were taken using the free software tool Image J I.44 (National Institutes of Health, USA) to quantify observed alterations. The measurement of TECH (in μm) was reported as the mean of four cardinal point estimates per follicle, i.e. the average of 40-68 follicular cell height measurements for each fish. The cardinal points were chosen based on having visible nuclei, indicating a proper cross section. The percentage of colloid depletion in a follicle was calculated and recorded, with values of 0 and 100% representing extremes of colloid-replete and colloid-exhausted follicles, respectively. Total colloid was calculated by subtracting the droplet area from colloid area + droplet area. Percentage of colloid depletion was calculated by dividing droplet area by total colloid. The TECA:colloid ratio was calculated as the area of the follicular epithelial cells divided by the area of the colloid assuming colloid was present.

Gene Expression—Gene expression data have been previously summarized by Hahn (2012). Briefly, gene expression analysis was performed on collected liver samples using nCounter Analysis® (NanoString Technologies, Seattle, WA). BLASTx searches of partial transcriptome databases for smallmouth bass (SRX156704, SRX199239) and brown bullhead (SRX199312, SRX148685) were used to identify a priori genes of interest. Selected genes were those with suspected modulation in response to contaminants including endocrine disrupting compounds, environmental stressors, as well as genes associated with neoplasia and/or presence of parasites. A total of 50 genes for each species were chosen for analysis. Annotated sequence data for each gene was orientated and trimmed to correspond to 102 coding regions using results from BLASTx searches. Custom CodeSets were designed by Nanostring Technologies (Seattle, WA).

Prepared liver samples were sent to University of Pittsburgh Genomics and Proteomics Core Laboratory (Pittsburgh, PA) for mRNA expression analysis on the Nanostring Technologies platform. Approximately 17 – 23 mg of liver tissue was combined with 400 μl of TRK lysis buffer (Omega Biotech, Norcross, GA) and homogenized on a tissue lyser (Qiagen, Valencia, CA) with 5 mm stainless steel balls (Boca Bearings, Boynton Beach, FL). Samples were then centrifuged at 13,200 RCF for 10 minutes and the supernatant was removed. Clarified homogenates were stored at -80°C until shipment. CodeSets for appropriate species were utilized in the nCounter analysis system workflow as per the manufacturer and core lab protocols. Generated fills were utilized for quantitative interrogation.

The nSolver Analysis Software (v2.0) was used for quality control and normalization of gene expression data. Negative controls, as included by Nanostring quality control protocol, with high average counts (values ranging from 7-37) were removed. The geometric mean plus three

standard deviations of negative control probes was subtracted from each sample to account for background signal. To eliminate genes with low count data this value was defined as the reporting limit of this assay and genes with expression data below this calculated value were removed from subsequent analysis. Next, a scaling factor was applied to all samples of the average geometric mean of positive spike-in samples across all lanes. Finally, samples were normalized by tissue mass.

Plasma hormone levels—these will be assessed once Objective 2 & 3 are accomplished.

Objective 2: I am in the process of validating the multiplex and ELISA methods for TH detection for our species. To do this, a charcoal stripping protocol was employed to test if the assay is free of potential interfering substances such as endogenous matrix components. Plasma was pooled, charcoal stripped following an established protocol, and spiked with the analytes for use in standard curves. The charcoal stripped standard using plasma as the biological matrix was compared to the standard provided by the kit. More experimentation must be done before confirming that this method will work for our species. Charcoal stripped standards will also be used for validation of the commercial ELISA in the upcoming month. Once these methods are validated, the hormone levels will be assessed and used in comparison to thyroid histology data and gene expression data.

**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.

Progress

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 analysis is still on-going and results are not yet available.

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 8-10 μ m 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.



CONSERVATION ASSESSMENT OF WEST VIRGINIA CRAYFISHES

Principle Investigators: Zac Loughman and Stuart Welsh

Years Ongoing: 2007-2018

Expected completion: 2020

Funding Source: WVDNR

Objectives:

1. Identify species in need of conservation
2. Document distribution ranges of invasive species
3. Document range expansion and conservation standing of “common” species
4. Conduct surveys for new state records and undescribed forms
5. 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.

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

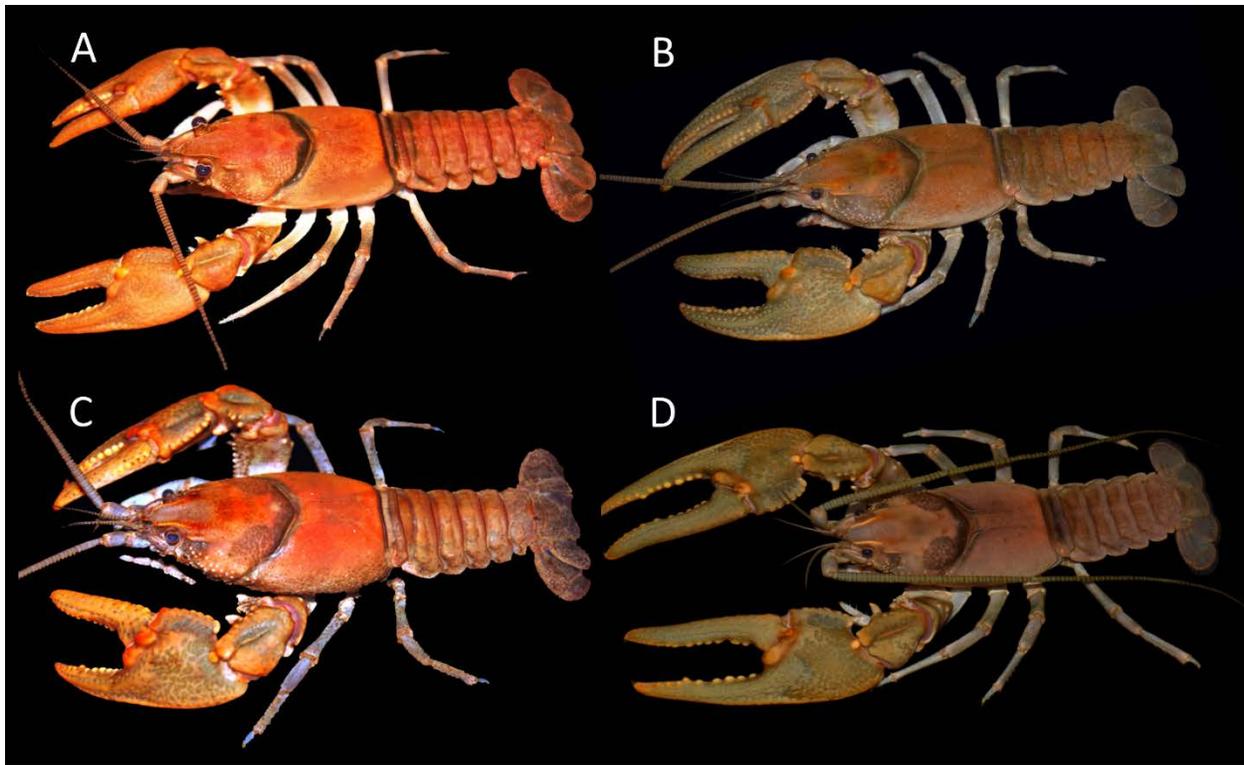


Figure 1. Four crayfish species described during this study: A. *Cambarus smilax*, B. *Cambarus thepiensis*, C. *Cambarus hatfieldi*, and D. *Cambarus appalachiensis*.

**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.

Benthic macroinvertebrate samples from sites located along the Parsons to Kerens alignment were collected twice during 2017 (fall and spring). The 2017 samples will be sub-sampled and identified in the lab during 2018. Construction began in the watersheds related to the Parsons to Kerens alignment in the fall of 2017. Samples from before construction were sub sampled and identified during 2017. These data were entered into a database and used to calculate a multi-metric index. Six metrics (EPT taxa, total taxa, % EPT, % chironomidae, % top 2 dominant taxa, and Hilsenhoff Family Biotic Index, [HBI]) were used to calculate the West Virginia Stream Condition Index (WV SCI). The WV SCI scores are as follows: 78.1 to 100 = Very Good; 68.1 to 78 = Good; 45.1 to 68 = Fair; 22.1 to 45 = Poor; and 0 to 22 = Very Poor.

The majority of sites along the Parson to Kerens alignment were in “good” to “very good” biotic condition based on samples collected before construction (Table 1) Sites PK-1, PK-3, PK-4, PK-9, and PK-11 received “good” scores, and sites PK-2, PK-5, PK-6 and PK-12 received “very good” Scores. These sites had high percentages of EPT taxa (benthic macroinvertebrates in the families Ephemeroptera, Plecoptera, and Trichoptera) and low percentages of Chironomidae (Table 1). The EPT taxa are sensitive to aquatic pollution, and the Chironomidae family is pollution tolerant. The “good” to “very good” WV SCI scores are indicative of sites with adequate riparian habitat and substrate. Riparian habitat refers to the vegetation along the stream bank. Stream banks that are greater than 70% covered in native vegetation are considered optimal. The preferred substrate for benthic macroinvertebrate is a cobble and gravel substrate. The flow over this substrate provides adequate oxygen and food particles.

