West Virginia Cooperative Fish and Wildlife Research Unit

FY 2016

ANNUAL REPORT

1 October 2015 - 30 September 2016

COOPERATING AGENCIES:

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

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Cover Graphics: Photo by Laura Farwell
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Introduction and History: The West Virginia Cooperative Fish and Wildlife Research Unit

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

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

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

Graduate education is also an important mission of the Coop Unit. Cooperating with West Virginia University, the Coop Unit contributes to the quality education and training of graduate students in fisheries and wildlife at West Virginia University. Coop Unit scientists achieve educational goals by chairing graduate committees, serving on graduate committees, teaching graduate level courses and delivering guest lectures and seminars. As of September 30, 2016, 109 students have completed their degree requirements: 87 Masters and 22 Ph. D. The Unit scientists are currently supervising 6 Master's students and 15 Ph.D. students.

WEST VIRGINIA COOPERATIVE FISH AND WILDLIFE RESEARCH UNIT
U.S. GEOLOGICAL SURVEY
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UNIT STAFF

UNIT LEADER

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

ASSISTANT LEADERS

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

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

STAFF

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PROJECT COOPERATORS

UNIVERSITY

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Scott Warner, Division of Natural Resources

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Todd Fearer, Appalachian Mountains Joint Venture
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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

STUDENT	DEGREE	GRADUATION DATE	ADVISOR
Kyle Aldinger	Ph.D.	Expected Dec 2017	Petra Wood
Ryan Braham	Ph.D.	Expected Dec 2017	Pat Mazik
Camille Concepcion	Ph.D.	Expected May 2017	Todd Katzner/Petra Wood
Laura Farwell	Ph.D.	Expected My 2017	Petra Wood
Steve Foster (NCTC)	Ph.D.	Expected Dec 2017	Stuart Welsh
Mack Frantz	Ph. D.	Expected May 2017	Petra Wood
Cassidy Hahn	Ph.D.	Completed May 2016	Pat Mazik
Cory Hartman	M.S.	Expected Aug 2019	Pat Mazik
Carlos Martinez (NCTC)	M.S.	Expected Dec 2018	Pat Mazik
Eric Margenau	Ph. D.	Expected May 2020	Petra Wood
Gretchen Nareff	Ph. D.	Expected May 2017	Petra Wood
Mark Paulson	M.S.	Expected May 2017	Todd Katzner/Petra Wood
Austin Rizzo	Ph. D.	Expected Dec 2017	Stuart Welsh
Brian Rolek	Ph. D.	Expected May 2017	Petra Wood/Cyndy Loftin
Jim Sheehan	Ph.D.	Expected Dec 2016	Petra Wood
Dustin Smith	Ph.D.	Expected Dec 2016	Stuart Welsh
Daniel Sparks (NCTC)	Ph.D.	Expected Dec 2017	Pat Mazik
Jeff Thomas (NCTC)	Ph.D.	Expected Dec 2018	Stuart Welsh
Patricia Thompson	M.S.	Expected Dec 2016	Stuart Welsh
Thomas Waldrop (NCTC)	M.S.	Expected Dec 2017	Pat Mazik
Heather Walsh	Ph. D.	Expected Dec 2017	Pat Mazik
Kelsey Young	M.S.	Expected Dec 2019	Pat Mazik

UNIT STAFF COURSES TAUGHT

Patricia M. Mazik, Adjunct Professor of Fisheries
Fish Physiology Fall 2016 3 credits

Stuart A. Welsh, Adjunct Professor of Fisheries
Advanced Ichthyology Fall 2015 3 credits

Petra B. Wood, Adjunct Professor of Wildlife
Wildlife and Fisheries Graduate Seminar Spring 2016 1 credit 26 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

DEVELOPMENT AND APPLICATION OF QUANTITATIVE GENE EXPRESSION ASSAYS IN NON-MODEL FISH SPECIES

Student Investigator: Cassidy Hahn
Principal Investigators: Dr. Patricia Mazik
Collaborators: Dr. Vicki Blazer, Dr. Luke Iwanowicz - USGS Leetown Science Center
Years Ongoing: 2011 - 2016
Degree Program: PhD
Completed: May 2016
Funding Source: U. S. Fish and Wildlife Service (RWO 55, 61)

Objectives:
The primary objectives of this study were:

1.) The development of partial transcriptomes for three wild-caught, resident fish species

2.) The utilization of these sequence databases in the development of quantitative transcript abundance assays

3.) The application of these assays in environmental analysis.

Transcriptome Discovery in Non-Model Wild Fish Species for the Development of Quantitative Transcript Abundance Assays.

Background:

Environmental studies increasingly identify the presence of both contaminants of emerging concern (CECs) and legacy contaminants in aquatic environments; however, the biological effects of these compounds on resident fishes remain largely unknown. High throughput methodologies were employed to establish partial transcriptomes for three wild-caught, non-model fish species; smallmouth bass (*Micropterus dolomieu*), white sucker (*Catostomus commersonii*) and brown bullhead (*Ameiurus nebulosus*). Sequences from these transcriptome databases were utilized in the development of a custom nCounter CodeSet that allowed for direct multiplexed measurement of 50 transcript abundance endpoints in liver tissue. Sequence information was also utilized in the development of quantitative real-time PCR (qPCR) primers. Cross-species hybridization allowed the smallmouth bass nCounter CodeSet to be used for quantitative transcript abundance analysis of an additional non-model species, largemouth bass (*Micropterus salmoides*). We validated the nCounter analysis data system with qPCR for a subset of genes and confirmed concordant results. Changes in gene biomarkers between sexes and seasons were evaluated to provide baseline data on gene modulation for each species of interest.

Results:

Transcriptome sequencing

The initial databases for brown bullhead (BBH) and smallmouth bass (SMB) were created using wild caught individuals from multiple geographic areas and laboratory fish that had been exposed to 17β-estradiol in a previous study (Robertson et al., 2009). White sucker (WHS) transcriptome sequencing was performed using tissue composites from male and female wild fish collected from the Great Lakes region.

Wild fish sampling

We attempted to collect 20 mature (defined as greater than 250 mm in length) fish of each species at each sampling site and season. A comprehensive necropsy-based assessment was conducted on each fish collected. Small sections of liver were collected and preserved in RNA later® (ThermoFisher, Waltham, MA) for transcript abundance analysis.

Transcript abundance analysis

Transcriptome shotgun assembly sequence databases (SMB: SRX156704, SRX199239; LMB: SRX1436666, SRX1339139; BBH: SRX199312, SRX148685) were searched by Blastx to identify *a priori* genes of interest. Genes of interest included those suspected or known to be modulated in response to CEC exposure including endocrine disrupting compounds, heavy metals, dioxin-like molecules and other environmental stressors. In each species, 50 genes including, 5-7 housekeeping genes were chosen for analysis (Table 1). Collected liver samples were prepared and sent to the University of Pittsburgh Genomics and Proteomics Core Laboratory (Pittsburgh, PA) for mRNA expression analysis on the nCounter analysis system.

Sex-related differences in transcript abundance

A number of genes were identified as differentially expressed between males and females (Figure 1). The greatest number of differentially expressed genes between males and females was observed in the *Micropterus* species. In spring collections, five genes (VTG, estrogen receptor α (ERα), CHG, WAP65, MT) in SMB, five genes (VTG, CHG, transforming growth factor βR1 (TGFβR1), androgen receptor (AR), and GLK) in LMB and four genes (ERα, catalase (CAT), proliferating cell nuclear antigen (PCNA), and WAP65) in BBH were identified as differentially expressed. In fall collections, five genes (VTG, CHG, ERα, HEP2, and TGFβ) in SMB, two genes (CHG and VTG) in LMB and one gene (ERα) in BBH were identified as differentially expressed between the sexes. In WHS, there were no statistically significant differences in transcript abundance between the sexes with the exception of VTG expression, which was higher in females than males in both seasons.

Seasonal variation in transcript abundance

Differentially expressed genes between spring and fall varied between species (Figure 2). In males, six genes (HEP2, VTG, CHG, MT, WAP65, and ERα) in SMB, four genes (DOI1, CHG, GLK, and VTG) in LMB, seven genes (cytochrome c oxidase (COI), granulin B (GRNB), 17β hydroxysteroid dehydrogenase (17βHD), CAT, glutathione peroxidase 1 (GPX1), WAP65 and estrogen receptor β (ERβ) in BBH and three genes (11β hydroxysteroid dehydrogenase (11βHD), VTG and PCNA) in WHS were reported with differential expression between seasons. In females, one gene (HEP2) in SMB, five genes (arginase (ARG), DOI1, androgen receptor β (ARβ), WAP65 and VTG) in LMB, one gene (GPX4) in BBH and one gene (GPX) in WHS were reported with differential expression between seasons.

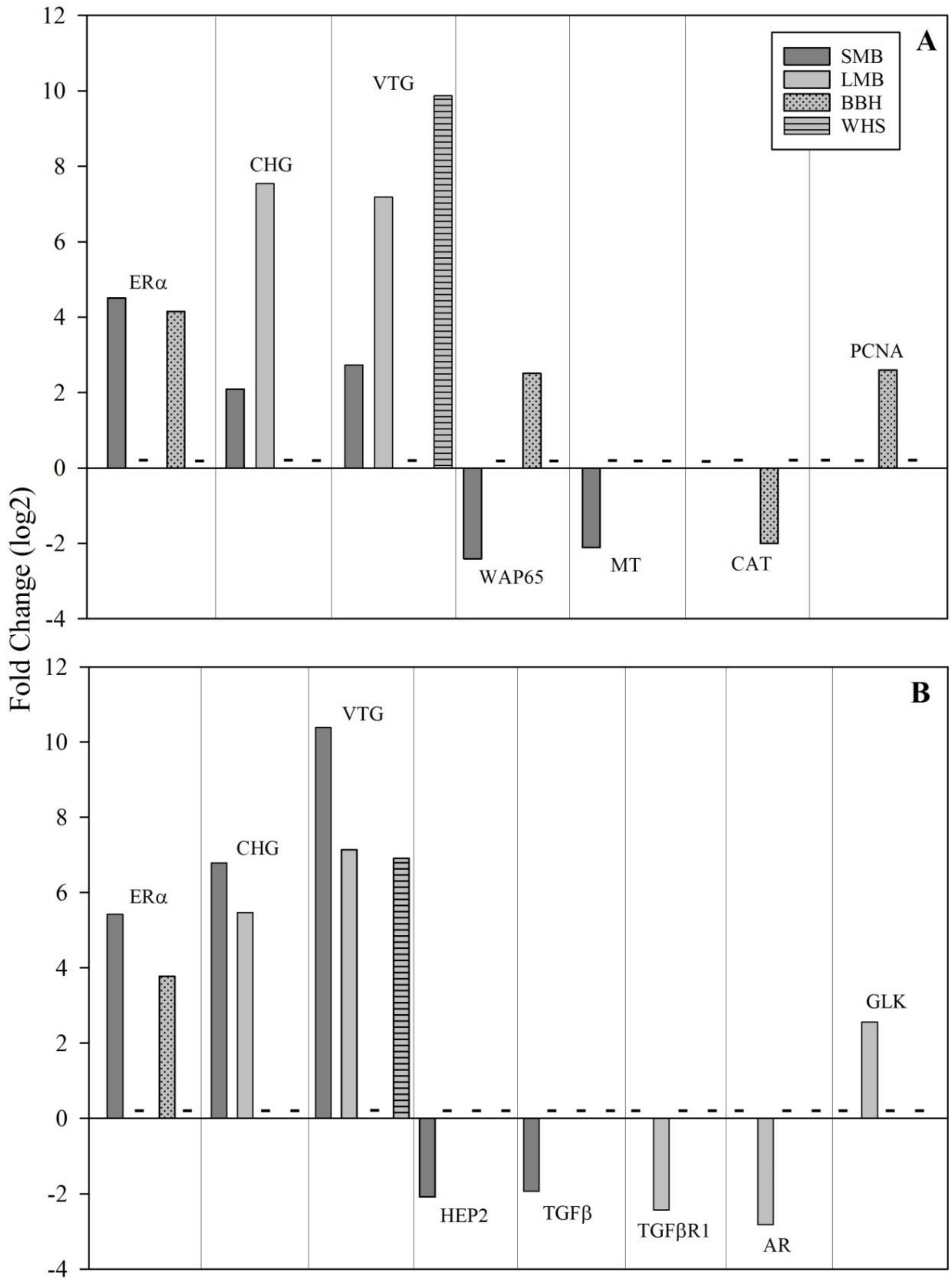

Figure 1. *Fold change (log2) of genes reported with differential abundance between males and females during (A) spring and (B) fall. Dashes indicated species in which no significant modulation of a gene was observed.*

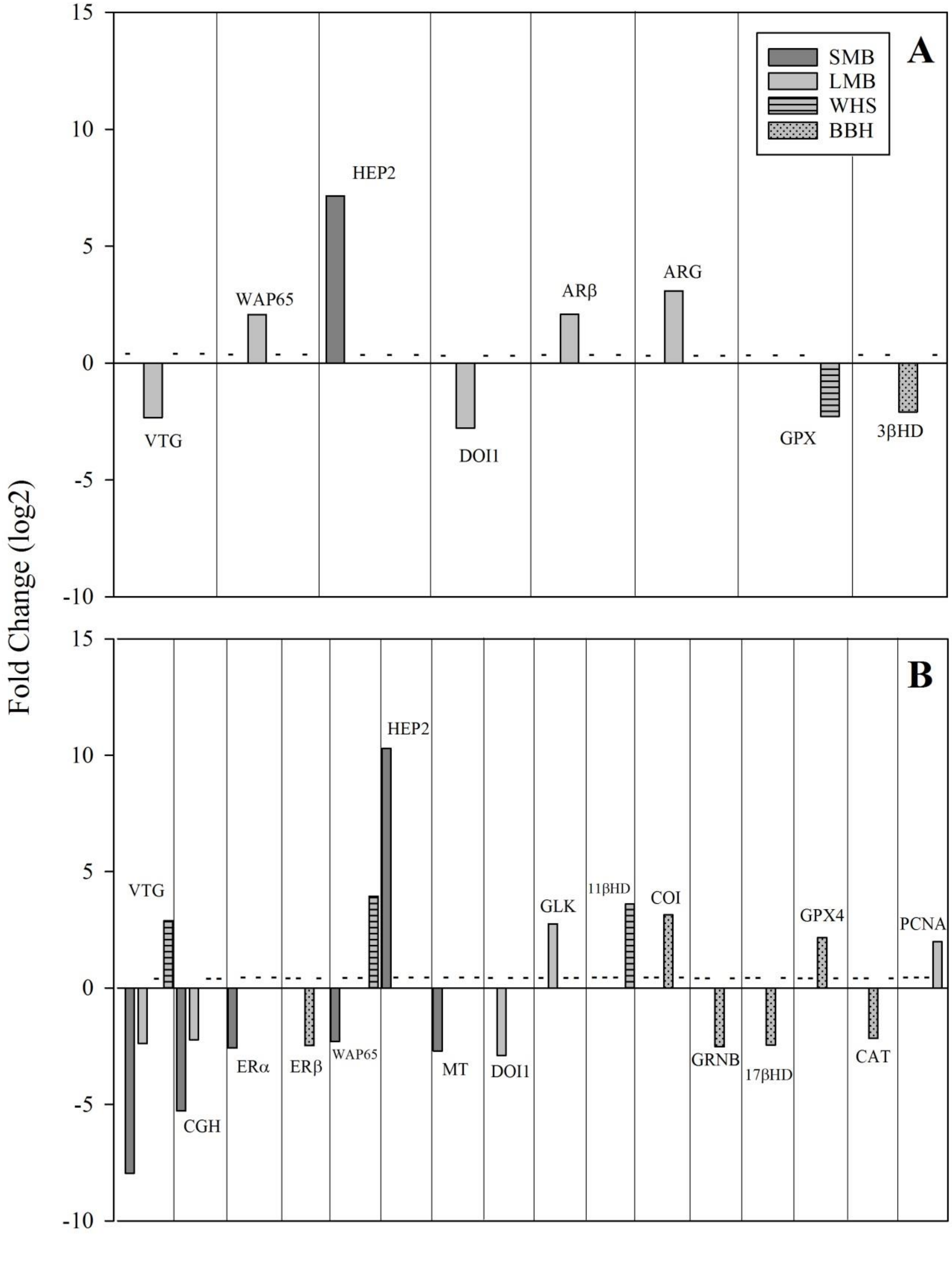

Figure 2. *Fold change (log2) of genes reported with differential abudance between each season in (A) females and (B) males. Dashes indicated species in which no significant modulation of a gene was observed.*

Table 1. Gene expression endpoints chosen for analysis with Nanostring nCounter Assay. * denotes housekeeping genes.

Micropterus species	***Catostomus commersonii***	***Ameiurus nebulosus***
03β Hydroxysteroid Dehydrogenase (3βHD)	11β Hydroxysteroid Dehyrdogenase (11BHD)	03β Hydroxysteroid Dehydrogenase (3βHD)
17β Hydroxysteroid Dehydrogenase (17βHD)	17β Hydroxysteroid Dehydrogenase (17βHD)	17β Hydroxysteroid Dehydrogenase (17βHD)
Actin β (βACT)	Androgen Receptor (AR)	Androgen Receptor (AR)
Androgen Receptor (AR)	Apolipoprotein A1 (APA1)	Apolipoprotein A1 (APA1)
Apolipoprotein A1 (APA1)	Aryl Hyrdrocarbon Receptor (AHR)	Arginase (ARG)
Arginase (ARG)	Catalase (CAT)	Aryl Hyrdrocarbon Receptor (AHR)
Aryl Hyrdrocarbon Receptor (AHR)	CTNNB1	Catalase (CAT)
Catalase (CAT)	CYP11C1	Catenin β (βCAT)
Catenin β (βCAT)	CYP1B2	CYP17
Choriogenin (CGR)	CYP3A	CYP19A1A (Aromatase)
CYP17	Elongation Factor 1α (EF1a)*	CYP1A
CYP19A1A (Aromatase)	Epidermal Growth Factor Receptor (EGFR)	CYP3A
CYP1A	Epoxide Hydrolase (EH)	Cytochrome C Oxidase (COX)
CYP3A	Estrogen Receptors α (ERα)	Elongation Factor 1α (EF1a)*
Cystenin-Rich Protein (CRP)	Estrogen Receptors β1 (ERβ1)	Epidermal Growth Factor Receptor (EGFR)
Elongation Factor 1α (EF1a)*	Eukaryotic Translation Initation Factor 3D (ETIF3D)*	Epoxide Hydrolase (EH)
Epidermal Growth Factor Receptor (EGFR)	Ferritin (FRT)	Estrogen Receptors α (ERα)
Epoxide Hydrolase 1 (EH1)	Fibroblast Growth Factor (FGF)	Estrogen Receptors β1 (ERβ)
Estrogen Receptors α (ERα)	Follicle Stimulating Hormone Receptor (FSHR)	Eukaryotic Translation Initation Factor 3D (ETIF3D)*
Estrogen Receptors β1 (ERβ1)	Glucocorticoid Receptor (GR)	Ferritin (FRT)
Estrogen Receptors β2 (ERβ2)	Glutathione Peroxidase 1 (GP)	Fibroblast Growth Factor (FGF)
Eukaryotic Translation Initation Factor 3D (ETIF3D)*	Glutathione S-Transferase (GST)	Follicle Stimulating Hormone Receptor (FSHR)
Ferritin (FRT)	Granulin (GRN)	Glutathione S-Transferase (GST)
Fibroblast Growth Factor (FGF)	Heat Shock Proteins 70 (HSP70)	Glucocorticoid Receptor (GR)
Follicle Stimulating Hormone Receptor (FSHR)	Heat Shock Proteins 90 (HSP90)	Glucokinase (GLK)
Glucocorticoid Receptor (GR)	Hepatitis B PreC Antigen	Glutathione Peroxidase (GP)
Glucokinase (GLK)	Hepcidin (HEP)	Granulin 1 (GRN1)
Glutathione Peroxidase 1 (GP)	Hypoxia-inducible factor (HIF)	Granulin Precursor b (GRNb)
Glutathione S-Transferase (GST)	Insulin-Like Growth Factor (IGF)	Heat Shock Proteins 70 (HSP70)
Heat Shock Proteins 70 (HSP70)	Keratin 8 (KRT8)	Heat Shock Proteins 90 (HSP90)
Heat Shock Proteins 71 (HSP71)	Metallothionein (MT)	Hepcidin (HEP)

Heat Shock Proteins 90α (HSP90α)	MUS81*	Hypoxanthine Phosphoribosyltransferase 1 (HPRT1)*
Hepcidins 1 (HEP1)	Peroxisome proliferator-activated receptor (PPAR)	Hypoxia-inducible factor (HIF)
Hepcidins 2 (HEP2)	Phosphoenolpyruvate Carboxykinase (PEPCK)*	Insulin-Like Growth Factor (IGF)
Hypoxanthine Phosphoribosyltransferase 1 (HPRT1)*	Proliferating cell nuclear antigen (PCNA)	Interferon (IFN)
Insulin-like Growth Factor 1 (IGF1)	RBMX*	Interleukin (IL)
Metallothionein (MT)	Ribosomal Protein L8 (RPL8)*	MYXO Parasite (MYXO)
Phosphoenolpyruvate Carboxykinase (PEPCK)*	Steroidogenic acute regulatory protein (STAR)	Peroxisome proliferator-activated receptor (PPAR)
RBMX2*		Proliferating cell nuclear antigen (PCNA)
Ribosomal Protein L8 (RPL8)*		RBMX*
Superoxide Dismutase (SOD)		Ribosomal Protein L8 (RPL8)*
Tata Box Binding Protein (TBP)*		Transforming Growth Factor β1 (TGFβ1)
		Thyroid Hormone Receptor α (THRα)
		Thyroid Hormone Receptor β (THRβ)
		Tumor Protein p73 (TP73)
		Tumor protein p53 (TP53)
		Superoxide Dismutase (SOD)
		v-K-ras
		Vitellogenin (VTG)
		Warm Temperature Acclimation Protein 65 (WAP65)

Brown bullhead (photo by Cassidy Hahn)

BIOLOGICAL MONITORING OF AQUATIC COMMUNITIES OF CHEAT LAKE AND CHEAT RIVER DOWNSTREAM OF THE LAKE LYNN HYDRO STATION

Student Investigator: Dustin M. Smith
Principal Investigator: Stuart A. Welsh
Cooperators: Frank Jernejcic and Dave Wellman
Years Ongoing: 2011 – 2015
Degree Program: PhD
Completion: December 2015
Funding Source: West Virginia Division of Natural Resources, FirstEnergy Corp.

Objectives:
A five-year biomonitoring project was initiated March 2011 for Cheat Lake and its tailwaters. The project (partitioned into nine tasks) was a continuation of previous work by West Virginia Division of Natural Resources. Three tasks of the proposed work focused on Cheat River and Cheat Lake tailwaters (Tasks 1 – 3), and 6 tasks focused on Cheat Lake (main lake and embayments). Work for all objectives was successfully completed and a completed project report was distributed to funding agencies. The nine tasks included in the study are listed below.

1. Fish biomonitoring downstream of Cheat Lake
2. Benthic macroinvertebrate resource biomonitoring downstream of Cheat Lake
3. Water quality biomonitoring downstream of Cheat Lake
4. Fish biomonitoring of Cheat Lake and embayments
5. Walleye population monitoring and stock assessment
6. Monitoring of adult walleye movement
7. Physical and chemical water quality characteristics of Cheat Lake
8. Aquatic vegetation mapping of Cheat Lake
9. Bathymetric mapping of Cheat Lake

Progress:
For this study, Cheat Lake was divided into three major study areas: embayments (Rubles Run – 56 acres, and Morgan Run – 37 acres); lower Cheat Lake, downstream of I-68 bridge to Lake Lynn hydro station; and upper Cheat Lake upstream of the I-68 bridge to the head of the lake. The 3.7-mile section of Cheat River downstream from the hydro station was defined as the Cheat tailwater area located in the first 1.1 miles, and Cheat River between the Cheat tailwater area and the confluence of Cheat River with the Monongahela River (lower 2.6 miles).

The water quality of the Cheat Lake tailwaters and Cheat River was monitored bi-monthly from 2011-2015 to assess any impacts from hydropower operations and/or existing acid mine drainage inputs on downstream water quality. The Cheat Lake tailwater section consistently maintained water quality supportive of aquatic organisms with an average pH of 6.6, average dissolved oxygen of 8.7 mg/l, and average specific conductivity of 109 µs/cm. In contrast, water quality in Grassy Run, an acidic tributary to the Cheat River, had comparably poor water quality with an average pH of 3.1 and conductivity of 1422 µs/cm. In general, Cheat River water quality downstream of Grassy Run reflected impacts of acid mine drainage (AMD) from Grassy Run with an average pH of 5.5 and an average conductivity of 220 µs/cm.

Physical and chemical water quality profiles were conducted monthly (except during periods of ice cover) from 2011-2014. The primary focus of these limnological profiles was to monitor the pH of Cheat Lake which is still impacted by upstream AMD sources, and to monitor the stratification of water temperature and dissolved oxygen within the lake. Depressions in pH (less than 6.0) within the lake occurred occasionally in 2011, primarily in the early spring. This trend of early spring pH depression has been documented since the initiation of lake profiles by WVDNR in 2005. However, in 2012, 2013, 2014, and 2015 Cheat Lake did not experience pH depressions below 6.0, possibly due to increases in AMD treatment efforts upstream in the Cheat River watershed. Stratification of water temperature and dissolved oxygen historically occurred in lower Cheat Lake from approximately June-September. During these months, the upper 6-8 meters of the water column was typically characterized by warmer water with suitable dissolved oxygen levels (above 5.0 mg/L), while the lower portion of the water column was typically characterized by much colder water with increasingly less dissolved oxygen (less than 5.0 mg/L). Summer stratification occurred primarily in the lower portion of Cheat Lake which on average has greater depths than other lake areas. However, Cheat Lake did not experience strong stratification during 2013 and 2014, likely due to increased precipitation, higher incoming river flows and cooler summer air temperatures.

Night boat electrofishing and gill netting were conducted during May and October 2011-2015 in Cheat Lake. The primary focus of these surveys was to monitor the health of the fish communities of Cheat Lake and compare data from our study to data from prior biomonitoring studies on Cheat Lake (1990-2008). In total, we captured 839 fishes with gill nets, while 7,499 were collected using electrofishing. Statistical analyses using non-metric multidimensional scaling (NMDS) and permutational analysis of variance (PERMANOVA) suggested that fish communities were significantly different across different lake zones (i.e., upper lake, middle lake, lower lake, embayments). Largemouth bass and spotted bass were the most abundant in embayment areas, while smallmouth bass were more abundant in the upper lake. Green sunfish, bluegill, and pumpkinseed were most abundant in the lower lake. Walleye, yellow perch, white bass, and channel catfish were typically most abundant in the upper lake. Smaller forage species abundance also differed dependent on lake zone. Mimic and emerald shiners were very abundant in the upper lake and fairly abundant in the lower lake, but were uncommon in embayments. Conversely, logperch and brook silversides were most abundant in the embayments and lower lake. Statistical results from NMDS and PERMANOVA also suggested that the fish community of Cheat Lake has significantly changed over time represented by increased fish abundance and species diversity. Improvements to Cheat Lake water quality are likely responsible, in part, for these temporal changes in the fish community.

Night boat electrofishing, tow barge (pram) day electrofishing, and gill netting were conducted during June/July, September (pram only), and October during 2011 and 2014 in the tailwaters and river downstream of Cheat Lake. In total, 1,903 fishes were captured with boat electrofishing, 195 with gill nets, and 1,055 with pram electrofishing. An abundance of small forage fish primarily represented by mimic shiners, emerald shiners, and bluntnose minnows were collected in both the tailwater and river sections. In the tailwaters, mimic shiner was the most abundant forage fish, while in the river emerald shiners were more abundant. Smallmouth bass and channel catfish were the most abundant game fishes collected, although largemouth bass and sauger were quite abundant near the mouth of the Cheat River. In addition, benthic macroinvertebrate sampling was completed in July and November of 2011 and 2014. A total of 6,388 benthic macroinvertebrates were collected during these surveys. The tailwater area just below the dam had a relatively low abundance of macroinvertebrates, likely due in part to the variation in outflow from the upstream dam. The family Chironomidae (midges) accounted for most of the invertebrates in the tailwaters just downstream of the dam. Two sites were sampled for macroinvertebrates approximately one mile downstream of the dam, and supported a much greater abundance of macroinvertebrates. However, the macroinvertebrate community at these sites had low diversity mainly comprised of tolerant taxa. Macroinvertebrates from the families Chironomidae and Hydropsychidae (net-spinning caddisflies) accounted for most of the macroinvertebrates at these downstream stations. Despite the dominant abundance of tolerant taxa, pollution sensitive mayfly and stonefly taxa (i.e., *Isonychia sp*., *Taeniopterx sp*.) were also collected during sampling efforts.

Additionally, WVSCI scores (West Virginia Stream Condition Index, a multimetric index that measures benthic macroinvertebrate community health) were calculated for samples during our study and indicated improvements to the benthic macroinvertebrate community compared to prior biomonitoring studies (1998-2008) in the Cheat tailwater.

Research on adult walleye movement was started in early December 2011. We implanted 50 adult walleyes (31 males, 17 females, 2 undetermined, 432-708 mm TL) with acoustic transmitters. Data collection on tagged individuals was completed in April 2015 and stationary receivers were removed from the reservoir. Fish locations were determined using both submerged, stationary receivers and active tracking. During winter months, tagged fish normally remained near their original capture locations until late February (2012)/mid-March (2013-2015) when fish usually began upstream movements, likely to reach spawning habitat. By early March (2012) to early April (2013-2015), most tagged fish moved to upper Cheat Lake to spawn. Analysis of the data suggests that upstream spawning movements were primarily driven by elevated water temperatures (Figure 2). Thirteen tagged fish periodically occupied the area upstream of the first riffle of Cheat Lake, possibly to spawn in the river upstream of the lake. Several tagged fish continued to use this area upstream of the first riffle during the spring and summer months. Also, during non-spawning periods (i.e., summer/fall), increases in river discharge and water temperature often triggered upstream movements of many tagged fish. Walleye usage of the upstream riverine reaches increased during summer when main lake water temperature increased and dissolved oxygen decreased.

Walleye population monitoring and stocking assessment surveys were conducted in Cheat Lake during March/April and November of 2012, 2013, 2014, and 2015. Gill nets were used to capture walleyes throughout the lake to assess the status of the population and the success of walleye stocking efforts. Catch per unit effort (CPUE) of walleyes was only 0.5-0.6 fish per net night during spring (2012-2014). However, it is likely that the walleye population was underrepresented during our spring surveys. Most adult walleye were likely in the upper lake, upstream of our netting areas. Information from our acoustic tagged walleyes indicated that most fish were occupying this upper lake area, presumably in preparation for spring spawning. Supporting this assumption, CPUE during fall 2012, 2013, 2014, and 2015 was much higher with 1.3-3.3 fish captured per net night.

Figure 1. *Dustin Smith (left) and volunteer David Smith (right) with two large walleyes captured during walleye population surveys.*

An aquatic vegetation map of Cheat Lake was created using sonar mapping, underwater cameras, and physical observations. Areas of Cheat Lake harboring aquatic vegetation were assessed using these methods to determine species composition and relative abundance. This information was incorporated into a lakewide map using GIS, and depicts species presence/abundance information. Areas of highest aquatic vegetation abundance were embayment habitats such as the Morgans Run and Rubles Run embayments. Dominant vegetation taxa found in Cheat Lake included *Potamogeton* spp., *Vallisneria americana*, and *Najas* spp. This information will be useful for future management efforts aimed at determining areas that likely represent import nursery habitat for larval/juvenile fishes of Cheat Lake.

A bathymetric map of Cheat Lake was created using sonar, GPS technology, and GIS-based interpolation techniques. Depth data with GPS coordinates were recorded from transects using boat-mounted sonar gear. These data were imported into a GIS, where interpolation and contour line mapping techniques were used to produce a bathymetric map of Cheat Lake. This component of the study was used to help determine habitat preferences of walleye and also areas that are vulnerable to water level fluctuations.

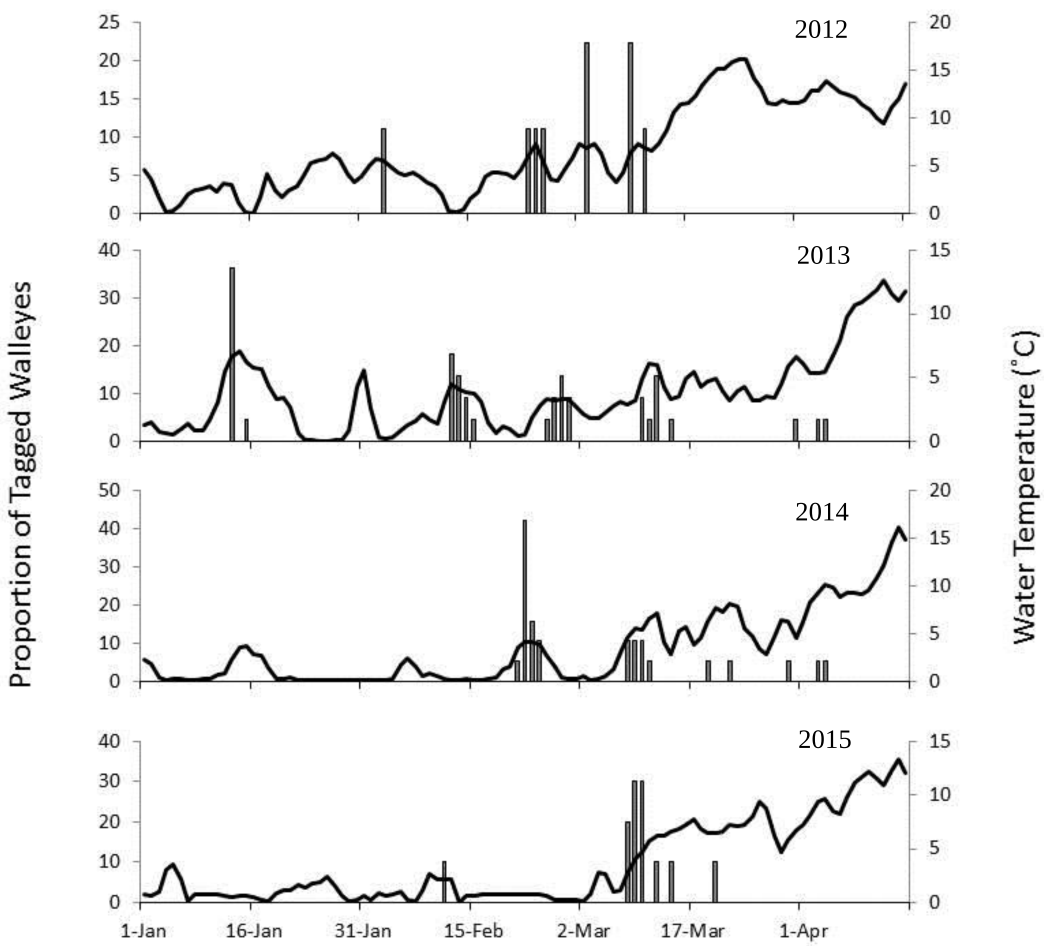

Figure 2. *Daily proportion of tagged walleyes migrating toward spawning areas (gray bars) during the pre-spawn period and associated water temperature (black line) data for 2012-2015.*

CONTINUING PROJECTS

AQUATIC
FISH ECOLOGY AND MANAGEMENT

INFLUENCE OF HIGH WATER DISCHARGES FROM FLOOD REDUCTION LAKES ON SPATIAL AND TEMPORAL TEMPERATURE CHARACTERISTICS AND THE RESULTING POTENTIAL TO ALTER FISH ASSEMBLAGES

Student Investigator: Steven W. Foster
Principal Investigator: Stuart A. Welsh
Years Ongoing: 2014 – 2016
Degree Program: PhD
Expected completion: December 2017
Funding Source: U.S. Army Corp of Engineers

Objectives:

1. Establish how environmental flow and flood control discharges from reservoirs influence downstream spatial water temperature characteristics (addressing hypolimnion and epilimnion water withdrawal)

2. Evaluate the influence of selective withdrawal outlet structures on the downstream occurrence of fishes, with emphasis on species with limited ability to avoid acute water temperature and dissolved oxygen changes, such as *Etheostoma spp.*

3. Experimentally examine how magnitude and rate of temperature change influence fish behavior, with emphasis on *Etheostoma spp.*

Progress:

Environmental flow is the process of managing water quantity, timing, and quality to sustain ecological integrity of riverine ecosystems. Currently it is difficult to measure how changes in flows will influence abiotic conditions such as temperature and sediment but even harder to determine how subtle changes in abiotic factors will change biotic communities. Ecologists understand the benefits of diversity in an ecosystem, yet most reservoirs operate with mandated and engineered uniform flow guidelines through minimum and maximum flows. During dry years minimum flow regulations often lead to tailwaters of reservoirs being maintained at minimum flows for months with no variation in discharge creating a highly stable ecosystem. This highly stable ecosystem results in streams below reservoirs becoming dominated by equilibrium strategists and tends to select against opportunistic and periodic strategists.

Burnsville Lake, Braxton County, WV; East Lynn Lake, Wayne County, WV; and Sutton Lake, Braxton County, WV are operated by the Huntington District, US Army Corps of Engineers (USACE) and vary in designs and capacities. Each lake was impounded under the authority of the Flood Control Act of 1962 and can include project purposes of flood control, recreation, fish and wildlife conservation, low flow augmentation, and water quality. All three dams are equipped with selective withdrawal intake systems that vary in degrees of complexity, allowing for some control of where water is withdrawn from the lake. High-level intakes located in the epilimnion and bottom-level sluice gates located in the hypolimnion are designed to be operated to pass high quality water from the lake. Therefore, it is possible, depending on flow conditions, to regulate both quality and quantity of water passing through the dam. Optimal outflow temperature guidelines (Figure 1) and minimum flow regulations were developed by the West Virginia Division of Natural Resources (WVDNR) and the US Fish and Wildlife Service when the lakes were designed. The goals of selective withdrawal system operations are to stay within these guidelines when possible while minimizing impacts of hypolimnetic discharges.