Site PK-8 has a “poor” WV SCI score, and site PK-10 has a “fair” WVSCI score. Site PK-8, which is located at a road crossing, downstream of a culvert, is heavily embedded with sediment. Site PK-10 is located on Lazy Run at the Kerens Post Office. This site also has a large load of sediment, and the substrate is mainly clay. The embeddedness of these sites do not provide good benthic macroinvertebrate habitat. Smaller substrate such as sand and fines clay do not provide adequate interstitial space, and sites tend to have higher turbidity. Turbidity is detrimental to benthic macroinvertebrates that depend on plant material for food, or use visual location of prey.

Table 1. Benthic macroinvertebrate metrics calculated from Before Construction samples from sites located along the Parsons to Kerens proposed alignment.

Site	% Top 2 Dominant Taxa	% Chironomidae	% EPT Taxa	HBI	EPT TAXA	TOT TAXA	WV SCI Score	Stream Condition
PK-1	46.68	24.01	62.16	9	4	16	76.52	Good
PK-2	52.79	13.31	77.83	11	3	18	83.98	Very Good
PK-3	48.19	17.00	65.46	8	3	15	77.30	Good
PK-4	78.33	2.54	91.52	9	3	14	75.77	Good
PK-5	43.50	15.76	64.69	11	4	17	82.37	Very Good
PK-6	60.03	8.76	80.41	9	3	17	81.20	Very Good
PK-7	48.42	23.51	46.82	11	4	19	77.86	Good
PK-8	78.13	59.90	14.82	5	5	13	42.55	Poor
PK-9	48.63	27.11	49.89	9	4	18	75.45	Good
PK-10	69.65	29.18	22.37	5	5	14	54.62	Fair
PK-11	47.48	26.21	50.13	11	4	20	77.67	Good
PK-12	47.49	18.08	67.38	9	4	18	80.48	Very Good

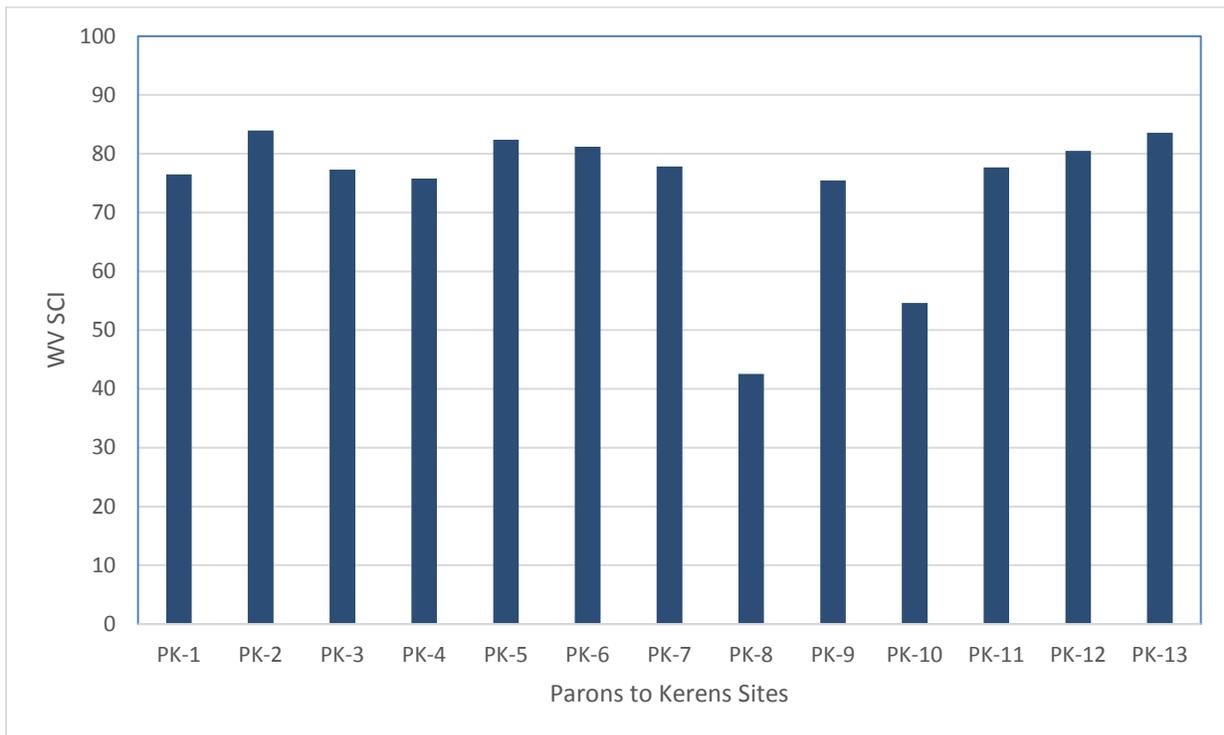


Figure 1. West Virginia Stream Condition Index Scores for sites located on in the Parsons to Kerens alignment before construction began in Fall 2017.

WILDLIFE

EFFECT OF IMIDACLOPRID TREATMENT OF HEMLOCKS ON AQUATIC ECOSYSTEMS

Student Investigator: Sara Crayton

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

Cooperators: John Perez, Christine Arnott

Years Ongoing: 2017-present

Degree Program: MS

Expected Completion: May 2019

Funding Source: USGS/NPS Water Quality Partnership Program (RWO 72)

Objectives:

1. Quantify the concentrations of imidacloprid present in stream water and stream sediment.
2. Compare the concentrations of imidacloprid in stream water with the abundance and diversity of stream salamanders and benthic macroinvertebrates.
3. Investigate whether imidacloprid is bioaccumulating in stream salamanders and benthic macroinvertebrates

Progress:

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

From late April-mid July 2018, we sampled 24 stream sections within the Monongahela National Forest in West Virginia. Twelve of these stream segments were exposed to imidacloprid and 12 were not. These 24 stream sites were paired with each pair having 1 control and 1 treatment site. The pairs were similar in geographical location, stream flow, number of cover objects, and surrounding vegetative community. Within each stream site, we laid out three 3.3 x 2 m subplots in stream areas with abundant cover objects. Salamanders were sampled within these plots using standard active searching methods. We flipped every cover object greater than 50 mm in size and searched through packs of leaves and debris. We measured total length, snout-vent length, and weighed all captured salamanders. Each site was sampled either 6 or 7 times between April and mid-July. Methods were the same as in 2017 when 24 streams on NPS lands in WV were sampled. Final analyses

Across all sites and sampling periods, we identified 894 individuals of 8 salamander species. Fairly comparable numbers of salamanders overall were detected in control and treatment streams, although some species had substantially more captures in control streams. In control streams, 471 individuals were captured or observed; in treatment streams, 423 individuals were captured or observed (Figures 1 and 2).

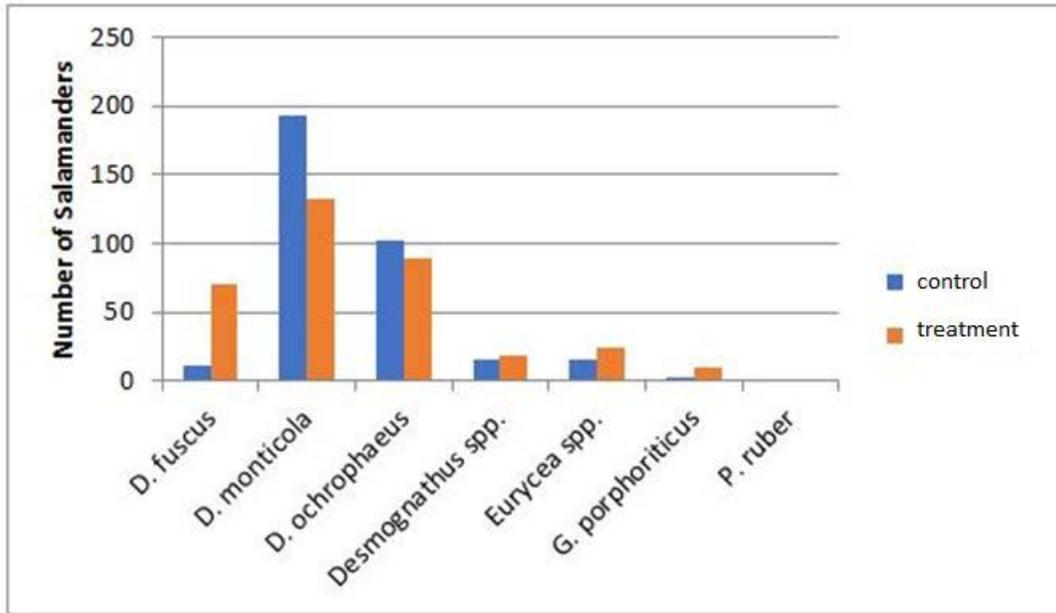


Figure 1. Number of adult and subadult (combined) salamanders detected in control and treatment streams.