Selective water withdrawal capabilities at all three dams allow for limited mitigation of temperature alterations. Water releases from a combination of intakes can provide downstream water temperature that is similar to the natural conditions of an unregulated stream. Dams are currently operated to achieve release temperatures that fall between or reasonably close to temperature guide curves (Figure 1). Because temperatures vary from year to year efforts are focused on following the slope of the curves (i.e. rate of increase or decrease in temperatures) rather than matching them exactly. When available, operational changes are used to ensure that outflow temperatures do not inappropriately deviate from guide curves. When large rain events occur in spring and summer, releases are based on the amount of available storage in the lake. If the lake is below summer pool, the rain events are stored with minimum releases below the dam until summer pool is reached. Otherwise, rain events are released not to exceed 80% of inflow. Flood reduction becomes the top priority as the downstream channel approaches capacity. Large pulses in flows are traded for sustained periods of higher than normal flows with little to no control over temperature during flood conditions. Increased discharges from a lake due to flood reduction efforts or implementation of environmental flow recommendations have the potential to significantly alter temperature characteristics for substantial distances downstream of the dam. A full understanding of how lake operation influences temperature would help to minimize extremes when operating for flood reduction or environmental flow.

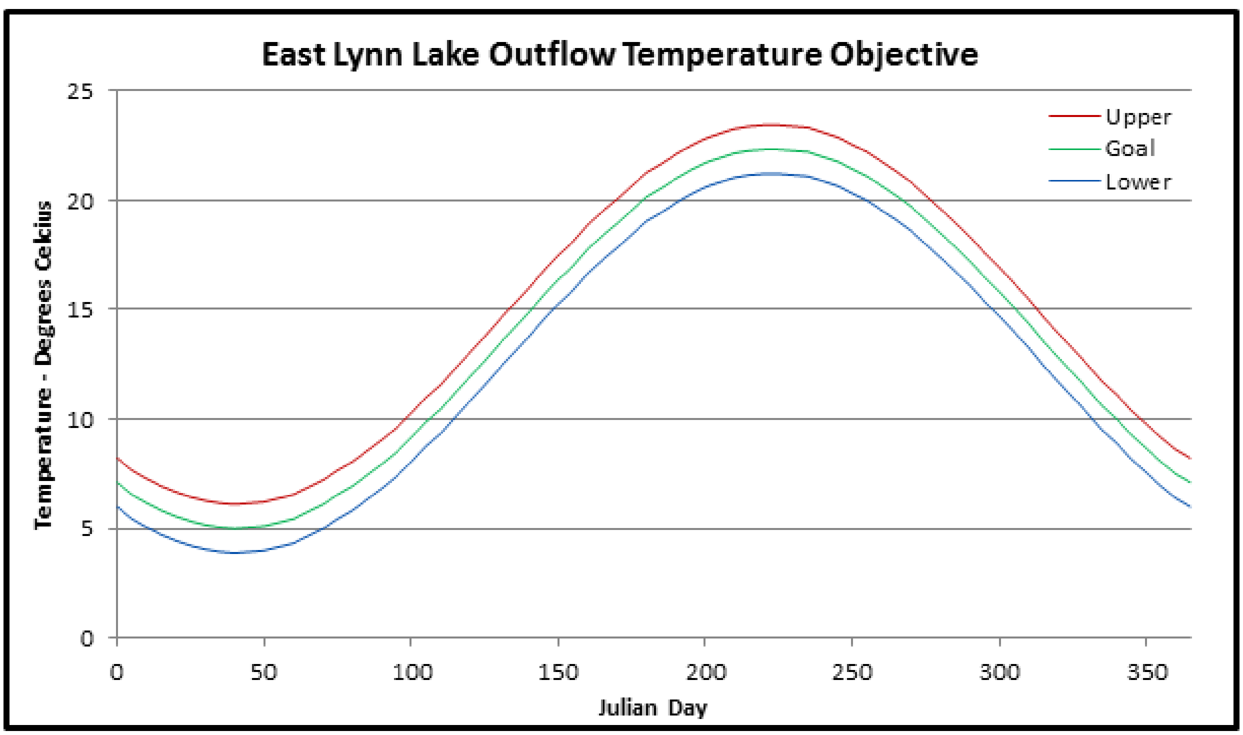

Figure 1. *East Lynn Lake outflow temperature guideline curve.*

FISH USE OF MISSISSIPPI RIVER DREDGE AND DISPOSAL SITES

Student Investigator: Cory Hartman
Principal Investigator: Kyle Hartman, Patricia Mazik
Years Ongoing: 2015-2017
Degree Program: MS
Expected Completion Date: May 2017
Funding Source: USGS (RWO 67)

Objectives:

The objective of this pilot study is to identify the impacts of dredging and disposal upon the fish use (larval fish through adults) of the Mississippi River. We will conduct immediate before and after surveys at the time of the cuts as well as monitor fish use through the rest of the year. Following these sites through the year will allow us to consider changes in habitat use by different fish life stages (e.g. larval, juvenile, adult) under different environmental conditions, as well as their use of the cut and disposal sites as these sites age. Additional locations representing the Middle and Lower Mississippi River will be included in year 2 and 3 to characterize dredge/disposal effects on fish in those reaches.

Progress:

Progress to date has focused on preparation to conduct the pilot study. This was originally scheduled to occur during fall-winter 2015-16. However, the dredging schedule and record flooding and river levels from fall 2015 through July 2016 have thus far prevented sampling. In July 2016 we evaluated an ARIS 3000 multibeam sonar as a possible tool to assess fish use of the bottom at both dredge and disposal sites for continuation of this study. The sonar was tested on the Monongahela River and collected data is currently awaiting analysis to determine feasibility of this gear for the Mississippi River study.

The M.S. student on the project (Cory Hartman) has changed. He has the research vessel and all research equipment for use on this project ready for deployment. Current dredge schedules for the middle Mississippi River suggest our pilot study will begin in mid-September 2016. We will use a before/after and reference/treatment design. We will select 3 dredging/disposal sites and nearby reference areas. At each site we will conduct hydroacoustic surveys and benthic sled samples to quantify fish and macroinvertebrate use of dredge and disposal areas and use that information to assess the impacts of dredging operations upon fish and lower trophic levels in the middle and lower Mississippi River. This research will form the thesis of the MS student who will present their research at professional conferences and as peer-reviewed publications in journals such as River Research and Applications.

Grand Towers on the Mississippi River (photo by Kyle Hartman)

ASSESSING THE STATUS OF THE FEDERALLY ENDANGERED DIAMOND DARTER IN THE ELK RIVER, WEST VIRGINIA

Student Investigator: Austin Rizzo
Principal Investigator: Stuart Welsh
Years Ongoing: 2014-2016
Degree Program: PhD
Expected completion: December 2017
Funding Source: NiSource

Objectives:

1. Document Diamond Darter presence and abundance in glide vs. pool habitats relative to gas pipeline crossings.
2. Evaluation of a photographic technique for estimating body length of benthic darters.
3. Evaluation of season and diel variation of Diamond Darter occupancy and abundance within glide habitats.
4. Mapping Diamond Darter habitat at three pipeline crossing sites in the lower Elk River.
5. Determining the impact that the 1,000 year flood (June 2016) had on the Diamond Darter population.
6. Developing an occupancy model for Diamond Darters in the lower 50 km of the Elk River.

Background:

The Diamond Darter (*Crystallaria cincotta*) was designated as an endangered species by the U.S. Fish and Wildlife Service in 2013 (Figure 1). Museum specimens indicate the Diamond Darter was once distributed throughout the Ohio River Basin, but it is now believed to be extirpated from the Muskingum River in Ohio, the Ohio River in Ohio, Kentucky and Indiana, the Green River in Kentucky, and the Cumberland River drainage in Kentucky and Tennessee. Currently, it is known to exist only within the lower 50 km of the Elk River in West Virginia.

Prior to 2011, researchers had difficulties locating Diamond Darters in the Elk River using conventional sampling methods (electrofishing, kick-seining, and bag-seining); however, the development of a new sampling method has led to much higher sampling success rates. This method employs the use of spotlighting at nighttime with flashlights within wadeable sections of the river. This method has proven to be most useful in glide habitats (those areas of the river immediately upstream of riffles). These areas are shallow enough for a person to wade transects and they have a smooth water surface which usually allows the spotlighter to see through the water column to the substrate. While this new sampling method has led to much higher sampling success rates, our complete understanding of Diamond Darter habitat use is restricted by its limitations. The spotlight method has proven successful only during the hours between dusk and dawn; Diamond Darters have not been detected during daylight hours within glide areas. Two possibilities exist that account for this non-detection: either Diamond Darters use other habitat during the day (pool or riffles) or they bury into the sand during the day within glide habitats and escape detection.

Very little information regarding the life history, habitat use, behavior, and general ecology of this fish is known compared to other species of darters. The lack of information is due, in part, to the species rarity, its nocturnal behavior, its patchy distribution within the river, and to a low rate of detectability. Much of this may have also contributed to its late discovery as a species. Our current knowledge regarding the Diamond Darter stems from a captive propagation study and field studies which have been conducted using the new search method. While some information regarding the ecology of the Diamond Darter is known, there are still many unanswered questions that must be addressed to better aid in management and conservation decisions. More specifically, data will aid planning and decision makers regarding uses of specific sections of the lower Elk River relative to gas pipeline crossings.

Progress:
One study objective is to determine if Diamond Darters are using pool habitat near gas pipeline crossings. This study focuses on three areas within the lower Elk River where NiSource gas pipelines cross the Elk River. Different search techniques must be employed in order to search pool and glide habitat. Glide habitat is searched using the spotlight search method. Pool habitat is more difficult to search because it is too deep for searchers to wade and see the substrate. Presence and abundance is assessed in pool habitat using boats and underwater cameras. During 2015 and 2016, nighttime searches of glide habitat have occurred during the late spring, summer, and early fall months. Diamond Darters have been found at all three study sites within the glide habitat. Daytime searches at these glides have revealed no Diamond Darters. Daytime and nighttime searches during spring, summer, and fall have not detected Diamond Darters in pool habitat.

A second research objective is to evaluate a photographic technique (i.e., photogrammetry) for estimating length of benthic darters underwater. This approach to obtaining fish length measurements is applicable to endangered fishes, such as the Diamond Darter, because it is non-intrusive and provides important data without the need to capture individuals. Surrogate species (Greenside and Variegate darters) were used to test the methodology. For photogrammetry, digital photographs from the dorsal view of each individual were taken using a waterproof camera paired with two parallel lasers. Photogrammetric measurements were conducted with ImageJ software (Figure 2). Agreement between direct and photogrammetric measurements was examined with concordance correlation coefficients (CCCs). Precision and accuracy of measurements were evaluated using Pearson’s correlation coefficient and the bias correction factor, respectively. The CCCs were similarly high for both surrogate species, indicating that the photogrammetric technique is an effective method for measuring benthic darter species. In addition to the accuracy and precision study, we also demonstrate an application of paired-laser photogrammetry to the Diamond Darter. A total of 199 photographs of Diamond Darters were taken during five seasonal sampling events in May through October of 2015. The number of Diamond Darters photogrammetrically-measured per season ranged from 16 individuals (October) to 63 individuals (Late August). Decomposition of length-frequency histograms by the program FiSAT II identified one distinct cohort during each sampling event (Figure 3). Based on the modal distribution and progression through time this cohort appeared to persist in the glide habitat through all five seasonal sampling events; with mean length increasing throughout the sampling season (May-October) at a rate of approximately 4.05 mm/month.

The third research objective involves evaluation of seasonal and diel variation of Diamond Darter occupancy and abundance within glide habitats. The primary objective of this study was to determine if there are seasonal and diel patterns in Diamond Darter detectability during population surveys. In addition to temporal factors, we also assessed 5 habitat variables that might influence individual detection. We used *N*-mixture models to estimate site abundances and relationships between covariates and individual detectability, and ranked models using Akaike’s information criteria. During 2015, three known occupied sites (associated with gas pipeline crossings within the lower Elk River) were sampled fifteen times each between May and Oct. The best supported model included water temperature as a quadratic function influencing individual detectability, with temperatures around 22° C resulting in the highest detection probability. Detection probability when surveying at the optimal temperature was approximately 6% and 7.5% greater than when surveying at 16° C and 29° C, respectively (Figure 4). Time of Night and Time of Year were not strong predictors of Diamond Darter detectability. By determining the relationship between water temperature and Diamond Darter counts at a site, adjustments can be made in future abundance estimates to account for incomplete individual detectability. The results of this study will allow researchers and agencies to maximize detection probability when surveying populations, resulting in greater monitoring efficiency and likely more precise abundance estimates.

A fourth research objective includes mapping potential Diamond Darter habitat in the lower Elk River. This objective focuses on three areas within the lower Elk River where NiSource gas pipelines cross the Elk River. Microhabitat variables which will be mapped are water depth and dominant/subdominant substrate. Measurements are taken when discharge is between 150 and 300 cubic feet per second (cfs). Microhabitat data are collected at strategically located points which will be used to interpolate habitat at locations that are not physically sampled. Field measurements are currently being taken this summer (2016) and interpolated maps will be created from these measurements following the collection of all field data.

The fifth research objective, recently added following a 1,000-year flood in late June 2016, during which discharge increased 184 fold, was to determine if the population of Diamond Darters had been negatively impacted by the catastrophic event. Prior to this flood, we had begun surveying sites for an occupancy study [described below] that was supposed to take place during the 2016 sampling season. Only fifteen of 20 designated sites to be included in the occupancy study were searched prior to the flood – each was sampled once. Pre-flood data at these 15 sites was used to run an analysis to determine if this one population cohort of Diamond Darters (using the glide habitat) had been negatively impacted. These 15 sites were sampled again after water levels and turbidity had returned to pre-flood levels. Pre and post-flood abundances at each site were used in analysis by running a randomized test with paired sites to determine if the abundances were statistically significantly different following the flood. A P-value of 0.296 indicated that the abundances were not different. This result supports the argument that the flood did not have a statistically significant impact on this cohort of Diamond Darters; however, subsequent research will need to be conducted in the next two years (at least) to determine if other cohorts may have been impacted.

A final research objective is to develop an occupancy model for Diamond Darters in the lower 50 km of the Elk River and to eventually determine the distribution of the species within the Elk River. By using site covariates in the occupancy model, we will be able to determine variables which influence site occupancy and abundance. Specific outcomes of this study will include (1) documenting site covariates which influence occupancy and abundance at a glide, and (2) current status information for the Diamond Darter in the lower 50 km of the Elk River.

Figure 1*. A Diamond Darter photographed at night with underwater camera within Elk River, near Elk View, WV during July 2012.*

Figure 2*. Photogrammetric measurement of a Diamond Darter using digital imaging software.*

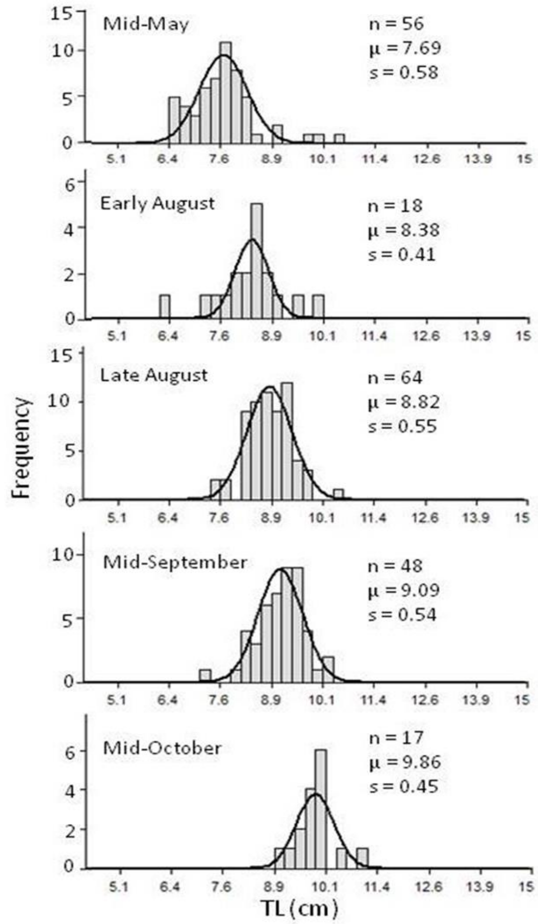

Figure 3. *Length frequency histograms for Diamond Darters based on photogrammetry.*

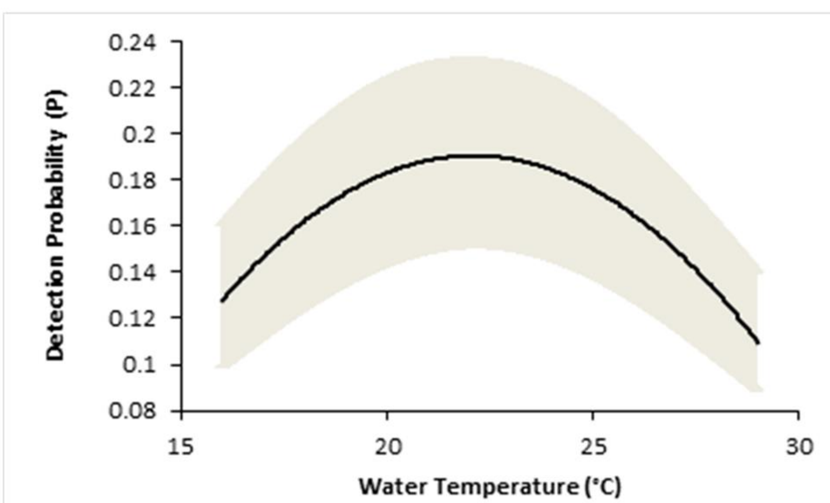

Figure 4. *Relationship between water temperature and detection probability of the Diamond Darter based on 45 surveys at 3 sites in the Elk River, West Virginia.*

SUBSTRATE SELECTION AND HABITAT USE OF EASTERN AND WESTERN SAND DARTERS IN THE ELK RIVER, WEST VIRGINIA

Student Investigator: Patricia Thompson
Principal Investigator: Stuart Welsh
Years Ongoing: 2015
Degree Program: MS
Expected completion: December 2016
Funding Sources: Currently not funded

Objectives:

Research objectives include documenting upstream and downstream distribution limits of the Eastern Sand Darter and Western Sand Darter in the Elk River, determining the Western Sand Darters benthic habitat use, and examining if substrate selection differs between the two species within the same environment.

Progress:

The Western Sand Darter (*Ammocrypta clara*) and the Eastern Sand Darter (*A. pellucida*) are the only sympatric sister species of *Ammocrypta*, and the Elk River in West Virginia is one of the few remaining places where both species occur. They are slender, sand-dwelling fish that were once broadly distributed, but have since undergone range-wide population declines, presumably owing to habitat loss. Habitat use studies have been conducted for the Eastern Sand Darter, but literature on the Western Sand Darter remains sparse, and is an essential element for the conservation of the species. The existing extent of the Western Sand Darter in the Elk River occurs within the designated critical habitat for the endangered Diamond Darter, another sand burying species. Historically, the Western Sand Darter and the Diamond Darter have experienced a similar pattern of range decline, while still both maintaining populations in the Elk. Thus, the Elk River presents a unique opportunity to study both species of sand darters where they are sympatric, to gain further insight on their status and microhabitat use. The information from this project will increase our knowledge of the species, and aid in the planning and decision making process regarding these vulnerable sand burying species and anthropogenic riverine uses of the Elk River.

Thus far, we sampled 63 sites throughout the lower 190 rkm of the Elk River (Fig. 1). Eastern Sand Darters were detected at 47 sites from Mink Shoals (7.8 rkm) to Frametown (133 rkm), while Western Sand Darters were detected at 8 sites from Blue Creek (19 rkm) to Clendenin (36 rkm). The species occurred together at 6 sites. Depth, velocity, and substrate composition were measured at each sand darter sampling location. The low sample size of Western Sand Darter limits our ability to make statistical inferences on microhabitat use.

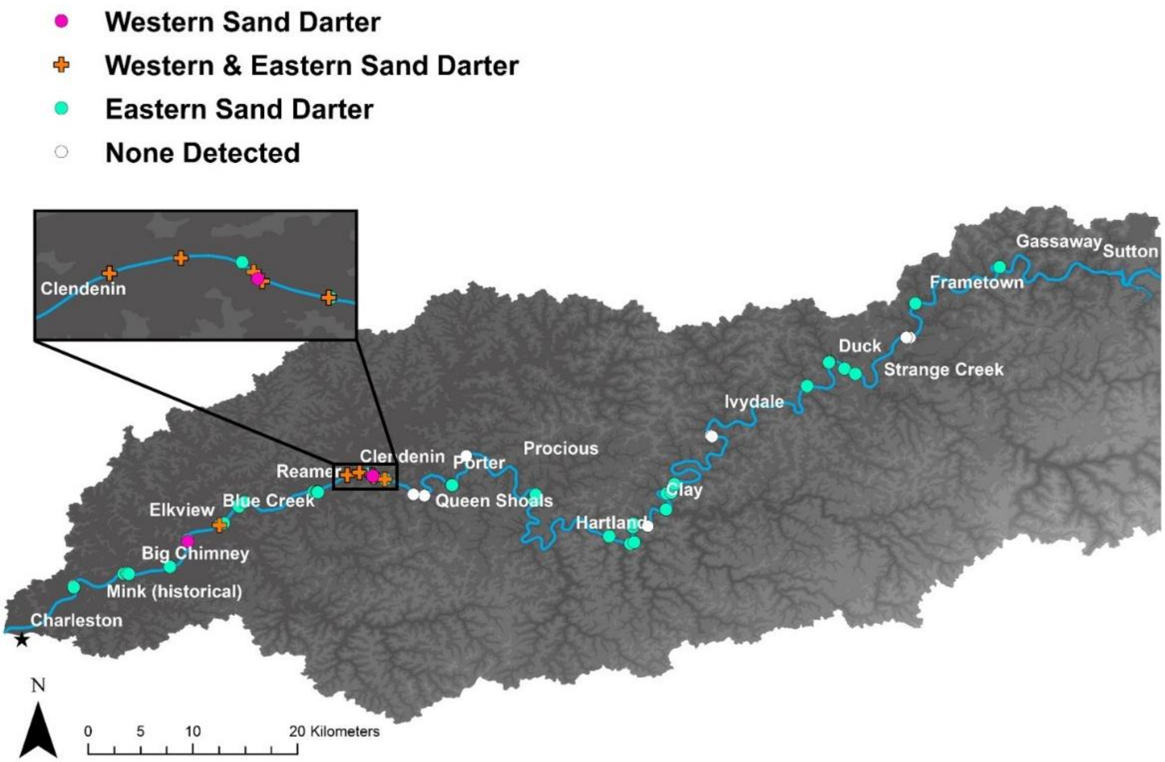

Figure 1. *Map of the Elk River watershed shaded by elevation with sand darter sampling sites.*

To supplement the lack of field data, an aquaria based experiment was conducted to examine sand darter benthic habitat use. Substrate selection was evaluated by conducting 15 laboratory trials in four aquaria. Two aquaria contained six Western Sand Darters in each, while the other two held a combination of both species, three Eastern Sand Darters and three Western Sand Darters. The sand darters were given the choice to bury into five equally available and randomly positioned substrates: fine sand (0.12-0.25 mm), medium sand (0.25-0.5 mm), coarse sand (0.5-1.0 mm), very course sand (1.0-2.0 mm), and granule gravel (2.0-4.0 mm). Western sand darters selected for coarse and medium sand, while the eastern sand darters were more of a generalist selecting for fine, medium, and coarse sand (Fig. 2). Substrate selection was significantly different (p-value = 0.02) between species in the same environment, where the western sand darter selected for coarser substrate more often compared to the eastern sand darter. The results from this study suggest that Western and Eastern sand darters respond differently to fluctuations in substrate composition, with the Western Sand Darters selecting for a narrower range of grain sizes.

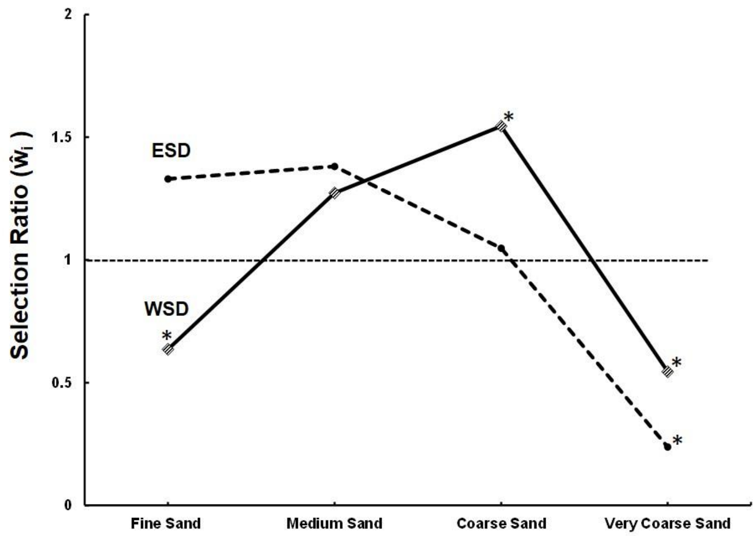

Figure 2. *Substrate selection ratios for the Western Sand Darter (WSD) and the Eastern Sand Darter (ESD). An asterisk (*) represents significant selection for a substrate (>1) or against (<1).*

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

Principal Investigator: Stuart A. Welsh
Years Ongoing: 2009 – 2016
Expected Completion: 2016
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). This ladder has passed over 24,000 eels during the period of 2003 to present. Eel ladders have also been installed upstream at Warren and Luray dams, but these 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, Figure 3). Eels often use the ladder during time periods near the new moon or periods of increasing river discharges (Figure 4). 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.

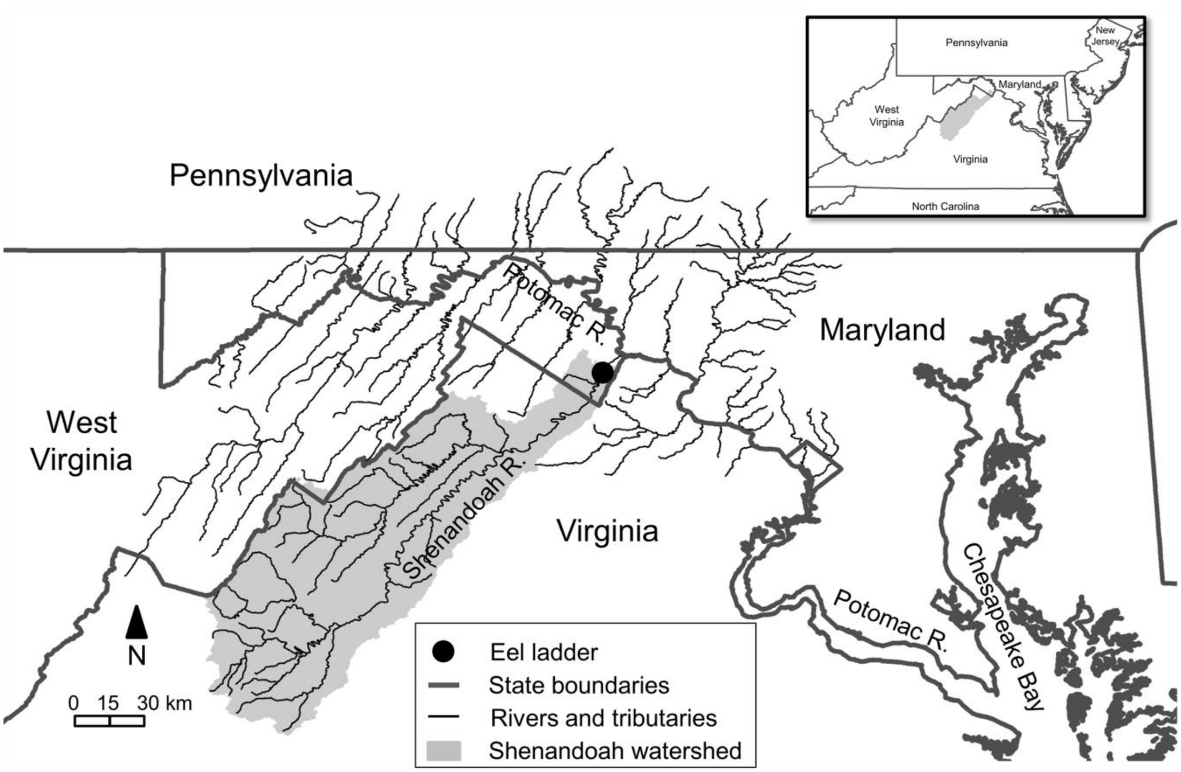

Figure 1*. Map of the Potomac River drainage and the location of the Millville Dam eel ladder on the Shenandoah River.*

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

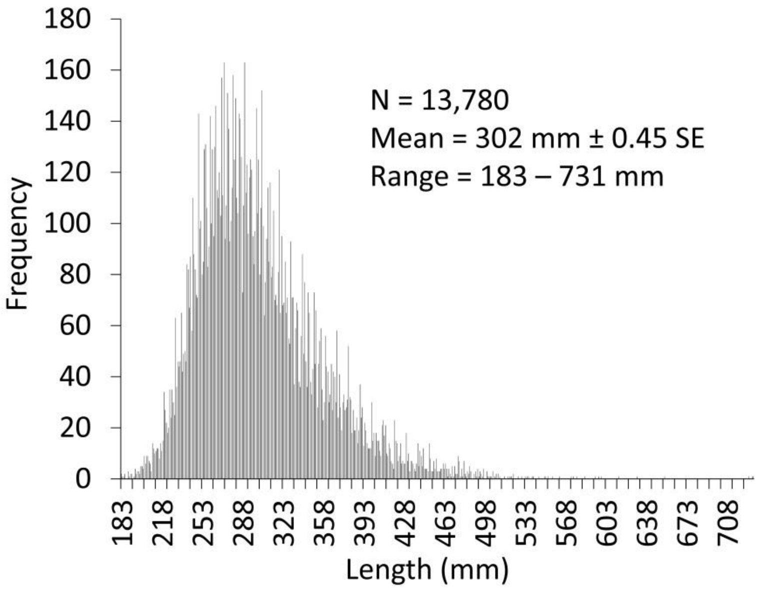

Figure 3. *Length-frequency histogram of 13,780 American Eels from the Millville Dam eel ladder on the Shenandoah River. The mean length of American Eels using the ladder is 302 mm with a range of 183–731 mm, although individuals >500 mm rarely use the ladder.*

AQUATIC
FISH HEALTH PROJECTS

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

Student Investigator: Ryan Braham
Principal Investigators: Dr. Patricia Mazik
Collaborators: Dr. Vicki Blazer - USGS Leetown Science Center, Jim Hedrick - WVDNR
Years Ongoing: 2013 - 2017
Degree Program: PhD
Expected Completion: December 2017
Funding Source: West Virginia Division of Natural Resources

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 redhorse 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 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.

3. Quantify changes in hepatic genes of smallmouth bass (*Micropterus dolomieu*) at one selected sites in West Virginia. Examine molecular endpoints which may indicate exposure to biologically relevant concentration of microcystin toxin.

4. Quantify the effects of exposure of microcystin toxin on immune function *in vitro*. Examine molecular endpoints in the anterior kidney result from exposure to microcystin toxins. Examine change in immune function in the anterior kidney resulting from exposure to microcystin toxin.

Progress:

Objective 1: A total of 175 water and periphyton samples were collected between June 26, 2013 and June 18, 2014 from the 15 selected in West Virginia, Maryland, and Virginia for analysis. Samples were collected and analyzed for total microcystin toxin present in the filtered water column, unfiltered water column and periphyton. During FY 2015, 3 sites were identified to have filtered water, unfiltered water, and periphyton samples and collected bimonthly for total microcystin quantification. A total of 141 samples were collected between June 24, 2014 and December 14, 2015 and analyzed for total microcystin toxin present in the filtered water column, unfiltered water column and periphyton. Analysis is ongoing. In addition, 117 of those samples were collected and analyzed for total nitrogen, total phosphorous, and total dissolved phosphorous in the water column and total nitrogen and total phosphorous in the sediment. Preliminary analysis has shown the presence of microcystin toxins present in the filtered water, unfiltered water, and periphyton at relatively low levels. The total concentration of microcystin in the water column is inversely correlated to discharge. Analysis is ongoing. A total of 260 smallmouth bass were collected among 4 sites between March 29, 2013 and August 11, 2015. A total of 294 golden redhorse suckers were collected among 2 sites between April 4, 2013 and August 12, 2015. Samples of liver were retained for quantification of microcystin present. Preliminary analysis has shown the presence of microcystin toxins present in the livers of both species at relatively low levels. Analysis is ongoing.

Objective 2: A total of 175 water and periphyton samples were collected between June 26, 2013 and June 18, 2014 from the 15 selected in West Virginia, Maryland, and Virginia for analysis. Samples were collected and analyzed for total estrogenicity present in the filtered water column, unfiltered water column and periphyton. During FY 2015, 3 sites were identified to have filtered water, unfiltered water, and periphyton samples and collected bimonthly for total estrogenicity quantification. A total of 141 samples were collected between June 24, 2014 and December 14, 2015 and analyzed for total estrogenicity toxin present in the filtered water column, unfiltered water column and periphyton. Analysis is ongoing. In addition, 117 of those samples were collected and analyzed for total nitrogen, total phosphorous, and total dissolved phosphorous in the water column and total nitrogen and total phosphorous in the sediment. Preliminary analysis has shown peaks in total estrogenicity in filtered water and unfiltered water in the summer months at relatively low levels. Total estrogenicity has also been identified in periphyton. Analysis is ongoing.

Objective 3: A total of 170 smallmouth bass were collected and livers retained for quantification of genes identified as either endocrine disrupting or possibly exposure to microcystins. Samples were prepared and shipped to NanoString Technologies for analysis. Transcriptome data has been returned. Analysis is ongoing.

Objective 4: A total of 18 smallmouth bass were collected on May 28, 2015 and November 10, 2015. Anterior kidneys were removed and exposed to microcystin-LR, as well as 5 immunostimulants *in vitro*. RNA was extracted and sent to the Institute for Genome Sciences at the University of Maryland for HiSeq-4000 analysis. Sequence results have been returned, annotated, and assembled. We are now running RNA-Seq exploring differences among the treatment group as compared to control and stimulant-challenged cells. Analysis is ongoing.

Ryan Braham with a small mouth bass

DEVELOPMENT AND APPLICATION OF MOLECULAR PATHOLOGICAL METHODS TO ELUCIDATE ETIOLOGICAL MECHANISMS OF DISEASE IN WILD FISHES

Student Investigator: Heather L. Walsh
Principal Investigators: Dr. Patricia Mazik
Collaborators: Dr. Vicki Blazer, Dr. Luke Iwanowicz - USGS Leetown Science Center
Years Ongoing: 2014 - 2017
Degree Program: PhD
Expected Completion: December 2017
Funding Source: U.S. Fish and Wildlife Service (RWO 55, 61), U.S. Geological Survey (RWO 60)

Objectives:

In past mortality events of smallmouth bass (SMB) in the Chesapeake Bay drainage, adult SMB from the Potomac River basin and young-of-the-year (YOY) SMB from the Susquehanna River basin have been affected. The primary objective of this study is to use in situ hybridization (ISH) and molecular pathology techniques for the detection of parasites, bacteria, and intersex and immune-functioning genes in smallmouth bass. The use of ISH with these biomarkers will assist in an overall evaluation of fish health from selected sites in the Potomac and Susquehanna River watersheds. Young-of-the-year SMB from the Susquehanna River Basin were found to exhibit infections of a myxozoan parasite, *Myxobolus inornatus*, in the connective tissue of the muscle below the epidermis. In some histology samples, observations of areas of inflammation are only observed and ISH can been used to determine if these areas are actually early stage infections of *M. inornatus*. It will also be used to try and determine the portal of entry into the fish for *M. inornatus* and whether there are instances of bacterial co-infections.

In addition to parasite infections, many YOY SMB also exhibit lesions and/or systemic infections. The cause of these infections remains unknown, but previous sampling has isolated species of *Aeromonas, Flavobacterium,* and *Vibrio* from bacterial swabs from both external and internal lesions. Largemouth bass virus is also commonly found in SMB from the Susquehanna River drainage and may also be a potential instigator of these symptoms. In order to determine the type of pathogen(s) present, sections of formalin fixed paraffin embedded (FFPE) tissue will be extracted from sections cut out with a laser microdissection microscope for DNA and downstream PCR. Additionally, skin will be taken from diseased and normal YOY SMB to isolate RNA for the assembly of a skin transcriptome. Skin is the first line of defense for fish, so understanding the types of genes that are turned on in response to a pathogen can provide useful information on a fish's susceptibility to external perturbations or pathogens. Normal skin will be compared with fish exhibiting various symptoms of disease in order to tease out the effect pathogens have on the immune functionality of skin.

Finally, intersex in smallmouth bass has been detected for many years in the Potomac and Susquehanna River watersheds, yet it still remains unknown when young fish become induced with this condition. In order to try and address this question, a gonad transcriptome will be sequenced and biomarker genes will be obtained to determine which genes are present in intersex males that are absent in normal males. Once biomarker genes are picked out, they can be used for qPCR or Nanostring in order to determine differences amongst sites, seasons, and age.