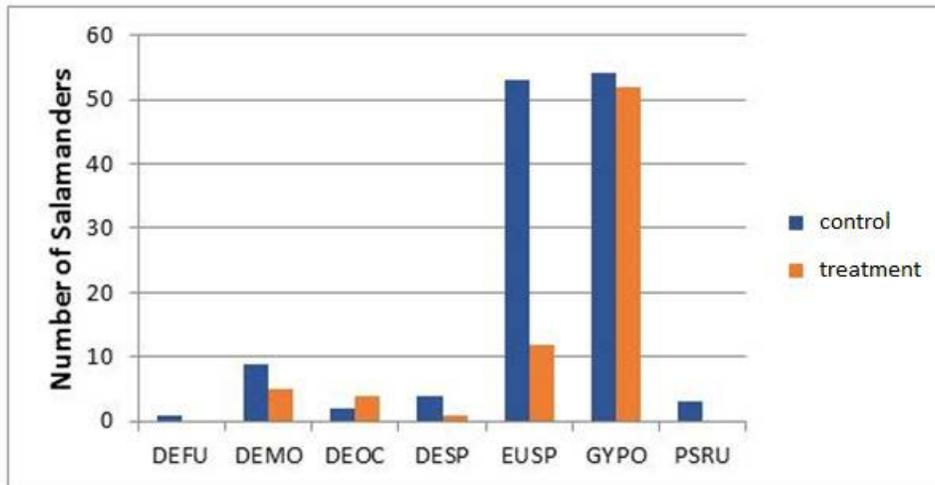


Figure 2. Number of larval salamanders detected in control and treatment streams.

Additionally, we collected benthic macroinvertebrates using a D-net from each subplot. In each subplot, we disturbed a 0.3 x 2 m area for approximately 1 minute to dislodge benthic invertebrates. The macroinvertebrates will be identified and we will calculate overall taxa richness, EPT richness, % of tolerant taxa, and % of intolerant taxa, and other metrics to evaluate benthic community composition. We will either dry the samples to measure biomass or measure individuals to obtain information about the prey available to stream salamanders. Benthic invertebrates will be collected from all treatment sites also and will be analyzed with light chromatography-mass spectrometry (LC/MS) to determine whether imidacloprid can be detected within the invertebrates' tissues.

During the fall of 2018, water and sediment samples from sites within the Monongahela National Forest and sites sampled last year in the New River Gorge and Gauley River areas will be tested with LC/MS to determine imidacloprid concentration. Additionally, salamanders of the genus *Desmognathus* which were captured from sites sampled last year will be tested using LC/MS to determine whether imidacloprid is being bioaccumulated within the salamander tissues.

Streams in hemlock forests provide critical habitat for stream salamander populations and benthic macroinvertebrate communities. Ultimately the results from this study will provide guidance for wildlife managers conducting hemlock woolly adelgid treatment programs.



Figure 2. Recently transformed red salamander (Pseudotriton ruber) captured during salamander surveys.

Figure 3. Research technician Allison Fulcher collecting morphometric measurements from a salamander.



Figure 4. Subplot in typical stream salamander habitat.

**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

Years Ongoing: 2016-Present

Degree Program: MS

Expected Completion: December 2019

Funding Source: National Park Service

Objectives:

- 1) Relate abundance and diversity of salamanders with the frequency and extent of imidacloprid treatment and invertebrate abundance.
- 2) Compare body conditions of salamanders found at imidacloprid treatment sites to salamanders found 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). These forests play critical roles in the ecology, aesthetics, and recreational aspects of each park, and compose approximately 5% of NERI, 8% of BLUE, and about 50% of GARI total forest cover. Hemlock-dominated forests create unique micro-climates providing favorable habitats for certain wildlife species and affecting understory plant species composition, biomass, and productivity among various hemlock stands. Hemlocks are threatened by an exotic insect pest, the hemlock woolly adelgid (Fig. 1). Although the effectiveness of imidacloprid on hemlock woolly adelgid (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 in the National Park System. The terrestrial salamander community research is one portion of a larger overall imidacloprid effects research project. Additional research includes studying the effects and extent of imidacloprid on non-target meso- and macro-soil dwelling invertebrates, movement or transfer of imidacloprid through terrestrial, riparian, and aquatic soils, and impacts of imidacloprid on water quality, benthic organisms, and the stream-associated salamander community.

Figure. 1. Hemlock woolly adelgid on eastern hemlock needles (photo by Layne Strickler, 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 and sampling began the following spring (Fig. 2). 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 will have been sampled at least five times.



Figure 2. Coverboard grid (Photo by Lenza Paul)

Captures in 2017 were dominated by two species: eastern red-backed salamanders (Fig. 3) and northern slimy salamanders. Eastern red-backed salamanders comprised 74% (n=162) of overall captures, while northern slimy salamanders accounted for 19% (n=42) of total captures. Control sites yielded 102 total captures from five species, while treatment sites had 116 total captures from six species. Preliminary data analysis has shown 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 ($p=0.907$ and $p=0.397$, respectively).



Figure 3. Eastern red-backed salamander (photo by Lenza Paul)

In 2018, along with continuing salamander surveying, habitat data is being collected for all 36 plots. Soil pH, foliage cover, soil moisture, litter and duff depth, live basal area, and tree species inventories are being completed for each plot for spring, summer, and fall. Data analysis for all years will be completed in winter/spring of 2019.

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.

LONG-TERM AVIAN RESPONSE TO LANDSCAPE AND CLIMATE CHANGE IN THE ALLEGHENY HIGHLANDS OF WEST VIRGINIA

Student Investigator: Hannah Clipp

Principal Investigators: Petra B. Wood and Christopher Rota

Cooperators: Cathy Johnson

Years Ongoing: 2018-Present

Degree Program: PhD

Expected Completion: May 2022

Funding: WVU Ruby Fellowship, NSF Graduate Research Fellowship Program

Objectives:

1. Compare avian community diversity and abundance on managed and unmanaged landscapes.
2. Evaluate avian species changes across time and elevational gradients.
3. Determine effectiveness of wood thrush (*Hylocichla mustelina*) and scarlet tanager (*Piranga olivacea*) habitat management guidelines.

Progress:

The central Appalachian Region is known for its diverse bird communities and is a focal area for avian research and conservation efforts. Beginning in 1992, a series of WV Unit research studies have quantified avian communities on the Monongahela National Forest (MNF) and nearby private lands. Additional avian monitoring has occurred in this area conducted by USFS, WVDNR, and WVU biologists. These existing datasets plus new additional survey efforts will be used to evaluate our research objectives. Data were collected using 10-minute point counts and recorded all species and individuals detected by sight and sound. Point counts on the MNF, a predominantly unmanaged landscape, will be compared with counts from nearby industrial private forest lands which is a heavily managed landscape.

Point counts sampled across multiple years and elevations can be used to model avian species response to climate change. We will focus on mature forest and young forest species of conservation concern, including species such as blue-winged warbler (*Vermivora cyanoptera*) and wood thrush as well as associated species such as scarlet tanager. We plan to evaluate potential altitudinal and/or latitudinal range shifts based on the long-term data sets.



Based on Breeding Bird Survey data gathered since 1966, wood thrush abundance has decreased throughout most of its breeding range including within its core breeding range in the central Appalachians. The persistent, negative trends prompted every state in the USFWS

Northeast Region to designate the wood thrush as a Species of Greatest Conservation Need (SGCN). Consequently, habitat management guidelines for wood thrush in the Northeast (Lambert et al. 2017) were recently developed and released. Recommendations in the guidelines were developed based on best available science from peer-reviewed journal articles. However, the recommendations have not been explicitly tested with empirical field data. Our point count data were collected within a wide variety of habitat conditions and will be used to evaluate expected responses of wood thrush to the recommended management practices and to suggest revisions to the guidelines if warranted. Although the wood thrush is an important SGCN focal species, it may also be a valuable indicator for a broader suite of forest species that will benefit from the wood thrush management guidelines. Because our point count studies sampled the entire avian community, our point count datasets will be used to evaluate response of the broader avian community. Further, Lambert et al. (2017) also provide habitat management guidelines for scarlet tanagers which our point count datasets can also evaluate.

Lambert, J. D., B. Leonardi, G. Winant, C. Harding, and L. Reitsma. 2017. Guidelines for managing wood thrush and scarlet tanager habitat in the Northeast and Mid-Atlantic regions. High Branch Conservation Services, Hartland, VT.

New data collection for this dissertation project will begin in the 2019 breeding season. Besides the three basic objectives listed above, additional research questions will be developed.

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: May 2020

Funding Source: West Virginia Division of Natural Resources

Objective:

Evaluate the response of the bird (songbirds, ruffed grouse [*Bonasa umbellus*], and American woodcock [*Scolopax minor*]) and salamander communities to the creation of young forest habitat throughout West Virginia on various landscape features (e.g. gas pipelines, transmission powerlines, and regenerated forest stands).

Progress:

West Virginia is approximately 80% forest cover, which is predominantly comprised of mid- and late-successional age classes. Aging forest conditions in the northeastern United States have had a negative impact on 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.