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Progress:

Young-of-the-year smallmouth bass were collected in the summers of 2013-present for the YOY smallmouth bass study. Primers previously made for *M. inornatus* for PCR were labeled with digoxigenin and are being used in an ISH method that has been successfully optimized to work for this parasite/probe pair. Fish preserved in Z-fix and PAXgene are being used to determine which preservative is optimal for hybridization. It has been determined that Z-fix samples decaled with a hydrochloric acid solution are unable to be used for ISH due to the destruction it causes to the DNA. Z-fix preserved samples must be decaled with EDTA in order to be adequate for use in an ISH assay. So far, early infections or areas where no mature spores are present show signs of hybridization with the probe which shows promise for the assay's use for detecting the myxozoan in areas where only inflammation is observed (Fig. 1). Fluorescently labeled probes were also made for *Flavobacterium* spp. in order to determine if co-infections exist. To determine the functionality of the probes, a fluorescent ISH (FISH) assay was conducted on Z-fix preserved skin of channel catfish and hybridization to the target was successful (Fig. 2). Using YOY SMB from 2014, 100 fish were used in a multiplex FISH with *M. inornatus* and *Flavobacterium* spp. probes. Out of 100 fish, only 3 (3%) exhibited co-infections of the myxozoan with *Flavobacterium* (Fig. 3). This information is useful because it indicates that *M. inornatus* does not seem to be a significant inducer of secondary *Flavobacterium* infections in YOY SMB from the Susquehanna River drainage.

Samples of skin from normal and diseased YOY SMB were taken from fish during this sampling season and will be taken during future sampling events as well. Currently, RNA is being isolated and prepared for shipment for Next-gen sequencing.

The gonad transcriptome was assembled with DNA Star using a de novo transcriptome assembly method and the medaka database from NCBI. Transcripts were annotated with Blast 2 Go in order to characterize gene ontology. Following annotation, an RNA Seq analysis was conducted to discriminate differences in gene expression amongst the four treatment groups. The four groups that were compared were immature females, immature males, intersex males, and normal males. Differences in gene expression amongst groups are still being analyzed in order to pick out adequate intersex biomarker genes.

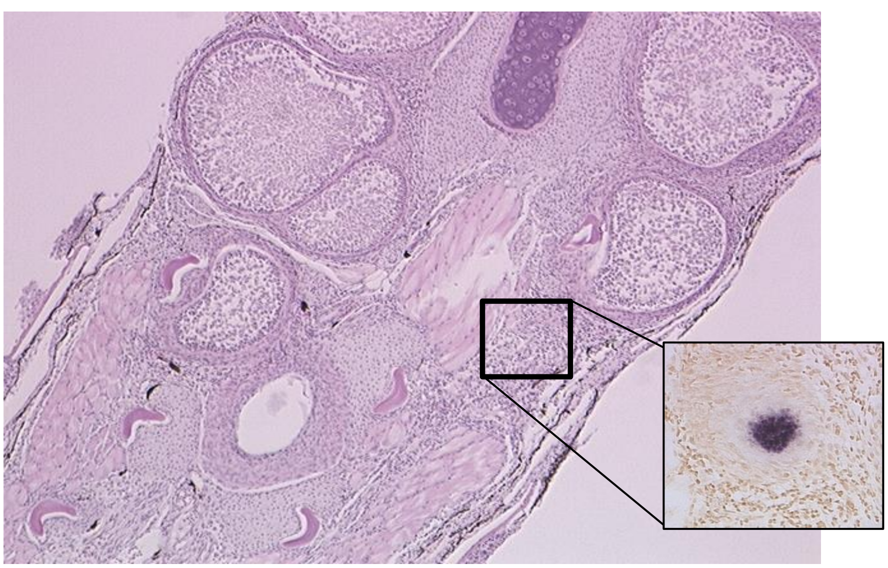


***Figure 1.** In situ hybridization of **M. inornatus** in the connective tissue of the muscle of a YOY SMB. Note the appearance of inflammation with routine histology (H&E stain) and the positive signal of **M. inornatus** (insert, dark purple) obtained with ISH.*

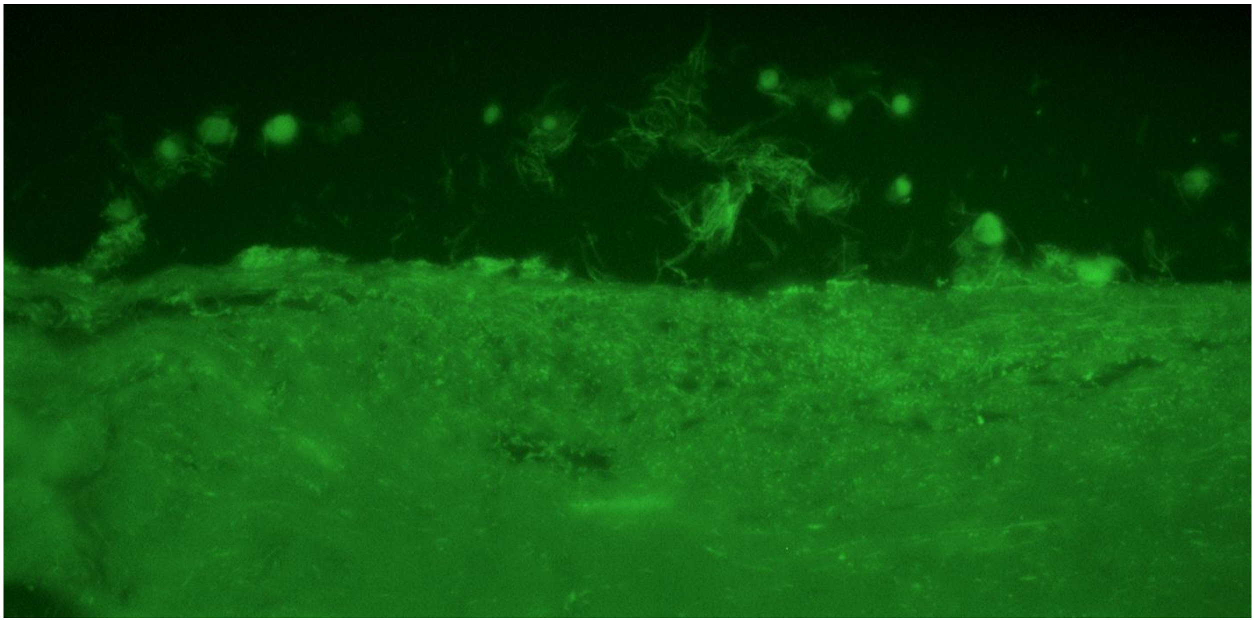


***Figure 2**. Fluorescence ISH of **Flavobacterium** sp. in the skin of a channel catfish.*

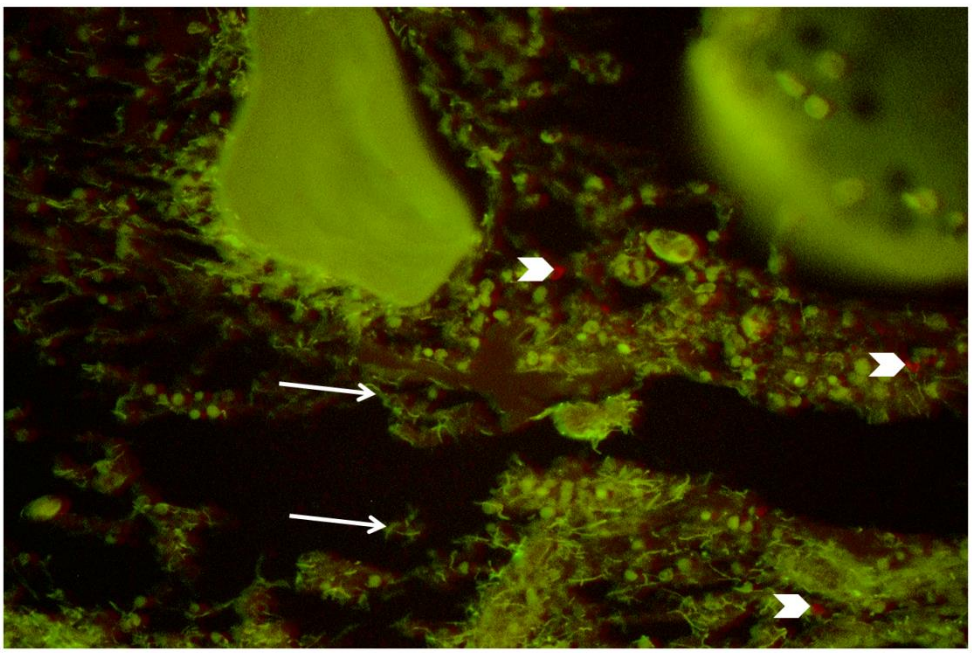


***Figure 3.** Fluorescence ISH exhibiting a dual-infection of **Flavobacterium** sp. (arrow) and **Myxobolus inornatus** (arrowhead; red fluorescence) in the muscle of a YOY SMB*

AQUATIC
OTHER PROJECTS

CONSERVATION ASSESSMENT OF WEST VIRGINIA CRAYFISHES

Principle Investigators: Zac Loughman and Stuart Welsh
Years Ongoing: 2007-2016
Expected completion: 2017
Funding Source: West Virginia Division of Natural Resources

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. Generate an interactive WV crayfish key and web site for public involvement and awareness

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.

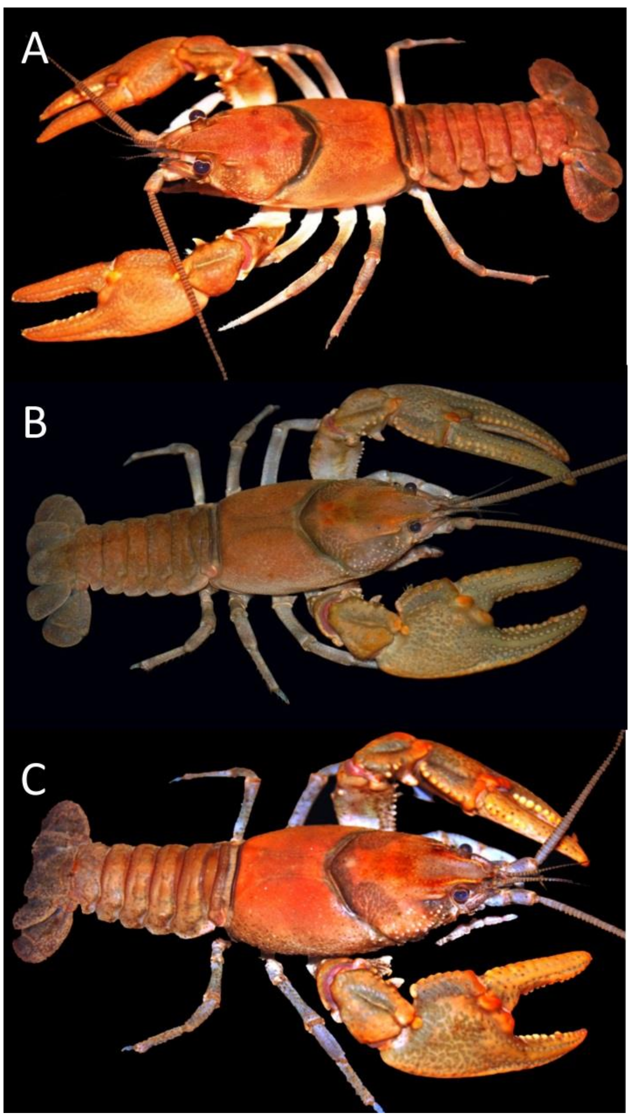
Efforts within the state to identify conservation threats, potential state records, and conservation concerns since the publication of Jezerinac et al. have been disjunct in nature. Key species (*Cambarus* (*H.*) *elkensis*, *Cambarus* (*P.*) *veteranus*), have received attention since Jezerinac's effort, 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 three new species of *Cambarus: Cambarus smilax*, *Cambarus theepiensis*, and *Cambarus hatfieldi*.

Cambarus veteranus (Big Sandy Crayfish) has been a focal species of this work due to its rarity when the state received its initial statewide census in the mid 1980's. Jezerinac et al. determined that *C. veteranus* likely would be extirpated due to land use practices and stream degradation in the West Virginia coal fields. Several investigators have focused on determining the conservation status of *C. veteranus* in the last decade in West Virginia; during these efforts zero *C. veteranus* were observed. In the summer of 2009, all historic locations (n = 17) and additional locations determined through a probabilistic site selection design, were surveyed in the Guyandotte, Bluestone, and Tug Fork river basins to determine if the West Virginia population had been extirpated. Resultant of this effort, *C. veteranus* was discovered at 1 historic station for the species, in Pinnacle Creek, Wyoming County. In addition to the rediscovery of the Pinnacle Creek population, another population was discovered in Dry Fork, a tributary to the Tug Fork River. This population represents a new basin record for the species in West Virginia and appears to be more stable than the Pinnacle Creek population.



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.

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

Finally, *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.

Currently, the first four research objectives of this study have been completed. The research has also expanded to a larger regional effort on crayfishes of the Central Appalachian region, where a book titled "Crayfishes of Central Appalachia" is currently planned for publication in 2018.

EFFECTS OF CORRIDOR H HIGHWAY CONSTRUCTION ON BENTHIC MACROINVERTEBRATE AND FISH COMMUNITIES

Principal Investigators: Stuart Welsh and Jim Anderson
Co-Investigator: Lara Hedrick
Years Ongoing: 2002-2016
Expected Completion: May 2017
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 and Waites 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.

New sites located in watersheds that will be impacted in the Parsons to Kerens alignment have been established and monitoring began in Spring 2013 (Figure 1). Sites are located on the following streams: PK-1 Smokey Hollow, Pk-2 Sugar Camp Run, PK-3 South Branch of Haddix Run, PK-4 Goodwin Run, PK-5 and PK-6 Haddix Run, PK-7 Baldlick Run, PK-8 and PK-9 Wilmoth Run, PK-10 and PK-11 Lazy Run, and PK-12 and PK-13 on Pleasant Run.

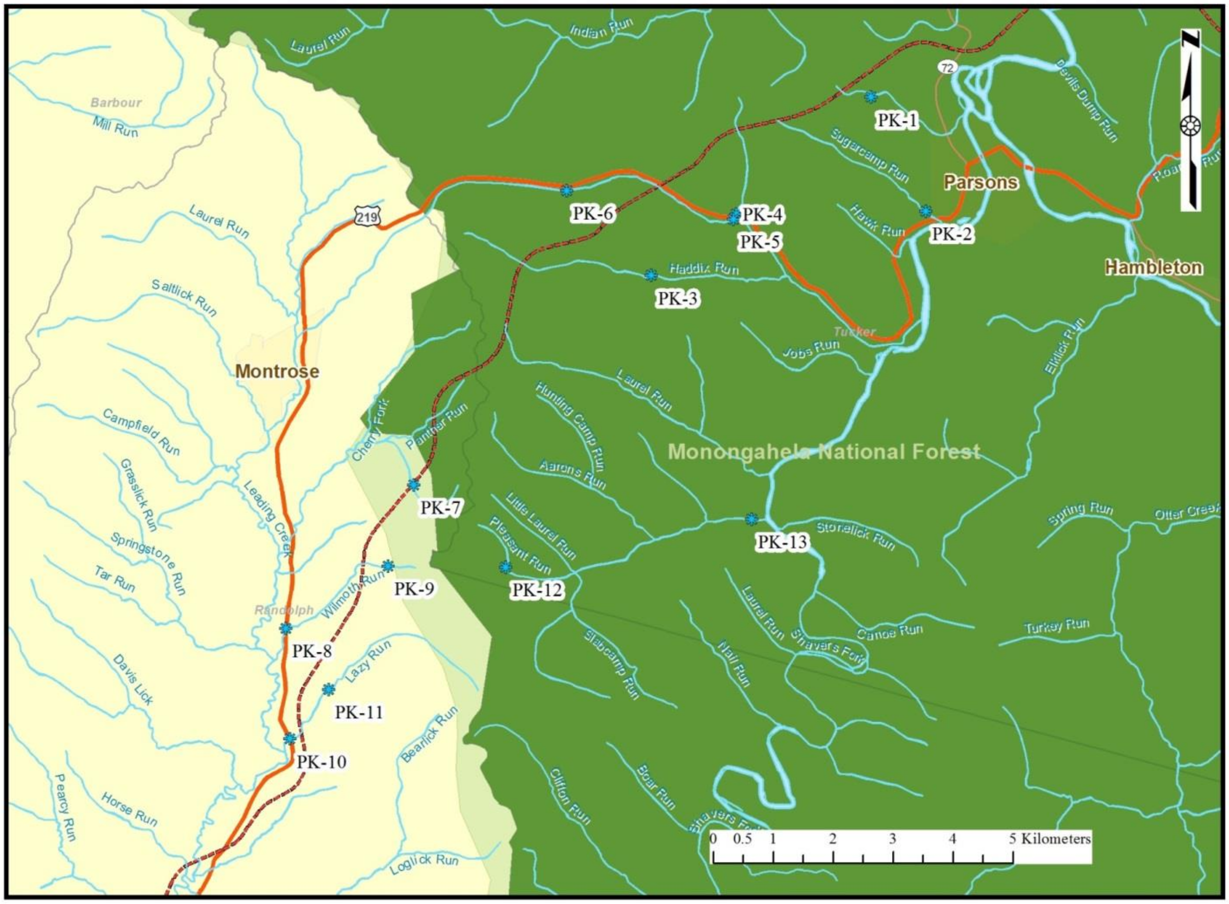


Figure 1. Location of sampling sites along the Parsons to Kerens, WV proposed Corridor H alignment.

In fall 2015 and spring 2016, benthic macroinvertebrate samples were collected in four areas, Patterson Creek, Walnut Bottom, Beaver Creek, and Parsons to Kerens. Benthic macroinvertebrate samples from 2014-2015 are currently being picked and identified in the laboratory. Samples collected in 2013- 2014 from the watersheds were sub-sampled, identified, and analyzed. The 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.

Work to be completed in 2016-2017 includes identification of benthic macroinvertebrate samples collected during 2014-2015. Benthic macroinvertebrate collections will be made in the fall of 2016 and the spring of 2017 at all sites. An annual report will be submitted to the WVDOH.

WILDLIFE

ECOLOGY AND MANAGEMENT OF GOLDEN-WINGED WARBLERS IN THE WEST VIRGINIA HIGHLANDS

Student Investigator: Kyle Aldinger
Principal Investigator: Petra B. Wood
Cooperators: Rich Bailey, Cathy Johnson, Jeff Larkin
Years Ongoing: 2008-Present
Degree Program: PhD
Expected Completion: December 2017
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:
To examine…
1. response of Golden-winged Warblers to habitat management.
2. site-fidelity, survival, and movements of banded males, females, and nestlings.
3. environmental correlates of Golden-winged Warbler and other bird species abundance at multiple spatial scales.

Figure 1. *Golden-winged Warbler nest (Kyle Aldinger).*

Progress:
The Golden-winged Warbler (*Vermivora chrysoptera*, GWWA) is one of 19 North American bird species on the "red watch list" in the 2016 Partners in Flight Landbird Conservation Plan. Red watch list species are extremely vulnerable due to small population size and range, high threats, and range-wide declines and are on a path toward endangerment and extinction without immediate conservation action. GWWA conservation is complicated by recent findings that the species shares 99.97% of its genome with the Blue-winged Warbler (*V. cyanoptera*, BWWA), which is generally considered to be a major threat to GWWA populations because of hybridization. Studies such as ours examining breeding ecology and demographics of GWWA, BWWA, and their hybrids in the West Virginia highlands ultimately will help to inform taxonomic revisions and decisions about Endangered Species Act listing. Our research has focused on potential breeding habitat throughout the West Virginia highlands and is part of two range-wide investigations of GWWA breeding ecology and one range-wide full life-cycle monitoring study.