Young forests were created by two different management practices: tree cutting and herbicide treatment in a variety of plot sizes, disturbance intensities, and surrounding matrices. Landscape matrices included right-of-ways (ROW; gas pipeline or transmission powerline), wildlife openings, or 10-acre harvests within surrounding forests. Tree cutting along ROWs and wildlife openings was done at three different depths into the forest (50, 100, and 150 feet) and two different harvest intensities (20 ft²/ba and 60 ft²/ba), along with a control plot. Tree cutting along ROWs and wildlife openings are referred to as cut-back borders. Within surrounding forests, we implemented three different management techniques on 10 acre plots, with stands either receiving a clearcut (clearcut-leave or clearcut-windrow) or a hack-and-spray herbicide technique, along with a control plot. Young forest management via harvests within surrounding forests are referred to as regeneration stands. 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 2018, we added our final sets of study plots to the project and finished the last year of sampling at plots that were established in 2016. We sampled 12 different study sites throughout West Virginia, and with the help of WVDNR personnel were able to conduct 2 rounds of ruffed grouse drumming surveys, 1 round of American woodcock singing-ground surveys, 1 round of songbird migration stopover surveys, 2 rounds of breeding songbird surveys, and 3 rounds of salamander surveys. Basic summaries are provided below. Statistical analyses will be completed after the 2019 field season.

Ruffed grouse drumming surveys were conducted twice every year from April 12 to May 20 between 0530-1000 EST on days considered optimal for sampling (e.g., no rain, no heavy or gusty winds, and minimal peripheral noise). In the data summaries below, we include all detections heard at the three sites sampled for three years (3 study sites, 4 block replicates). With limited replications thus far, it is difficult to draw conclusions but we expect to see more clear trends as more data are gathered. We did not detect any American woodcock on surveys this year again, but did observe them at study sites.

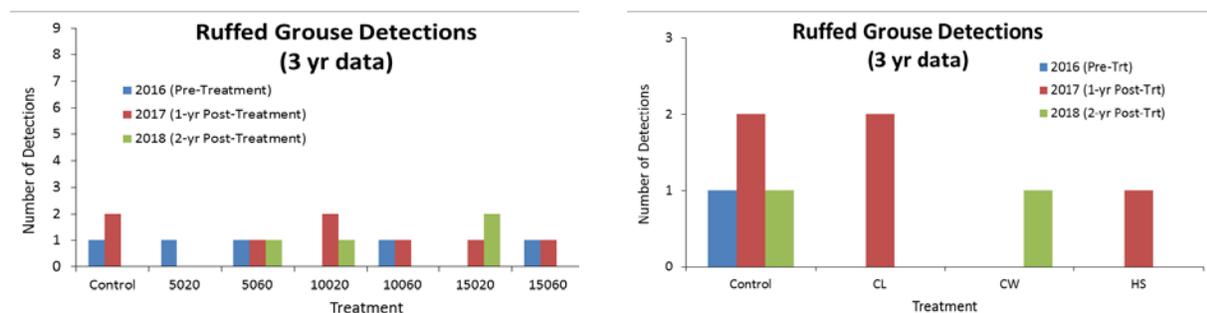


Figure 1. Number of detected ruffed grouse in cut-back borders (left) and regeneration stands (right) using sites that have had three years of data collected. Absence of a bar in a given year indicates no detections.

We assessed songbird responses to cut-back border and regeneration stands using a community approach which grouped species into one of four songbird guilds (edge, interior-edge, forest interior, and synanthropic). Initial summaries below are only on the sites sampled for

three years. For analysis, we used cumulative number of species detected within each guild at each point over both surveys during each year. We used a two-way analysis of variance to test for differences among treatments, years, and treatment x year interaction effects in species richness of each guild. Songbird results for regenerations stands is not included in this report due to limited sample size at this point in the study. Significance is reported at $\alpha = 0.05$ level.

Species richness of the edge guild in cut-back borders did not show any significant responses (treatment $p = 0.21$, year $p = 0.15$, treatment x year $p = 0.92$). Interior-edge guild richness did not have a significant response in cut-back borders to treatment, year, or treatment-year interaction ($p = 0.23$, $p = 0.16$, $p = 0.39$, respectively). Species richness of the forest interior guild in cut-back borders showed a significant response to year ($F_{2,12} = 8.5$, $p = 0.01$) but not treatment or a treatment-year interaction (Figure 2). Species richness of the synanthropic guild in cut-back borders showed a significant year response ($F_{2,12} = 2.58$, $p = 0.02$).

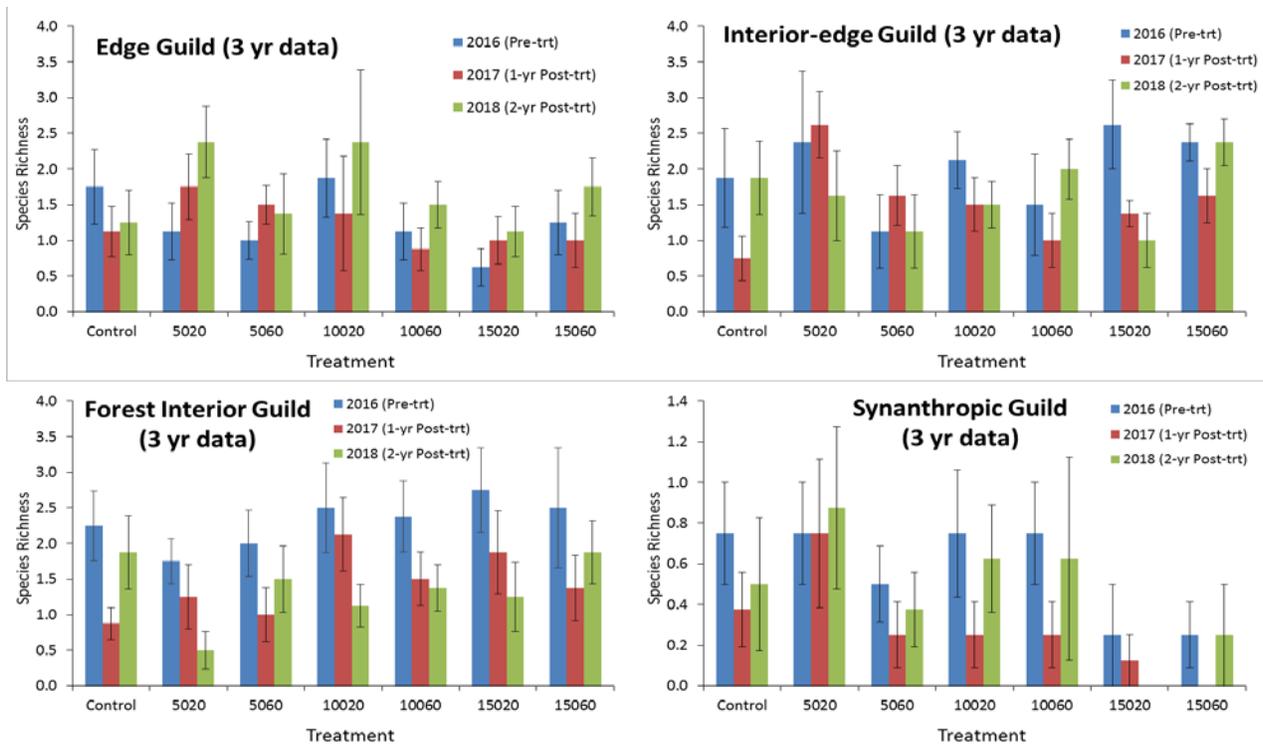


Figure 2. Species richness (mean and standard error) of songbird guilds in cut-back borders over time from sites that have three years of data collected (Bunsville Lake WMA, Little Canaan WMA, and Stonewall Jackson WMA; 4 block replicates total).

Salamanders were captured under coverboards and with area searches under natural cover within a 5-m radius around coverboards. In 2017, we captured 148 individuals (not including recaptures) of five different species (eastern red-backed salamander [*Plethodon cinereus*], slimy salamander [*Plethodon glutinosus*], mountain dusky salamander [*Desmognathus ochrophaeus*], northern red salamander [*Pseudotriton ruber*], and Wehrle's salamander [*Plethodon wehrlei*]). In 2018, we captured 170 individuals (not including recaptures) of the same species excluding northern red salamander (Figure 3).

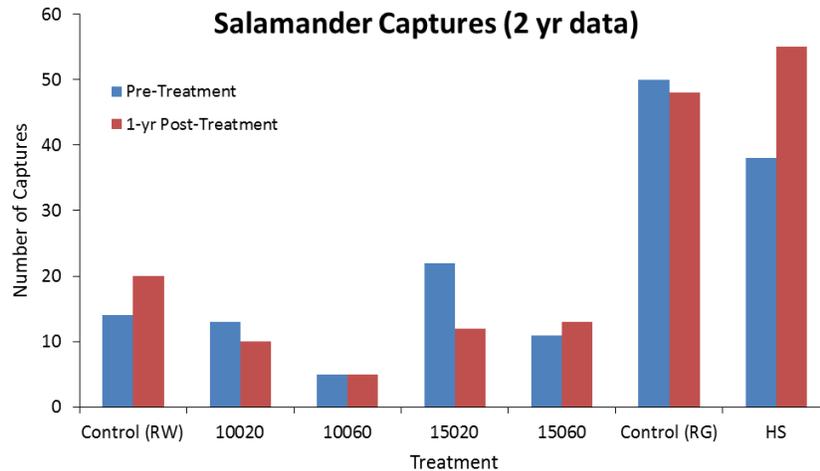


Figure 3. Number of salamanders captured at sites with pre- and post-treatment data (Beury Mountain WMA, Lewis Wetzel WMA). Cut-back border treatments (first 6 bars) and regeneration stands (Control (RG) and HS) are both included in graphic.