We found 196 *Vermivora* spp. nests (n=170 GWWA, n=26 other *Vermivora* spp.) during 2008-2014, of which, 142 GWWA and 22 other *Vermivora* spp. nests reached at least egg-laying (Fig. 1). Daily survival rate (DSR) of nests did not differ by species or year, but decreased sharply about 30 days into the nesting season, which started 12 May (± 1.4 days [SE]) each year (Fig. 2). Complete clutch and fledged brood size for *Vermivora* spp. nests were 4.6 ± 0.1 eggs and 3.9 ± 0.2 fledglings, respectively. Three nests were parasitized by Brown-headed Cowbirds.

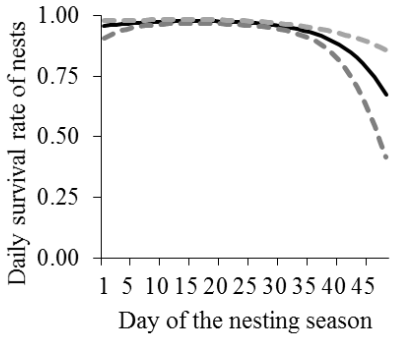


Figure 2. *Daily survival rate (± 95% CI) of Vermivora spp. nests.*

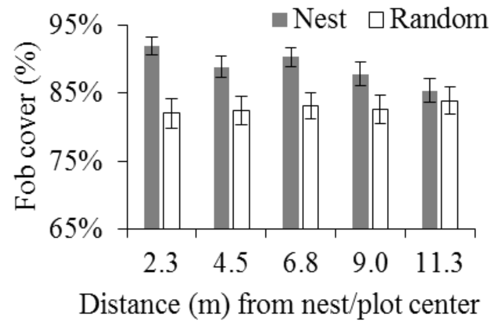


Figure 3. *Forb cover (± SE) as a function of distance from GWWA nest or random plot center.*

We measured detailed spatial arrangement of vegetation characteristics at 121 GWWA nests during 2011-2014 that reached at least egg-laying. GWWA selected areas with more forb and *Rubus* cover than random and their preference for these cover types varied spatially, such that forb and *Rubus* cover decreased as distance from the nest increased (Figs. 3-4).

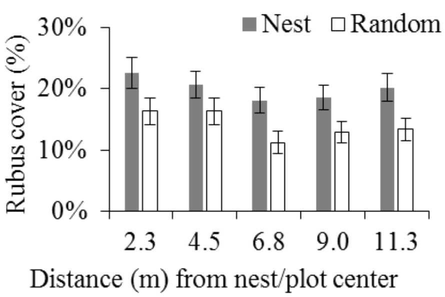


Figure 4. *Rubus cover (± SE) as a function of distance from GWWA nest or random plot center.*

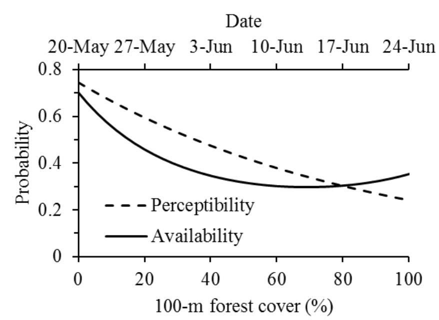


Figure 5. *GWWA male detection probability was negatively associated with date and forest cover.*

During May-June 2008-2016, we conducted 1,096 point counts at 273 point count locations on 122 sites to quantify avian abundance and community structure. Male GWWA availability (the probability that a bird is available for detection) was associated with date and perceptibility (the probability that an observer detects a bird given that it is available for detection) was associated with forest cover within 100 m (Fig. 5). Male GWWA density was associated with northing, minimum elevation, and shrubland cover (Fig. 6), while models with vegetation covariates measured in 0.04-ha plots had no support.

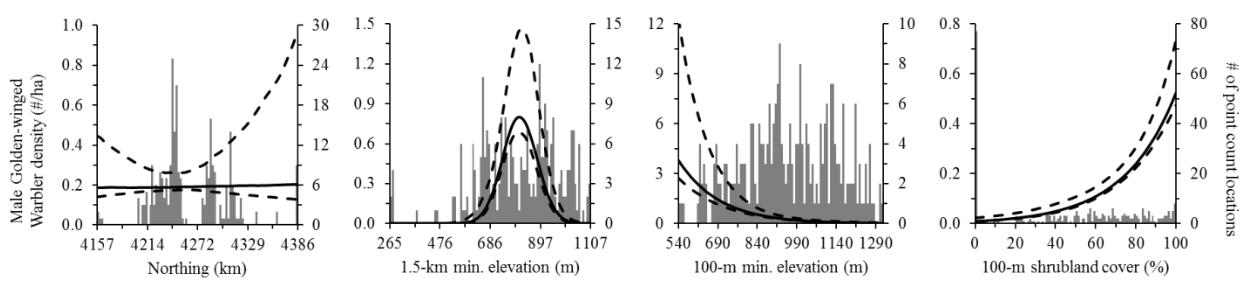


Figure 6. *The most-supported model of male GWWA density included covariates for northing, minimum elevation within 1.5 km, minimum elevation within 100 m, and shrubland cover within 100 m.*

Results from collaborative studies that included our data were used to develop the GWWA Conservation Plan and regional habitat management plans and evaluate the effectiveness of the NRCS Working Lands for Wildlife program. As part of another collaborative study, we fitted 21 GWWA, 1 BWWA, and 1 hybrid with light-level geolocators during May 2016 to track their year-round movements. We observed six Golden-winged Warblers with geolocators in May 2016 and retrieved five of them.

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

Postdoctoral Investigator: Zachary Loman
Principal Investigators: Petra Wood, Daniel Harrison, Cynthia Loftin
Years Ongoing: 2015-2016
Expected Completion: September 2017
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.

Progress:
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 are using 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 are evaluating transferability of the representative species' LC models to the species they are designed to represent. We are also evaluating LC models for two upland gamebird species Ruffed Grouse (*Bonasa umbellus*) and American Woodcock (*Scolopax minor*). From the projects' initiation in September 2015, we have made significant progress in achieving the project objectives soliciting and compiling the necessary data to perform the validation and have completed an assessment. We have completed objective 3 assessing LC predictions of game bird occurrence and abundance at multiple scales and have submitted a manuscript for publication (Loman et al. *in review*). We have completed analysis and have a working draft of a manuscript addressing objective 1 which we intend to submit pending revision of the LC models by the UMass developers based on preliminary results from our analyses.

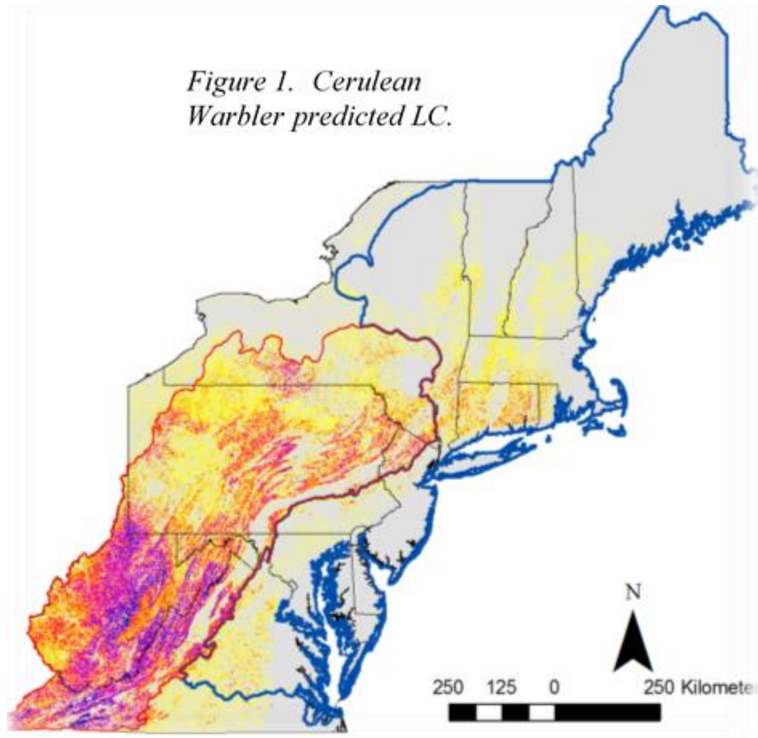
At a point-level for songbirds, LC is a moderate to excellent predictor of songbird occupancy for the seven songbird species modeled that we validated: Blackburnian Warbler (*Setophaga fusca*), Blackpoll Warbler (*Setophaga striata*), Cerulean Warbler (*Setophaga cerulea*). Northern Waterthrush (*Parkesia 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 (Table 1).

Table 1. Landscape Capability (LC) performance metrics as a predictor of occupancy at point-count scale for forest songbirds.

Species	Accuracy	Precision	Cohen's κ	Sensitivity	Specificity
Blackpoll Warbler	**90.2**	0.96	0.85	0.79	0.98
Louisiana Waterthrush	**79.3**	0.83	0.64	0.73	0.85
Northern Waterthrush	**79.6**	0.76	0.62	0.86	0.74
Blackburnian Warbler	**79.3**	0.76	0.61	0.85	0.73
Cerulean Warbler	**73.8**	0.72	0.52	0.79	0.69
Wood Thrush	**68.4**	0.63	0.39	0.89	0.48
Ovenbird	**61.2**	0.60	0.32	0.67	0.56

For point-level density corrected for incomplete detection probability, LC models were a significant positive predictor only for three species: Blackpoll Warbler (R^2 = 0.59, $F_{1,100}$ =146.4, P < 0.01), and Northern Waterthrush (R^2 = 0.09, $F_{1,187}$ = 17.82, P < 0.01), and Louisiana Waterthrush (R^2 = 0.03, $F_{1,1274}$ = 37.96, P < 0.01). These three species are somewhat more specialized in their ecological requirements within the region. LC models did not explain variation in abundance for Blackburnian Warbler (R^2 < 0.01, $F_{1,771}$= 0.59, P = 0.44), Cerulean Warbler (R^2 = 0.01, $F_{1,771}$ = 2.02, P = 0.16), and Ovenbird (R^2 < 0.01, $F_{1,1473}$ = 1.06, P = 0.30), and had a slight negative relationship with Wood Thrush abundance (R^2 = 0.02, $F_{1,201}$ = 4.92, P = 0.03). The Wood Thrush model is currently being recalibrated which is expected to improve model performance.

Our assessment of game bird LC models found that LC models performed as reliable predictors of occurrence, but were less informative as indicators of relative abundance at route (11.5-14.6 km) and point scales (0.5-1 km). LC models were designed as predictors of landscapes' potential to support focal animals, thereby emphasizing model predictive sensitivity. We found LC models as predictors of occurrence had high sensitivity (0.71-0.93), and were accurate (0.71-0.88) and precise (0.88 and 0.92 in best case scenarios for woodcock and ruffed grouse, respectively). Models did not predict point scale abundance independent of the ability to predict occurrence for either species. Because of their design, LC perform better at identifying suitable habitat where animals are likely to occur (i.e., potential habitat) rather than predicting non-occurrence.



Future work and current analysis underway will focus on the transferability of LC models from representative species to those species the models are intended to represent (objective 3).

WILDLIFE RESPONSE TO YOUNG FOREST HABITAT CREATION

Student Investigator: Eric Margenau
Principal Investigator: Petra Wood
Years Ongoing: 2015-Present
Degree Program: PhD
Expected Completion: May 2020
Funding Source: West Virginia Division of Natural Resources

Objective:

Evaluate the response of bird (songbirds, Ruffed Grouse, and American Woodcock) and herpetofaunal communities to the creation of young forest habitat along various landscape features (e.g. gas pipelines, transmission powerlines, and regenerated forest stands) throughout West Virginia.

Progress:

West Virginia is approximately 80% forest cover most of which is in mid- and late-successional age classes. Aging forest conditions in the northeastern US have had a negative impact on over 70 species of wildlife that require some form of disturbance mediated-habitat. Many of these species reside on state-owned wildlife management areas (WMA). Oftentimes, habitat management calls for integrating a variety of habitats thereby creating ecotones that benefit a wide array of wildlife species. This project focuses on a structured way to manage habitats along right-of-ways (transmission powerlines and gas pipelines) and wildlife openings to create young forest habitat that will benefit early-successional bird species and herpetofauna.

Cut-back borders aim to "feather" or "soften" distinct forest edges, increasing the usefulness for wildlife in these areas. Through varied harvest intensities (20 and 60 ft^2/ac basal area retention) and cut-back borders widths (50, 100, and 150 feet into the forest; Fig. 1), we will determine best management strategies to provide beneficial habitat for bird communities and herpetofauna. Additionally, we are looking at other young-forest habitat creation techniques through various regeneration treatments (clearcut-and leave, clearcut-and-windrow, and herbicide via hack-and-spray). While these treatments are not related to our ROW/cut-back border project, the objectives are similar (i.e. create young-forest habitat for bird and herp communities) and the hope is to determine best management strategies that benefit early-successional wildlife.

In 2016, pre-harvest sampling of the bird and herpetofaunal communities was conducted at three West Virginia Division of Natural Resource's WMAs (Burnsville Lake WMA, Little Canaan WMA, and Stonewall Jackson WMA). We surveyed Ruffed Grouse (*Bonasa umbellus*) and American Woodcock (*Scolopax minor*) from mid-April to early-May and the songbird community through point counts (72 points) with each point being visited three times between April – June. We sampled herpetofauna via coverboards (92 coverboard arrays) and drift fences (40 drift fences) during spring (April), summer (June), and late-summer (August) to capture variations in herpetofauna activity. We recorded 84 different species of birds during the breeding season at our study sites, and 7 herp species (Fig 2). Harvest treatments will be implemented in fall-winter 2016-17 and post-treatment sampling will begin on these sites in April 2017.

Figure 1. *Cut-back border layout planned along a transmission powerline at Little Canaan WMA.*

***Figure. 2**. Left: A spotted salamander (**Ambystoma maculatum**) found under a coverboard at Little Canaan WMA (Photo credit: Erin Smith). Right: Wildlife technician Kate H. holding a black rat snake (**Pantherophis obsoletus**) she caught at Little Canaan WMA (Photo credit: Katelyn Horn).*

In 2017, we will add two more study sites (Lewis Wetzel WMA and Buery Mountain WMA) to our project, which will be sampled for pre-harvest data. This will include gas pipelines and power transmission right-of-ways, as well as 4-6 additional regeneration treatments. Additional WMAs will be selected for inclusion to the study in 2018.

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
Degree Program: PhD
Expected Completion: May 2017
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 species whose core breeding range is in the hardwood forests of the Appalachian Mountains, uses heavily forested landscapes with heterogeneous vegetation structure. It is a focal species of management concern because its ~3% annual population decline is one of the steepest declines for any warbler species in North America. Understanding habitat selection on multiple scales and managing for cerulean warbler habitat preferences is critical in reversing this trend and conserving the species. This 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. The West Virginia team is completing global analyses.

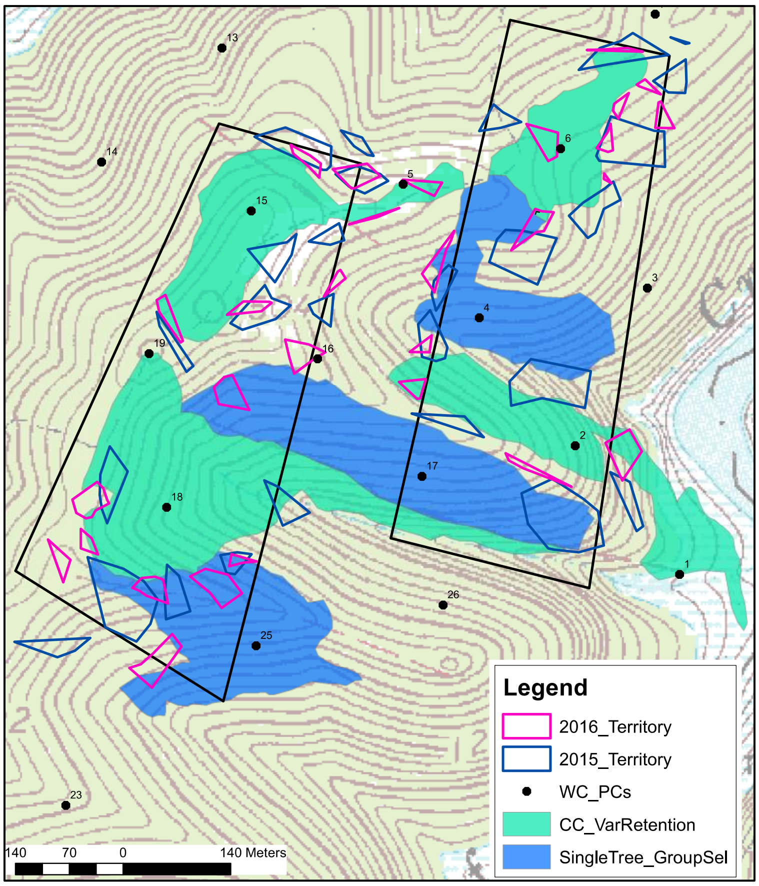


Figure 1. *Cerulean warbler territories from 2015 and 2016 (post-harvest) at the Wolf Creek Harvest Mosaic within the Elk River Wildlife Management Area in Braxton County, WV.*

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. Our results will be used to refine the existing breeding habitat management guidelines.

We quantified songbird 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 songbird abundance and community composition pre- and post-harvest, and spot mapping techniques (only in West Virginia) to evaluate changes in territory density pre- and post-harvest of six focal species (eastern towhee [*Pipilo* *erythrophthalmus*], indigo bunting [*Passerina cyanea*], hooded warbler [*S. citrina*], cerulean warbler, wood thrush [*Hylocichla mustelina*], and worm-eating warbler [*Helmitheros vermivorum*]) representing a range of preferred basal areas. Ten spot mapping plots 16-18 ha in size were placed over the harvest mosaics in West Virginia (Fig. 1) and visited 6-8 times per season to determine territory locations of the six focal species.

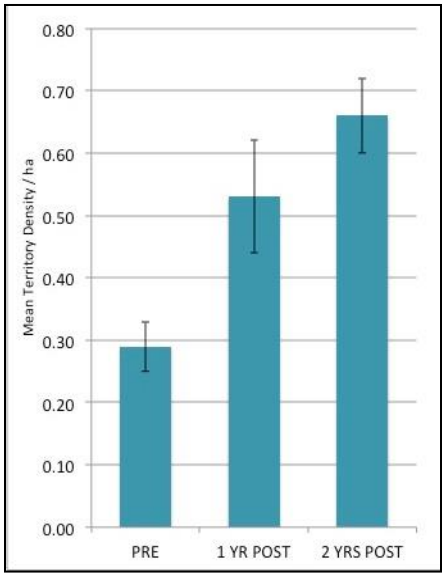
Cerulean results presented here summarize data from Kentucky, Virginia, and West Virginia where we have pre- and post-harvest data for comparison (ceruleans were not detected at the pre-post sites in Pennsylvania). Results for the other five focal species include Pennsylvania. For point count data summaries, we excluded flyovers, species that are not well sampled by point counts, and birds detected >50m from the observer.