CERULEAN WARBLER AND ASSOCIATED SPECIES RESPONSE TO SILVICULTURAL PRESCRIPTIONS 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

Expected Completion: December 2018

Funding Sources: U.S. Geological Survey (WV RWO 62), WV Department of Natural Resources, Pennsylvania Game Commission, 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 6 focal species to silvicultural harvest mosaics and assess using the cerulean warbler as an umbrella species for songbird management across a spectrum of conditions.

Progress:

The cerulean warbler (*Setophaga cerulea*; hereafter, cerulean), a late successional songbird with core breeding range in the hardwood forests of the Appalachian Mountains, uses heavily forested landscapes with heterogeneous vegetation structure. It is a species of management concern because of its ~3% annual population decline and projected population half-life of fewer than 30 years. Understanding habitat selection on multiple scales and managing for cerulean warbler habitat preferences is critical in reversing these trends and conserving the species. Our study is a region-wide, cooperative project with 15 study sites encompassing ~734 ha in Kentucky, Pennsylvania, Virginia, and West Virginia. Fieldwork was conducted within the host states by local teams and data management and analysis is an ongoing cooperative effort.



Our study expands on the Cerulean Warbler Management Guidelines for Enhancing Breeding Habitat in Appalachian Hardwood Forests by studying operational silviculture under a broad set of conditions and harvest types. Each state conducted timber harvests that incorporated the recommendations of the guidelines. We hope to explain how ceruleans select territories within harvest mosaics that offer structural diversity to the birds and how other songbird species may be managed under the umbrella of cerulean breeding habitat management.

We quantified cerulean warbler response to a range of forest management treatments in the four states during the 2013-2016 breeding seasons. We used 10-min point counts to evaluate changes in cerulean warbler abundance pre- and post-harvest, and spot mapping techniques (only in West Virginia) to evaluate changes in territory density pre- and post-harvest. We analyzed point count data using single-species hierarchical (N -mixture) models to estimate abundance while accounting for detection biases inherent in point count surveys. We tested models with topographic (e.g., slope position, aspect) or vegetation (e.g., mean basal area) variables deemed important to cerulean abundance.

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Cerulean results presented here summarize data from Kentucky, Virginia, and West Virginia where we have pre- and post-harvest data for comparison; ceruleans were not detected at the pre-post sites in Pennsylvania. Territory density results for ceruleans and five focal species come from all post-harvest sites in West Virginia. Focal species include: eastern towhee (*Pipilo erythrophthalmus*), indigo bunting (*Passerina cyanea*), hooded warbler (*S. citrina*), wood thrush (*Hylocichla mustelina*), and worm-eating warbler (*Helmitheros vermivorum*).

Treatment type (harvest interior, harvest edge, or reference), availability of preferred tree species (i.e., white oak species [*Quercus* spp.], sugar maple [*Acer saccharum*], hickories [*Carya* spp.]), and availability of large trees (≥ 40.6 cm dbh) were most influential on post-harvest cerulean warbler abundance. Population growth was predicted to occur when ~50% of residual basal area was preferred tree species or sawtimber. The models testing slope position ($\Delta AICc = 2.59$) and aspect ($\Delta AICc = 2.69$) on population growth rate had some support for the pre-post

data, but represented 18% and 17% of model weight, respectively, suggesting that population growth rate in response to harvest was less influenced by slope position and aspect than treatment alone. Thus, timber harvests in the central Appalachian region do not have to occur on preferred topographic conditions (e.g., ridges and northeastern aspects) to benefit ceruleans.

Cerulean warbler territory density increased from pre-harvest to 2 years post-harvest and then declined (Fig 1) likely because few sites had 3-years post-harvest data. Increase in territory density was greater in stands with low pre-harvest densities of ceruleans, whereas populations within stands with higher densities pre-harvest did not increase in territory density. Territory densities of eastern towhees, hooded warblers, and indigo buntings increased across time-post-harvest (Fig 1). Eastern towhee and indigo bunting response to harvests was steady over time, whereas hooded warblers showed a greater positive response 2-years post-harvest. These species took advantage of the thick *Rubus* and sapling regeneration following at least one full growing season. Wood thrush territory density initially decreased but stabilized. Worm-eating warblers were never common in the spot mapping plots, but defended territories throughout the study.

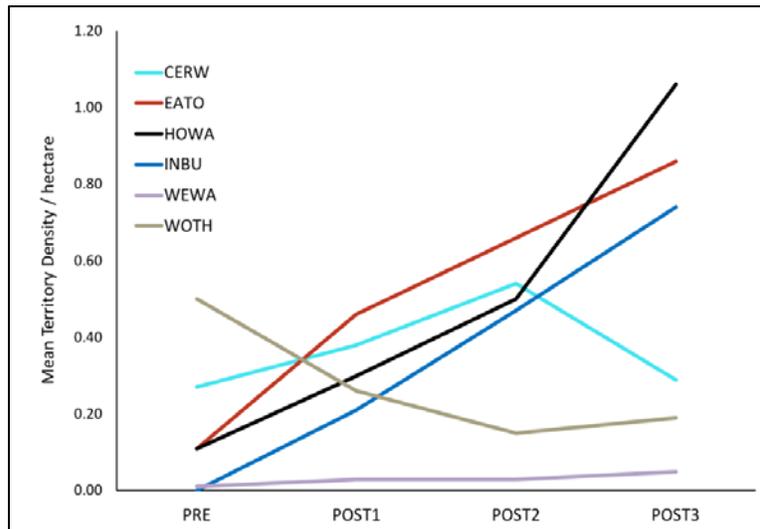
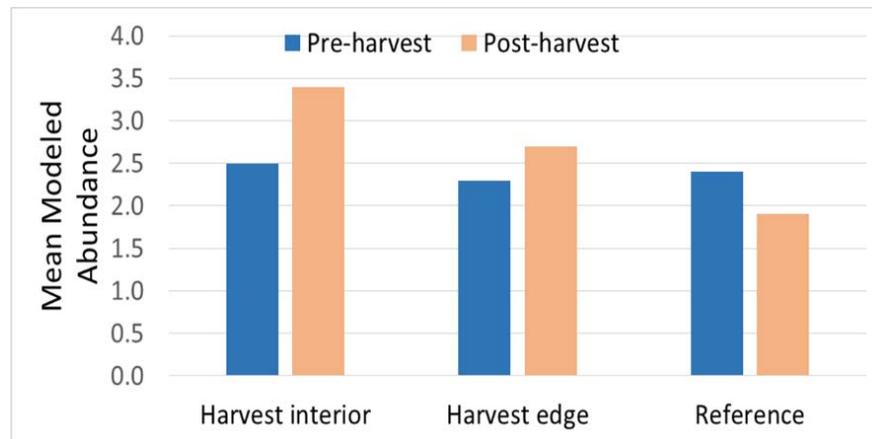


Figure 1. Mean territory density of six focal species pre-harvest and 1-, 2-, and 3-years post-harvest in West Virginia.

Cerulean warbler abundance remained similar at all treatment types post-harvest (Fig. 2), but occupancy at harvest interior points increased. Post-harvest, we detected ceruleans at 75%, 42%, and 36% of harvest interior, harvest edge, and reference points, respectively. We detected a change in occurrence at points post-harvest relative to treatment. Ceruleans were detected at 30%, 13%, and 10% of harvest interior, harvest edge, and harvest reference points where they had not been detected pre-harvest (Fig. 2).

Figure 2. Pre- and post-harvest Cerulean Warbler abundance by treatment (harvest interior, harvest edge, reference) at 93 sample points at five harvested study areas in Kentucky, Virginia, and West Virginia 2013-2016. Bars represent 95% confidence intervals. The hierarchical model used to estimate abundance included study area as a covariate for initial abundance, treatment as a covariate for population growth rate, and observer, ordinal date, noise, and time-since-sunrise for detection probability.



Final analyses will be completed by November 2018, with project completion expected in December 2018. We plan to determine which topographic and vegetation variables are most important to cerulean warbler territory selection by comparing data from territories to data from territory mapping plots where territories were not detected. We will update the breeding habitat management guidelines as needed. We will conduct the same analyses on abundance and territory density on the focal species listed above plus abundance analyses on scarlet tanager (*Piranga olivacea*).

PRE- AND POST-TREATMENT MONITORING ON WORKING LANDS FOR WILDLIFE AND REGIONAL CONSERVATION PARTNERSHIP PROGRAM SITES IN WEST VIRGINIA

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 Working Lands for Wildlife management on Golden-winged Warbler (*Vermivora chrysoptera*) occupancy and abundance in West Virginia
2. Quantify the effects of Appalachian Forestland Enhancement Project management on Cerulean Warbler (*Setophaga cerulea*) occupancy and abundance in West Virginia
3. Determine the longevity of effects of management for both species and provide guidelines for adaptive management on private lands

4. Compare management through NRCS programs to a commercial forestry operation
5. Evaluate management effects on the overall avian community

Progress:

Golden-winged Warbler (*Vermivora chrysoptera*; GWWA) and Cerulean Warbler (*Setophaga cerulea*; CERW) 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 although efforts are underway to implement targeted species-specific management on private lands. 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>) and CERW is the focal species of the Appalachian Forestland Enhancement Project (FEP) under the Regional Conservation Partnership Program (RCPP) (<http://www.nrcs.usda.gov/wps/portal/nrcs/detail/wv/newsroom/releases/?cid=STELPRDB1268019>). An extensive body of literature and recent research findings are informing on-the-ground management for these species. However, there is a need to monitor the outcome of these practices to document their effectiveness in increasing GWWA and CERW populations.