Across the three states, ceruleans were detected at 43% of points pre-harvest. Post-harvest, ceruleans were detected at 86% of harvest points, 57% of edge points, and 61% of reference points. Following harvests, ceruleans colonized 10 harvest interior points, 8 edge points, and 14 reference points, while they went extinct at only 1 harvest interior point, 4 edge points, and 5 reference points. Ceruleans exhibited a spatial shift at both the point and site level as seen in the colonizations and extinctions at points as well as a change in location of territories. Post-harvest territories tended to be clustered at the edges of harvests (Fig. 1). Cerulean territory density increased annually post-harvest (Fig. 2, Table 1). Ceruleans increased at harvest interior points one-year post-harvest and then decreased, but increased at edge points two-years post-harvest (Fig. 3). This response may be due to lighter, smaller harvests in Kentucky, which initially opened the canopy in the first year post-harvest, but then closed after two years, reversing the temporary increase in appropriate breeding habitat for ceruleans.

Table 1. Mean territory densities (territories/ha) for six focal species at Stonewall Jackson Lake WMA in Lewis County and Wolf Creek in Braxton County, West Virginia.

	Pre-harvest	**1 Year Post**		**2 Years Post**
Cerulean warbler	0.22	0.42		0.69
Eastern towhee	0.11	0.49		0.61
Hooded warbler	0.16	0.24		0.45
Indigo bunting	0.00	0.16		0.48
Worm-eating warbler	0.02	0.02		0.02
Wood thrush	0.37	0.25		0.26

Territory densities of Eastern towhees, indigo buntings and hooded warblers also increased annually post-harvest (Table 1) as did relative abundance, particularly at harvested points (Table 2). Eastern towhees responded immediately following harvests as they can take advantage of slash piles for nesting and foraging. Indigo buntings and hooded warblers showed a steadier increase over time (Tables 1 and 2), as they require regeneration of the understory and shrubs for nesting habitat. Worm-eating warblers were never abundant on the sites in West Virginia (they were never detected at Stonewall Jackson WMA) and despite increasing slightly at reference points one-year post-harvest (Table 1), ultimately they were nearly extirpated from the sites. Wood thrush decreased slightly, but exhibited a spatial shift to reference points and territories in reference areas or on the edges of harvests, rather than leaving the sites completely (Table 2).



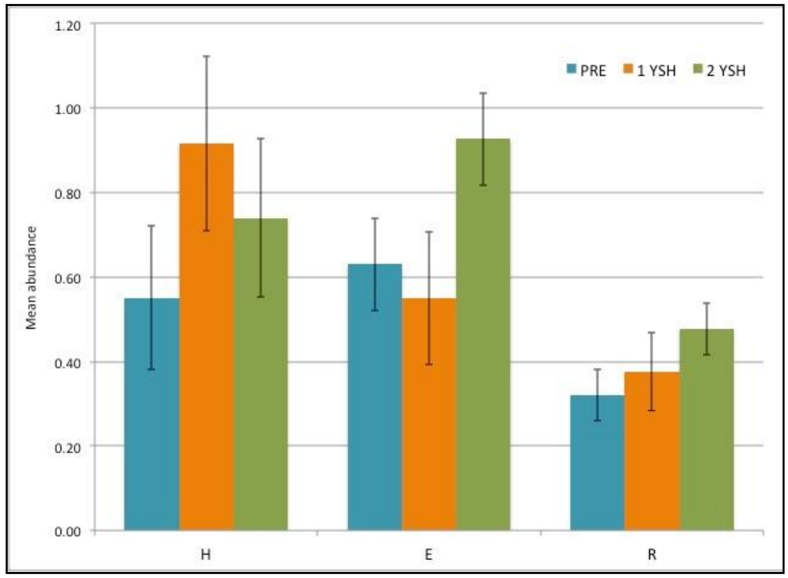


Figure 2 *(left). Mean cerulean warbler territory density pre- and post-harvest at Stonewall Jackson Lake WMA and Elk River WMA, WV.* ***Figure 3*** *(right). Cerulean warbler abundance (mean ± SE) pre- and post-harvest by years-since-harvest (YSH) in Kentucky, Virginia, and West Virginia for harvest interior (H), harvest edge (E), and reference (R) points.*

Table 2. Relative abundance (mean ± SE) from the 2013-2016 seasons for sites with pre- and post-harvest data in Kentucky, Pennsylvania, Virginia, and West Virginia. H = harvest interior and edge points combined, R= reference point

	Pre-harvest		**1 Year Post**		**2 Years Post**	
	H	**R**	**H**	**R**	**H**	**R**
EATO	0.44±0.07	0.57±0.09	0.97±0.11	0.66±0.13	1.13±0.13	0.52±0.12
HOWA	0.38±0.06	0.38±0.06	0.44±0.07	0.21±0.07	0.55±0.09	0.29±0.09
INBU	0.09±0.06	0.20±0.05	0.33±0.07	0.20±0.07	0.85±0.09	0.11±0.04
WEWA	0.30±0.05	0.28±0.65	0.14±0.04	0.27±0.07	0.10±0.03	0.15±0.04
WOTH	0.88±0.08	0.57±0.07	0.51±0.08	0.92±0.11	0.34±0.08	0.76±0.11

Final analyses will be completed by January 2017, with project completion expected in May 2017. We plan to estimate at which range of residual basal area ceruleans are most abundant and cerulean territories exhibit the highest density. We will look at the relationships between ceruleans and vegetation and landscape variables that are known to influence their occupancy and abundance in unharvested conditions (e.g., presence of preferred tree species, aspect, slope position) to determine if they are still important to habitat selection after opening the canopy. We will update the breeding habitat management guidelines as needed. This information can be passed on to foresters and landowners interested in managing for cerulean warblers and possibly the suite of wildlife species that responds positively to these particular silvicultural prescriptions.

EFFECTS OF FOREST MANAGEMENT ON AVIAN ABUNDANCE AND OCCUPANCY IN SPRUCE-FIR FORESTS OF NEW ENGLAND

Student Investigator: Lenza Paul
Principal Investigators: Yong-Lak Park, Donald Brown, Petra Wood
Years Ongoing: 2016-Present
Degree Program: MS
Expected Completion: May 2018
Funding Source: National Park Service

Objectives:

1. Determine the diversity and abundance of soil macroinvertebrates and salamanders relative to frequency and extent of imidacloprid treatments.

2. Investigating spatio-temporal dynamics of soil macroinvertebrate populations relative to imidacloprid treatment.

Progress:

Hemlock forests are major contributors to the ecological, aesthetic, and recreational values of New River Gorge National River (NERI), Gauley River National Recreation Area (GARI), and Bluestone National Scenic Area (BLUE). Hemlock-dominated forest creates unique micro-climates providing favorable habitats for certain animals and affecting understory plant species composition, biomass, and productivity among various hemlock stands. Hemlocks cover 11,690 acres in these three national parks in West Virginia and 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. This research is designed to determine the diversity and abundance of soil and benthic macroinvertebrates and salamander species (Fig. 2) in relation to imidacloprid treatment histories within hemlock stands.

Figure. 1. *Hemlock woolly adelgid on eastern hemlock needles (photo by John Perez)*
Figure 2. *Red-eft (photo by Matt Shumar)*

To assure that the imidacloprid-treatment history in the parks reflects the presence and 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 will be determined using a competitive Enzyme-Linked Immunosorbent Assay (ELISA) technique. Once these analyses are completed, final study sites will be selected. We will choose 20 research plots across the parks, which will comprise two levels of pesticide application (no and high imidacloprid inputs) and two different levels of soil moisture (mesic and hydric conditions) with five replications of each. In fall 2016, coverboards for sampling salamander populations will be placed in each plot and sampling will begin in spring 2017. Soil and invertebrate samples will be collected at the same locations.

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

EFFECTS OF FOREST MANAGEMENT ON AVIAN ABUNDANCE AND OCCUPANCY IN SPRUCE-FIR FORESTS OF NEW ENGLAND

Student Investigator: Brian Rolek
Principal Investigators: Petra Wood, Daniel Harrison, Cynthia Loftin
Years Ongoing: 2012-2016
Degree Program: PhD
Expected Completion: May 2017
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:

1) How does abundance of focal species respond to silviculture?

2) How does forest management influence forest attributes?

Progress:

Spruce-fir associated birds have recently declined (Fig. 1) and commercial harvesting within northern New England has a dominant influence on landscape structure and composition. We tested the influence of mature forest structure (quadratic mean diameter) and tree species composition (proportion of spruce-fir trees) on 14 focal bird species that use spruce-fir habitat and related mature forest structure to forest management treatments. Focal bird species included spruce-fir obligates and associates: Yellow-bellied Flycatcher (*Empidonax flaviventris*), Gray Jay (*Perisoreus canadensis*), Boreal Chickadee (*Poecile hudsonicus*), Red-breasted Nuthatch (*Sitta canadensis*), Golden-crowned Kinglet (*Regulus satrapa*), Ruby-crowned Kinglet (*Regulus calendula*),.Swainson's Thrush (*Catharus ustulatus*), Bay-breasted Warbler (*Setophaga castanea*), Cape May Warbler (*Setophaga tigrina*), Blackburnian Warbler (*Setophaga fusca*), Magnolia Warbler (*Setophaga magnolia*), Blackpoll Warbler (*Setophaga striata*), Palm Warbler (*Setophaga palmarum*), and White-winged Crossbill (*Loxia leucoptera*).

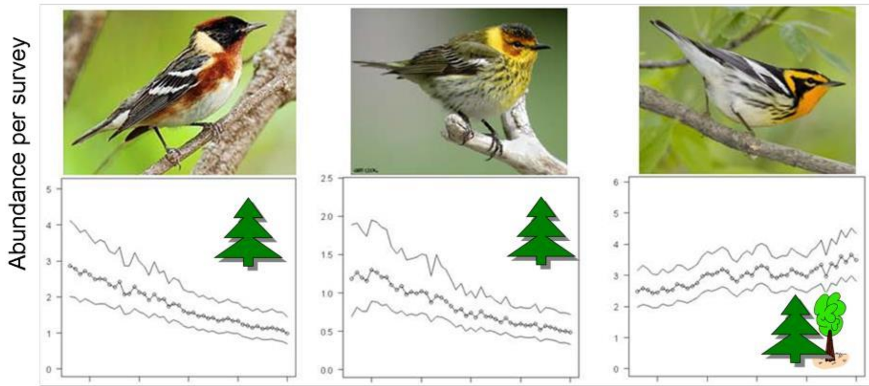


Figure 1. *Softwood bird trends, 1966-2010, in Bird Conservation Region 14 from Breeding Bird Survey data. Left to right are Bay-breasted, Cape May, and Blackburnian warblers.*

We sampled six silvicultural treatments including clearcut, clearcut with herbicide, precommercially thinned, precommercially thinned with herbicide, 1st stage shelterwood, and selection harvests along with mature forest reference stands to obtain samples from the diversity of harvest techniques and forest structures present on the landscape in Bird Conservation Region 14 within the northeastern United States (Fig. 2). We conducted bird surveys in 2013-2015 at 657 point count locations 3 times per year. Physiognomic and compositional vegetation characteristics were measured at each point in 2014.

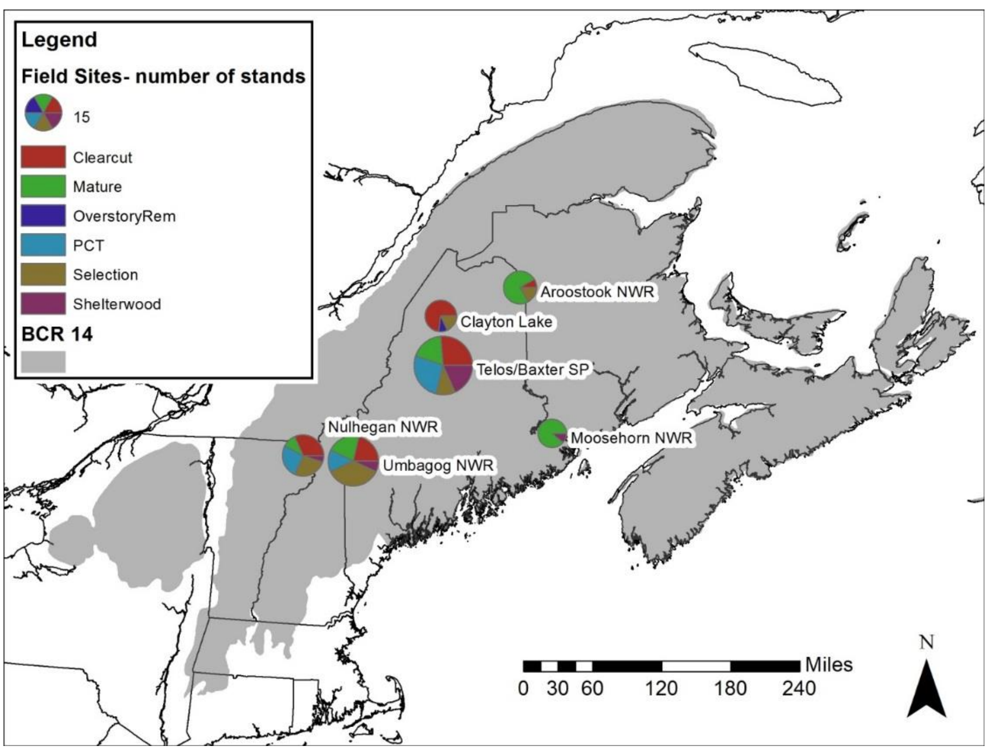


Figure 2. *Study stands were located throughout northern New England that were >30 acres and contained primarily softwood trees. Size of pie charts is proportional to the number of stands at each site.*

We used generalized linear models to compare vegetation variables among silvicultural treatments. Partial harvest treatments maintained mature forest structure (measured as quadratic mean diameter) similar to that of mature forests (Fig. 3). Post-harvest treatments (herbicide and pre-commercial thinning) shifted tree species composition toward spruce-fir.

We modeled avian occupancy corrected for detection probability in response to mature forest structure (quadratic mean diameter) and tree species composition (proportion of spruce-fir trees). We also modeled stand-level avian richness of spruce-fir obligates and spruce-fir associates and their response to mature forest structure and tree species composition using generalized linear models with Poisson distributions and correcting for stand area surveyed using the semi-log species area relationship because the number of point counts in each stand varied. Occupancy of spruce-fir obligates and associates responded positively or non-significantly to spruce-fir forests, suggesting management for spruce-fir species could target increased spruce-fir composition. In contrast, avian occupancy responses to mature forest structure were highly variable, with some birds showing nearly opposite occupancy responses (e.g. Yellow-bellied Flycatcher and Red-breasted Nuthatch in Fig. 4), suggesting management for mature forest structure will depend on the focal species of interest. Avian richness of spruce-fir obligates and associates largely agreed with single-species responses. Richness increased greatly in stands with higher spruce-fir composition and decreased slightly with more mature forest structure. Our results can directly inform management of spruce-fir forests and help identify important conservation areas for spruce-fir birds.

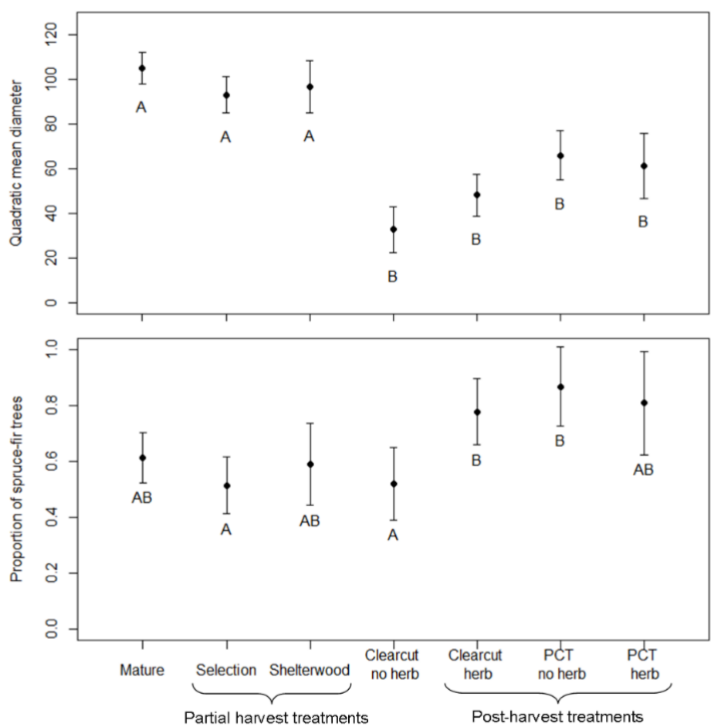


***Figure 3**. Generalized linear models predicting mean vegetation response and 95% confidence intervals to silvicultural treatments and mature reference stands. Statistically similar treatments are identified with the same letter.*

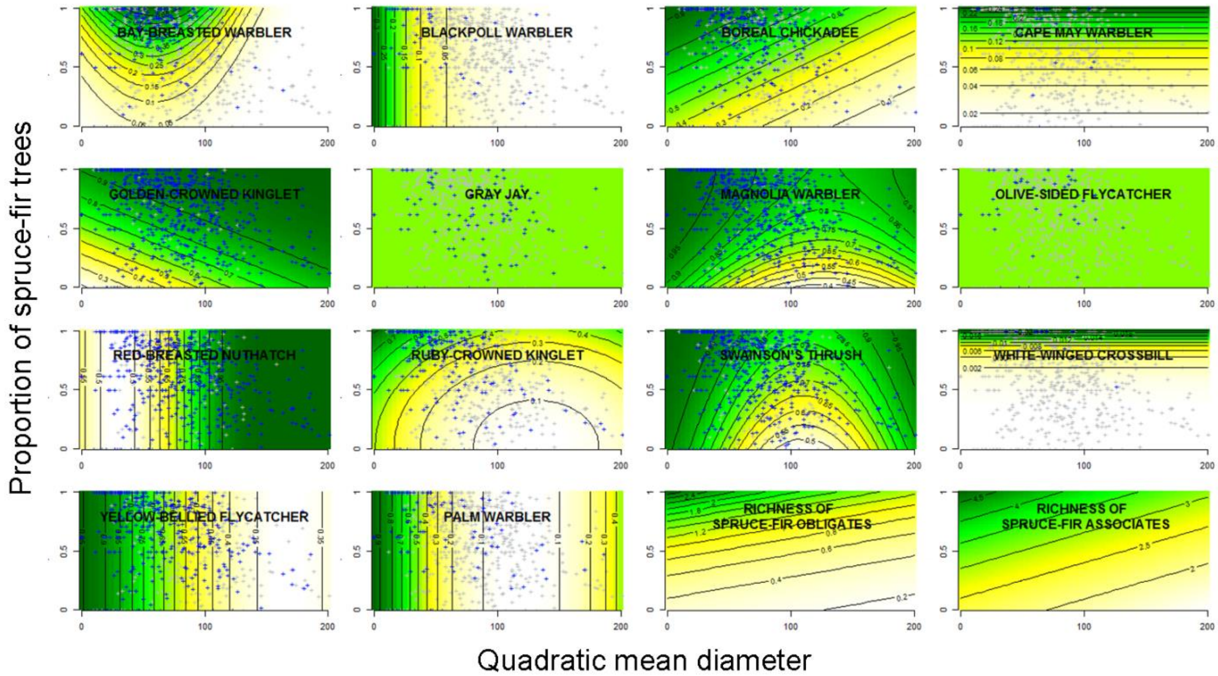


Figure 4*. Occupancy responses corrected for detectability by 14 spruce-fir obligates and associates (color gradient and contours) to mature forest structure (quadratic mean diameter) and tree species composition (proportion of spruce-fir trees). Also shown are richness responses by spruce-fir obligates and associates. Gray '+' are sampled sites where the species was absent and blue are sampled sites where the species was present.*

USE OF A HIGH RESOLUTION SATELLITE IMAGE AND DIGITAL ELEVATION MODEL TO PREDICT THE RIDGE FOREST BIRD COMMUNITY, AND FACTORS LEADING TO SONGBIRD SPECIES DISTRIBUTION PATTERNS AND CERULEAN WARBLER CLUSTERING AT THE LEWIS WETZEL WILDLIFE MANAGEMENT AREA, WV

Student Investigator: Jim Sheehan
Principal Investigator: Petra B. Wood
Cooperators: Harry Edenborn, Randy Dettmers, T. Bentley Wigley
Years Ongoing: 2010-Present
Degree Program: PhD
Expected Completion: December 2016
Funding Sources: Department of Energy, U.S. Fish and Wildlife Service, National Fish and Wildlife Foundation

Objectives:

1. Determine if remote sensing information from a satellite image and a digital elevation model can predict habitat characteristics and the avian community found on mature hardwood-forested ridges.