West Virginia is implementing both conservation programs which provides an opportunity to monitor response of GWWA and CERW populations. The state is at the core of both species' geographic range, and both species are listed as species of conservation concern by the West Virginia Division of Natural Resources (WVDNR) State Wildlife Action Plan (SWAP). We will monitor the effects of WLFW management on GWWA and RCPP management on CERW on sites throughout West Virginia to evaluate the



effectiveness of each program at creating, improving, or maintaining GWWA and CERW habitat, as well as the longevity of management for both species. We will also compare management implemented through these programs to responses on commercial logging areas on industrial private forests. Hypotheses include: GWWA and CERW occupancy and abundance will increase after WLFW and RCPP management. GWWA and CERW occupancy and abundance will remain relatively high, and stable, compared to surrounding areas at least 3 years after management is implemented. Management for GWWA and CERW will result in

predictable changes in the avian assemblage that should improve overall avian biodiversity. Habitat created for GWWA in this region will promote a diverse community of shrubland birds.

We will partner with NRCS and WVDNR biologists to identify enrolled private lands for both WLFW and RCPP and to obtain landowner permission for access. We will stratify sites into 4 categories; pre-treatment, immediate post-treatment, post treatment, and commercial harvest. Pre-treatment sites will be areas enrolled in the programs which have not had any management implemented. Immediate post-treatment sites will be areas where management occurred within the past 2 years. Post treatment sites will be areas where management occurred within the past 3-8 years. Commercial harvest areas will occur in Nicholas, Fayette, and Greenbrier counties. We will use multi-species unlimited-radius 10-minute avian point counts conducted during the peak breeding season (May 15 – June 30) at all sites. Counts will be stratified by time and distance bands. To estimate GWWA and CERW occupancy and abundance in Program R using package *unmarked*, we will visit points 3 times per year for two years (2019 and 2020). At each point surveyed, we will collect vegetation variables identified as important for each species in previous research. We will also evaluate management impacts on the overall avian community using multi-species occupancy models in program MARK.



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–present

Degree Program: PhD

Expected Completion: December 2018

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

Objectives:

1. Determine how shale gas well development activities influence Louisiana Waterthrush (*Parkesia motacilla*) territory density and length, nest survival and productivity, riparian habitat quality, apparent survival, site fidelity, and site fidelity factors.
2. Relate Waterthrush demographic response and nest survival to possible changes in its benthic aquatic prey due to shale gas development.
3. Using spatial stream network modeling (SSNM), determine a relationship between observation areas of waterthrush foraging and 1) water chemistry, 2) a waterthrush foraging score based on stream channel data, 3) shale gas land use based on reach contribution, and 4) multi-metric indices of biotic stream integrity at the family and genus level.
4. Determine how shale gas development influences Louisiana Waterthrush epigenetic (DNA methylation) variation between areas of disturbed and undisturbed streams, and to determine how DNA methylation varies among individuals.

Progress:

Shale gas development continues to outpace implementation of best management practices for wildlife affected by this development. We examined how shale gas development influenced demographic response of Louisiana Waterthrush (*Parkesia motacilla*) during 2009–2011 and 2013–2015 on 58.1 km of 14 predominately forested headwater streams. Across years, we saw annual variability in nest survival but with a general declining trend over time (Table 1). Riparian habitat quality scores, as measured by an EPA index and a waterthrush Habitat Suitability Index (HSI), were negatively related to the amount of each territory affected by shale gas development and generally declined over time. Territory density was not related to the amount of shale gas disturbance but decreased over time as territory lengths increased. Population-level nest productivity declined across years and individual level productivity was lower in areas disturbed by shale gas. Overall, our results suggest a decline in waterthrush site quality as shale gas development increased, despite a relatively small site-wide forest loss (~4%).

Table 1. Louisiana Waterthrush demography across six years in response to shale gas development at Lewis Wetzel Wildlife Management Area, WV. Population productivity is mean number of fledglings/successful nest/year multiplied by annual nest survival and average annual territory density/km of stream. A larger EPA or HSI score indicates better riparian habitat quality.

Year	Territory Density (territories /km)	Nest Survival (DSR ²⁹)	# of Nests	% Nests Parasitized	Population Productivity (per km) ± 95% CIs	EPA Index (range 0–200)	HSI (range 0–1)
2009	1.5 ± 0.1	34.3 ± 8.9	41	0.0	2.32 ± 0.59	171.8 ± 2.0	0.82 ± 0.02
2010	1.8 ± 0.1	59.8 ± 9.3	39	2.6	4.63 ± 0.33	154.8 ± 1.9	0.79 ± 0.02
2011	1.5 ± 0.1	38.0 ± 8.0	47	0.0	2.56 ± 0.25	158.6 ± 1.8	0.78 ± 0.02
2013	1.2 ± 0.1	28.5 ± 6.1	65	4.6	1.61 ± 0.25	148.9 ± 2.1	0.76 ± 0.02
2014	1.1 ± 0.1	25.7 ± 5.8	54	5.6	1.16 ± 0.35	165.6 ± 2.2	0.77 ± 0.02
2015	1.0 ± 0.1	31.9 ± 8.4	34	3.0	1.31 ± 0.42	*	*

Waterthrush initially had high site fidelity that declined over time. At the same time, the number of unpaired males defending territories increased as did natal fidelity. Females were less likely to return with increased number of breeding attempts, and females in shale gas disturbed areas had a higher number of breeding attempts and lower individual productivity. We saw a general but non-significant decline in annual male apparent survival. Apparent survival estimates for adult males (0.56) and females (0.44) were similar to those reported for other populations. Our study adds to previous evidence from our demographic study that shale gas disturbed areas may serve as sink habitats.



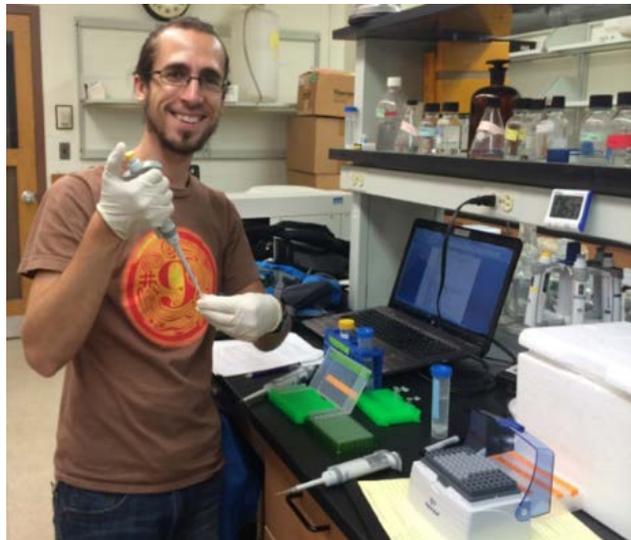
We collected 314 macroinvertebrate samples in 2013 and 2014 at foraging and nesting locations to compare shale gas disturbed and undisturbed areas. We additionally collected wetted perimeter stream channel and water chemistry data along a 50m fixed point stream grid. We related Louisiana Waterthrush (*Parkesia motacilla*) 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 general, most demographic responses to aquatic prey were variable and negatively related to aquatic prey in 2013 but positively related in 2014. Competing aquatic prey covariate models to explain nest

survival were not statistically significant but differed annually and in general reversed from negative to positive influence on daily survival rate. Collective evidence suggests there may be a shale gas disturbance threshold at which waterthrush respond negatively to aquatic prey community changes.

We made 948 observations of foraging waterthrush. Each stream had an average of 30.6 ± 7.2 foraging and 37.1 ± 6.2 non-foraging observations. Average foraging probability index (FPI) on each stream was 47.9 ± 4.5 % (range 20.3–80.4 %). The FPI index was greater in areas where family and genus-level multi-metric indices of biotic stream integrity were higher (i.e. WVSCI and GLIMPSS). SSNMs outperformed traditional regression models, and demonstrated the utility of SSNMs to be applied to make predictions on unsampled areas of streams for management prioritization.

During mist-netting, we collected 379 feather and 384 blood samples over three seasons that are being used for analysis of contaminants and epigenetic (DNA methylation) variation (Figure 1). Over 100 DNA fragment restriction sites across 320 individuals are currently being analyzed for differential methylation based on 40 replicates (12.5% of sample total). Barium and Strontium are two heavy metals associated with the fracking process, and heavy metals are known to interfere with methyl transfer. Once the epigenetic analysis is complete, it is hypothesized that individuals with higher Barium and Strontium contaminant loads would have a lower number of methylated DNA fragment restriction sites.

Figure 1. Preparing waterthrush blood samples in the lab for methylation-sensitive amplified fragment length polymorphism (MS-AFLP) analysis.



LONG-TERM SONGBIRD POPULATION RESPONSE TO SHALE GAS DEVELOPMENT

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

Principal Investigator: Petra B. Wood

Years Ongoing: 2008-present

Expected completion: May 2020

Funding Sources: West Virginia DNR, Department of Energy, US Fish and Wildlife Service

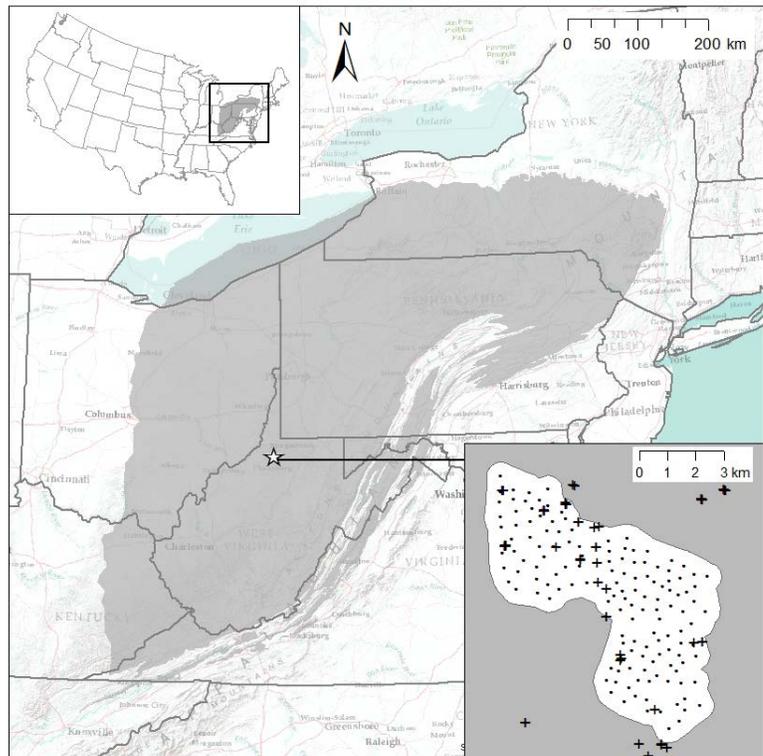
Objectives:

1. Quantify the 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 due to shale gas development.

Progress:

Over the last decade, unconventional drilling for natural gas from the Marcellus-Utica shale has increased exponentially in the central Appalachian region, a key conservation area for forest songbirds. The Lewis Wetzel Wildlife Management Area (LWWMA) in north-central WV (Fig. 1) has seen a substantial expansion of shale gas development beginning in 2008. We have monitored breeding bird abundances and land cover change annually at this site, since 2008.

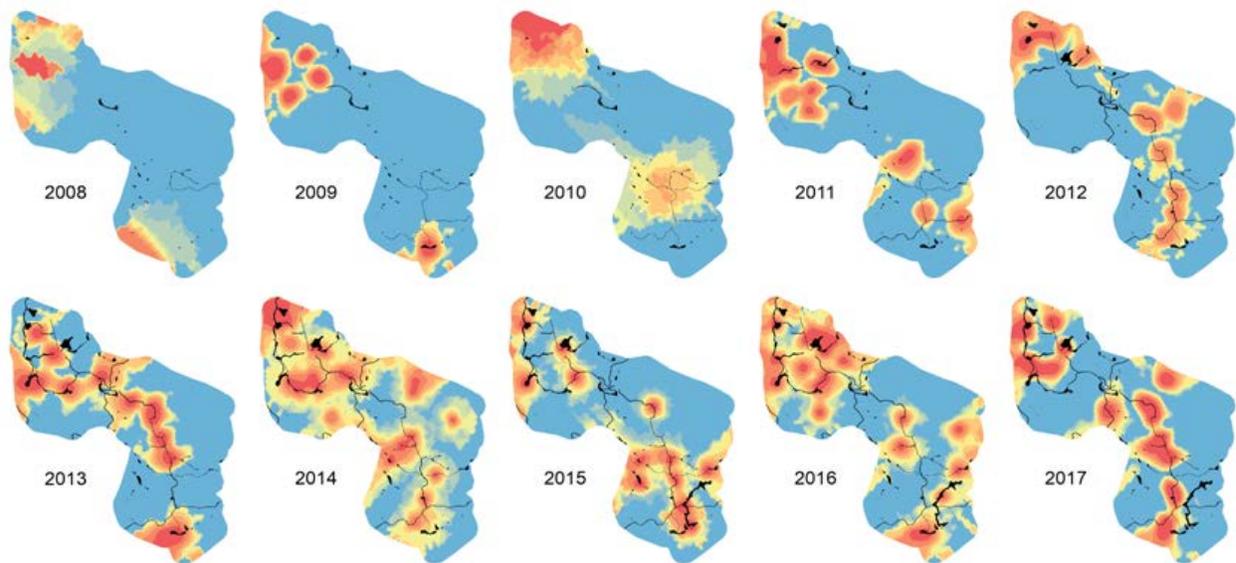
Figure 1. Star shows location of study site relative to the Marcellus shale formation, shaded in gray. Lower inset: black dots represent 142 point count survey locations within the focal study area (white area); black crosses show locations of completed unconventional shale gas wells within the study site and surrounding area.



Our previously published paper from this project documented the relationship between increasing shale gas development and avian species abundances and richness within three habitat guilds, from 2008-2015 (Farwell et al. 2016). Forest-interior guild species richness decreased over time across all points and at points impacted by shale gas within 100 m, but did not change at points unimpacted by shale gas development. Early-successional guild species richness increased over time across all points, and at points impacted by shale gas within 100 m, but did not show a significant change at points unimpacted by shale gas. Synanthropic guild species

richness also showed a significant site-wide increase over time across all points and at points impacted by shale gas, but did not show a significant change at points unimpacted by shale gas. Although we found variability in focal species responses, we observed similar trends among focal species within each of the three habitat guilds. These results suggest that shale gas development is fragmenting regional forests and altering avian communities, and that efforts to minimize new development in core forests will reduce negative impacts to forest interior species.

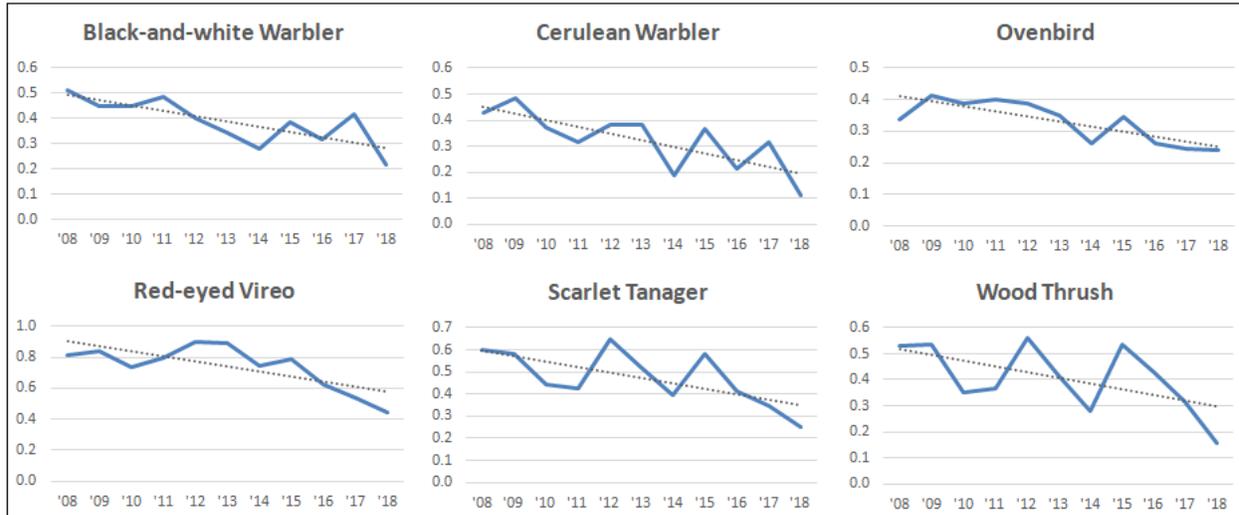
In our more recent study of changing distributions of focal species in response to shale gas development at LWWMA from 2008-2017, we found that more than half of the 27 focal species evaluated showed sensitivity to distance from shale gas infrastructure. Five forest interior species occurred in higher abundances with increasing distance from shale gas (Black-throated green warbler, hooded warbler, ovenbird, red-eyed vireo, and wood thrush), 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. Interpolated distribution maps (e.g., Fig. 2) help visualize spatial patterns of response for focal species to shale gas development, over time.



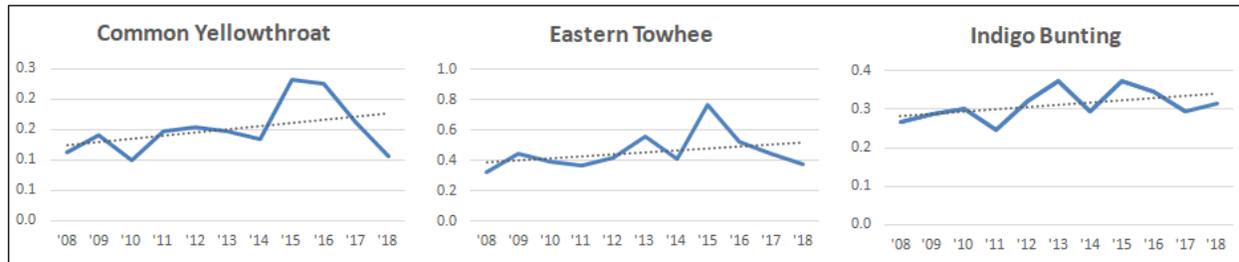
*Figure 2: Annual interpolated distribution probability maps for brown-headed cowbirds (*Molothrus ater*), based on indicator kriging. GLMMs showed brown-headed cowbirds occurred in higher abundances with increasing proximity to both shale gas well pads ($P < 0.001$) and shale gas linear infrastructure ($P < 0.001$). Darker red areas represent locations with higher indices of abundance. Unconventional shale gas infrastructure shown in black, for visual reference.*

After a lull in unconventional shale gas activity during 2014-17, clearing for pipeline and well pad development increased after the 2017 breeding season at LWWMA. Even with the lull in activity, overall declining trends in forest interior species abundance continued, particularly during 2018 (Fig. 3). Additionally, increasing trends in early successional and synanthropic species abundance have tapered off in recent years. Substantial year-to-year variation underscores the importance of long term data sets, such as this.

A.



B.



C.

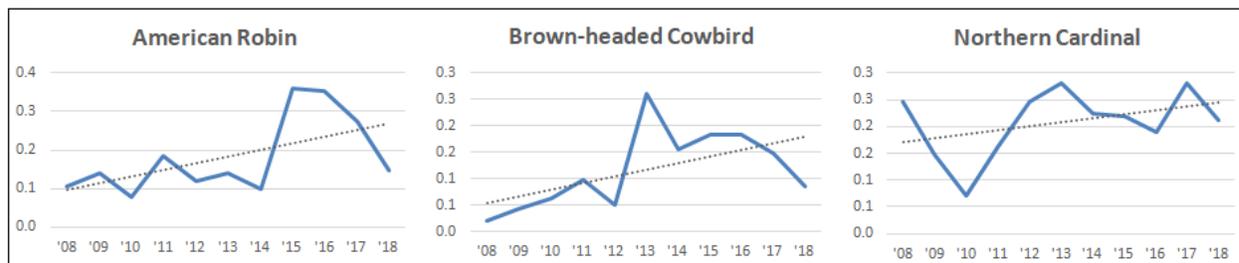


Figure 3. Line graphs of raw site-wide average annual abundance of focal species abundances from 2008-18 (50-m radius counts, max detections from 2 surveys per point, per year) for three habitat guilds: (a) forest interior, (b) early successional, (c) synanthropic.

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. Breeding bird and land cover monitoring efforts will continue on LWWMA through the 2019 breeding season and future analyses will include at least 12 years of data (2008–2019).

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

SCIENTIFIC PUBLICATIONS

- Aldinger, K.R., P.B. Wood, C.M. Johnson. 2017. Refined conservation strategies for Golden-winged Warblers in the West Virginia highlands with implications for the broader avian community. *Condor* 119:762-786. <https://doi.org/10.1650/CONDOR-17-49.1>
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- Blazer, V.S., H.L. Walsh, C.H. Shaw, L.R. Iwanowicz, R.P. Braham and P.M. Mazik. In Press. Indicators of exposure to estrogenic compounds at Great Lakes Areas of Concern: Species and site comparisons. *Environmental Monitoring and Assessment*
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- Frantz MW, Wood PB, Sheehan J, George G. 2018. Demographic response of a migratory songbird of conservation concern to shale gas development. *The Condor: Ornithological Applications*. 120:265–282.
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- Walsh, H.L., V.S. Blazer, G.D. Smith, M. Lookenbill, D.A. Alvarez, and K.L. Smalling. 2018. Risk factors associated with mortality of age-0 smallmouth bass in the Susquehanna River Basin, Pennsylvania, USA. *Journal of Aquatic Animal Health* 30: 65-80.

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- Aldinger, K.R. August 2018. Ecology and Management of Golden-winged Warblers (*Vermivora chrysoptera*) and associated avian species in the Allegheny Mountains of West Virginia. PhD Dissertation, West Virginia University, Morgantown.
- Braham, R.P. May 2018. An examination of cyanotoxins and environmental estrogens in the middle and upper Potomac River watershed, USA. PhD Dissertation, West Virginia University, Morgantown

Farwell, L.S. August 2018. Songbird response to forest disturbance due to unconventional shale gas development in the Marcellus-Utica region: a multi-scale analysis. PhD Dissertation, West Virginia University, Morgantown.

Young, K.T. May 2018. Assessment of Melanistic Lesions in Smallmouth Bass (*Micropterus dolomieu*) of the Chesapeake Bay, USA. MS Thesis, West Virginia University, Morgantown.

PRESENTATIONS

Brown, D.J., P.B. Wood, Y.L. Park, S. Crayton, L. Paul, A. Millikin. July 2018. Assessment of Potential Indirect Impacts of Using Pesticides for Hemlock Preservation on Terrestrial and Semiaquatic Salamanders. Joint Meeting of Ichthyologists and Herpetologists

Crayton, S, P.B. Wood, D. Brown, Y.L. Park. Feb 2018. Effects of Imidacloprid Treatment on Aquatic Systems. Southeastern Partners for Amphibian and Reptile Conservation, Helen, Georgia, 23 February 2018. poster

Crayton, S, P.B. Wood, D. Brown, Y.L. Park. Feb 2018. Effects of Imidacloprid Treatment on Aquatic Systems. Institute of Water Security and Science, 20 February 2018. poster

Farwell, L.S., Wood, P.B., Dettmers, R., Brittingham, M.C. March 2018. Region-wide assessment of Marcellus-Utica shale gas development impacts on forest songbirds. West Virginia Chapter of The Wildlife Society Annual Meeting, Morgantown, WV.

Frantz, M. W. April 2018. Response of Louisiana Waterthrush (*Parkesia motacilla*) and their Benthic Prey to Shale Gas Development. Washington and Jefferson College, Washington, PA. **Invited.**

Loman, Z., W.V. Deluca, D. Harrison, C. S. Loftin, B. W. Rolek and P. B. Wood. April 2018. Latent indicators and Bayesian model selection in identifying forest bird habitat modeling error. American Ornithological Society Annual Conference. Tucson, AZ, April 9-14.

Margenau, E.L. and P.B. Wood. 2018. Preliminary assessment of bird community responses to young forest management. West Virginia Chapter of The Wildlife Society Annual Conference.

Margenau, E.L. and P.B. Wood. 2018. Preliminary assessment of the bird community to young forest management. West Virginia Division of Natural Resources Joint Wildlife Resources Biologist Meeting. Elkins, WV. **Invited.**

Rolek, B.W., D. Harrison, C.S. Loftin, P.B. Wood. 23-27 September 2017. Regenerating clearcuts and postharvest treatments increase spruce-fir avian assemblages and richness in managed stands. The Wildlife Society 24th Annual Conference, Albuquerque, NM, USA.

Shaw, C.M., V.S. Blazer, L.R. Iwanowicz, H.L. Walsh and P.M. Mazik. In Press. Assessing the health of resident largemouth bass (*Micropterus salmoides*) and brown bullhead (*Ameiurus nebulosus*) from the Rochester Embayment of Lake Ontario, NY. J. Great Lakes Research

Walsh, H.L., V.S. Blazer, G.D. Smith, M. Lookenbill, D.A. Alvarez, and K.L. Smalling. 2018. Risk factors associated with mortality of age-0 smallmouth bass in the Susquehanna River Basin, Pennsylvania, USA. Journal of Aquatic Animal Health 30: 65-80.

Welsh, S.A. and Z.J. Loughman. A nonnative crayfish (*Faxonius virilis*) use of and eel ladder, Potomac River Drainage, USA. 22nd Meeting of the International Association of Astacology, Pittsburgh, PA, 10 July 2018

Wood, P.B., L. Farwell, and M. Frantz. Mar 2018. Avian response to shale gas development in central Appalachian forests. Davis and Elkins College, Elkins, WV. 29 Mar 2018. **Invited.**

Wood, P.B. and G. Nareff. Apr 2018. Avian response to implementation of cerulean warbler habitat management guidelines. American Ornithological Society Annual Conference. 11 Apr 2018.

Wood, P.B. and G. Nareff. Aug 2018. Avian response to implementation of cerulean warbler habitat management guidelines. Appalachian Mountains Joint Venture Technical Committee Meeting, Logan, WV. 8 Aug 2018.

AWARDS

Eric Margenau was awarded:

Stitzel Graduate Student Support Award in March 2018 from the *Davis College of Agriculture, Natural Resources, and Design - West Virginia University*

Roger Latham Memorial Scholarship from the *Appalachian Vegetation Management Association*

Sara Crayton was awarded the Carl del Signore Foundation Graduate Scholarship from the Davis College in support of her MS research

Brian Rolek was awarded a Summer Dissertation Writing Fellowship, June through August 2018. University of Maine Graduate School.

Hannah Clipp was awarded a WVU Ruby Fellowship and a National Science Foundation Graduate Research Fellowship in support of her Ph.D. program.

The Coop Unit was awarded the 2017 Excellence In Management Award from the Cooperative Research Unit Headquarters in Reston VA.