2. Use this remote sensing information along with habitat data to study the distribution patterns of territories of ridgetop Cerulean Warblers, Hooded Warblers, and Ovenbirds.

3. Investigate how territories of Cerulean Warblers cluster in relation to topography, forest structure and composition, and conspecific presence.

Progress:

The heavily forested ridgetops of northwestern West Virginia harbor a rich avian community that is an important component of the native biodiversity of the Central Appalachians. These ridgetops also are the focus of much energy industry activity, particularly the development of Marcellus Shale natural gas. The Marcellus well pads and associated roads and pipelines, as well as conventional oil and gas development, have the potential to affect a significant portion of the region's ridgetops and associated breeding bird species, including the sharply declining Cerulean Warbler. To see if remote sensing data can be used to predict the habitat use of forest songbirds potentially affected by this activity, a high-resolution, leaf-on 2009 Quickbird satellite image and a 3-meter digital elevation model (DEM) were used to analyze avian survey data and habitat measurements on ridgetops at the Lewis Wetzel Wildlife Management Area, WV.

For the first objective, image spectral brightness and several measures of image texture (the spatial variability of image pixel values) predicted a gradient in forest structure and composition, ranging from less complex, chestnut oak-dominated forest to more complex, sugar maple-dominated forest. The richness of canopy gap understory-associated bird species obtained with 2010-11 point count data was positively related to the strongest image texture measure, the standard deviation of panchromatic image pixel values (Fig. 1). These findings support the use of remote sensing data in lieu of intensive field surveys to provide valuable information on forest composition and structure at the site, and indicate that a number of bird species depend on structurally heterogeneous forests.

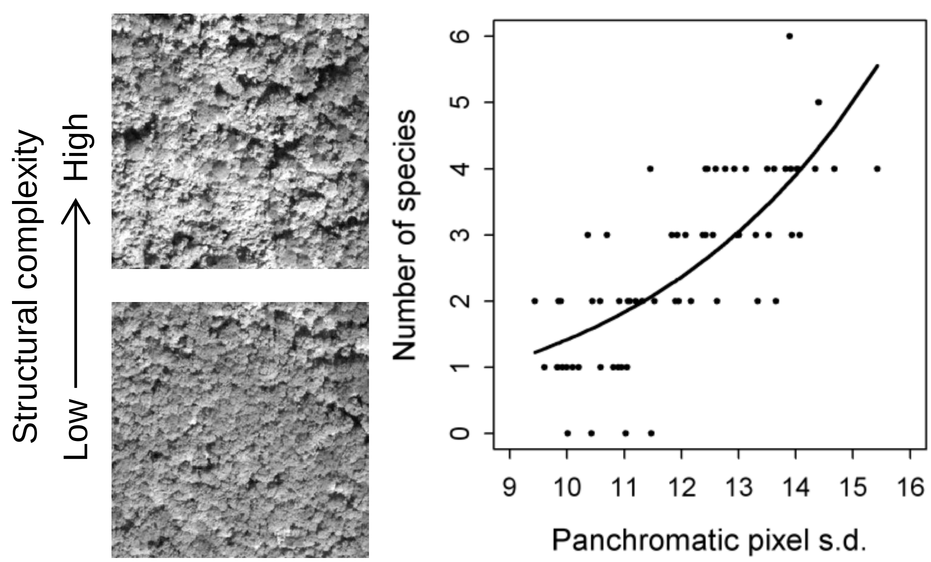


***Figure 1**. A gradient in forest structural complexity measured by panchromatic pixel s.d. (left). Richness of six forest gap understory-associated bird species increased in relation to a Quickbird satellite image texture measure of this gradient.*

For the second and third objectives, analyses focused on the distribution patterns of three territorial songbirds obtained from 2010-11 transect surveys placed on focal ridgetop. Cerulean Warblers, Hooded Warblers, and Ovenbirds (Fig. 2 top) are widespread and abundant at the site, and differ in the forest habitat components they require for breeding. Based on exploratory point pattern analysis at a local (within-ridgetop) scale, each species exhibited different patterns of territorial spacing. Clustering was the dominant pattern for the Cerulean Warbler, while the Hooded Warbler and Ovenbird showed inhibition (i.e., repulsion) at short distances, some indication of clustering, and then a mostly random distribution (Fig. 2).

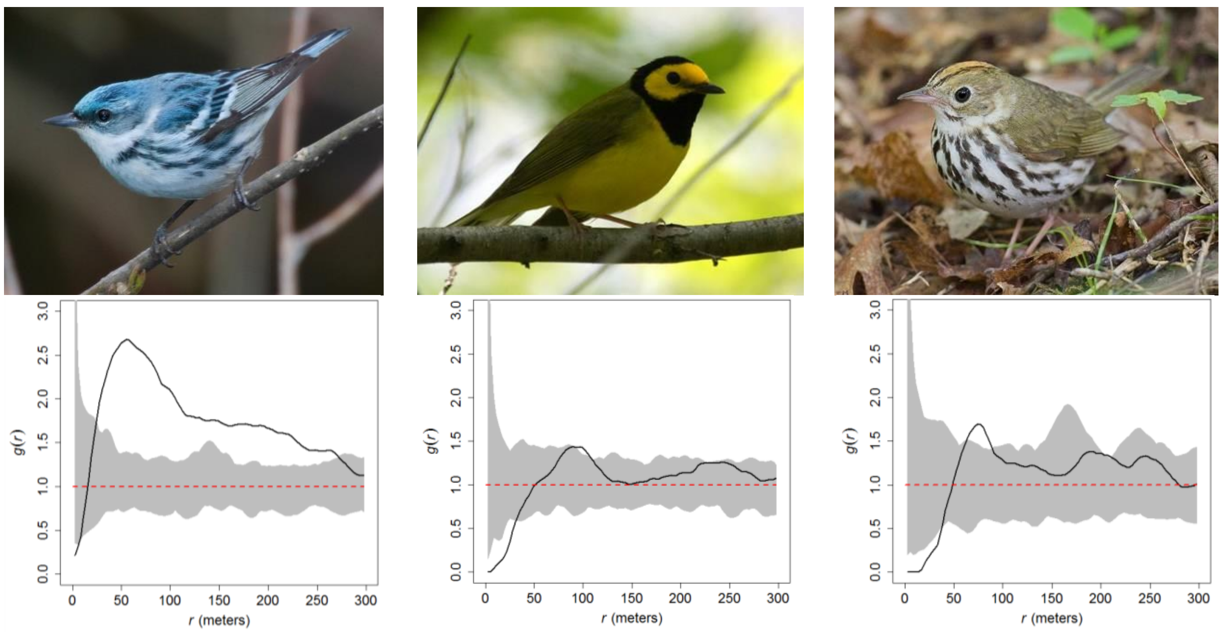


Figure 2. *Top: The upper canopy nesting Cerulean Warbler (left), shrub nesting Hooded Warbler (center), and ground nesting Ovenbird (right). Bottom: Pair correlation functions (thick black lines) and point-wise significance envelopes (gray bands) for complete spatial randomness of the species' territory point patterns on the focal ridgetops. Territorial clustering is indicated at distances where the black line is above the envelope and inhibition (i.e., repulsion) among territories is below the envelope.*

A multivariate analysis technique, redundancy analysis (RDA), was used to relate field-collected habitat data to satellite data and DEM-derived topographic information, and to map an index of forest structure and composition on the studied ridgetops. This index, when coupled with topographic aspect, differentiated the territories of Hooded Warblers, which occurred primarily on eastern aspects with high structural complexity, from those of Ovenbirds, which occurred primarily on western aspects with moderate structural complexity (Fig. 3; left). Cerulean Warbler territories, while primarily eastern in aspect and with moderate to high structural complexity, were also strongly associated with locally high elevations ("knolls") and anthropogenic interior forest edges such as a ridgetop pipeline (Fig. 3; right).

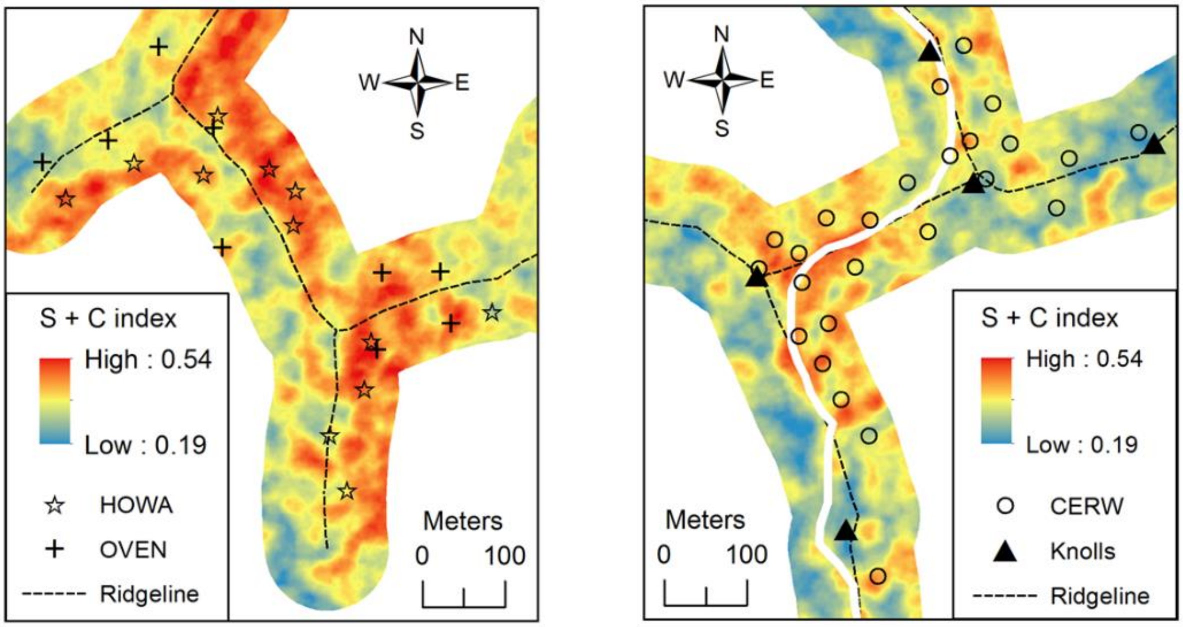


***Figure 3**. Examples of the ridgetop RDA index of forest structure and composition (S + C index) and the species' territory distributions. Left: Hooded Warbler (HOWA) and Ovenbird (OVEN) territories. Right: Cerulean Warbler (CERW) territories, topographic knolls, and a narrow ridgetop pipeline (white line).*

Based on these results, the remote sensing data is being incorporated in further point pattern analyses that control for environmental influences on the species' observed point patterns. This is an important step to better understand the potential influence of interactions between individuals (attraction and repulsion) on their territorial spacing. For the Cerulean Warbler in particular, determining if clustering includes a strong component of social attraction in addition to effects of habitat and anthropogenic forest edge has important implications for populations of this high conservation priority species. Overall, these analyses suggest the value of remote sensing-based approaches for fine-scale modeling of these species where their populations intersect with energy industry activities throughout the region.

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

Student Investigator: Laura Farwell
Principal Investigator: Petra B. Wood
Cooperators: Randy Dettmers, Todd Fearer, Margaret Brittingham
Years Ongoing: 2013-present
Degree Program: PhD
Expected Completion: May 2017
Funding Source: US Fish and Wildlife Service

Objectives:

1. Assess breeding songbird response to shale gas well and infrastructure development by comparing avian guild species richness, focal species relative abundance and spatial distribution at sites impacted by shale gas to comparable unimpacted control sites.

2. Determine how far edge effects on avian populations extend into adjacent forests from well pads and infrastructure, and identify potential thresholds of forest loss and fragmentation due to shale gas beyond which forest interior birds are negatively impacted.

3. Develop recommendations regarding well pad location, size, and density to minimize negative impacts to birds. Identify vegetation management approaches that will minimize fragmentation effects while maximizing habitat for early successional species.

Progress:

Extraction of natural gas from Marcellus-Utica shale has increased exponentially in the central Appalachians, yet much is still unknown about the potential biological impacts of this industry in forest ecosystems. To identify the response of breeding songbirds to forest disturbance due to the development of shale gas well pads and infrastructure, we sampled 191 study sites across the Marcellus-Utica region during 2014-2015, including 120 impacted sites and 71 control sites (Fig. 1). Sites were chosen using semi-random stratified selection to ensure a wide range of values across target gradients and to minimize correlations among predictor variables. Sites represented a gradient of forest cover (30-90%) and a range of well pad sizes, ages, densities, and placements on the landscape. Sites consisted of 1-km radius plots (314 ha) centered on a shale gas well pad, a cluster of pads, or a pipeline. Control sites had no shale gas development within a minimum 4-km radius, and were selected across similar gradients of forest cover and levels of human disturbance that were unrelated to shale gas.

We conducted avian point count and vegetation surveys at 2,583 sampling points within these 191 study sites (averaging 13.5 points per site). At least 2 points per site were placed in habitat directly disturbed by shale gas development (or other types of human development at control sites), with remaining sampling points radiating outwards into surrounding forested areas. Since we are most interested in the impacts of relatively fine-resolution well pads and infrastructure, we are currently hand-digitizing land cover at each site using high-resolution satellite and aerial imagery. We categorize land cover by specific type of shale gas activity (e.g., well pad, pipeline, access road) and also within broader categories of land use (e.g., forest, non-forest related to shale gas disturbance, and non-forest unrelated to shale gas). We are digitizing land cover within a 2-km radius (1,257 ha) of the center of each site, to assess the extent of the influence of shale gas development, as well as surrounding landscape matrices, on breeding birds (Fig. 2).

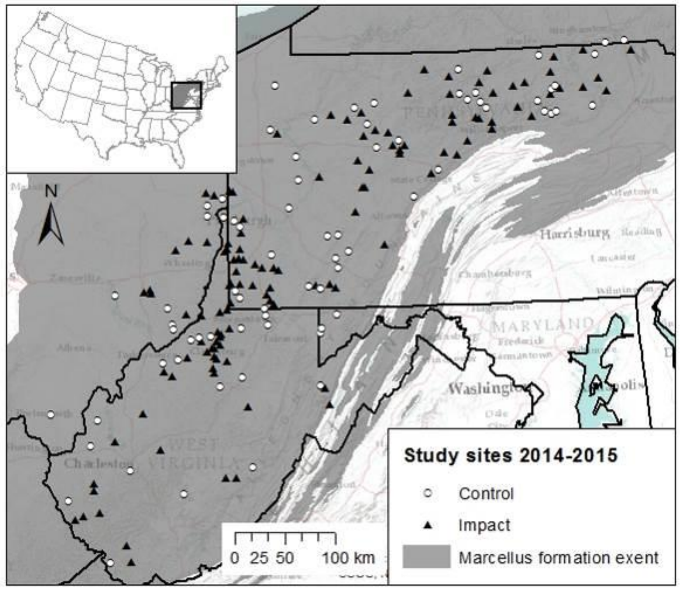


***Figure 1**. Field site locations from 2014-2015, in WV, OH, PA, MD, NY: black triangles represent shale gas impacted sites (n = 120), white circles are reference sites (n = 71).*

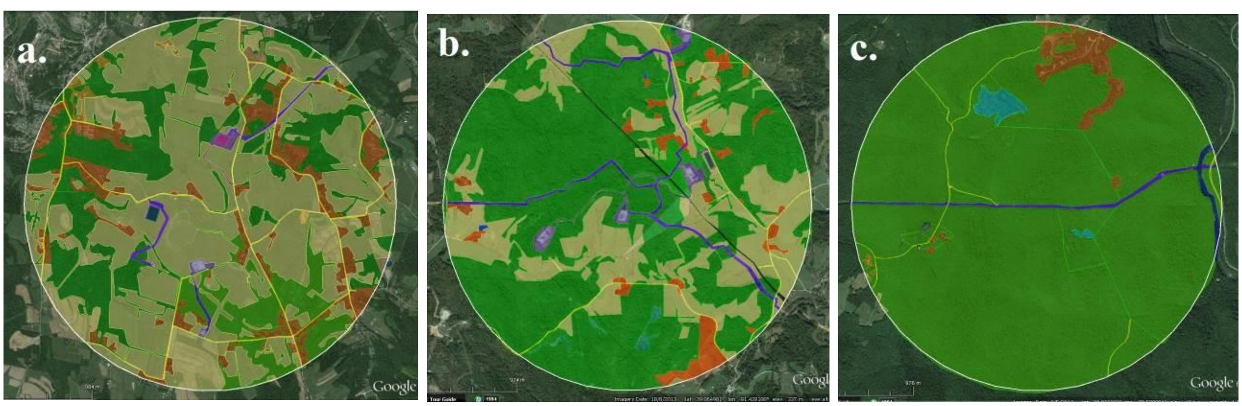


***Figure 2**. Hand-digitized, 2-km radius land cover maps, at three sites representing a gradient of forest cover: (a) low, (b) moderate, and (c) high. Forest cover shown in green, shale gas in purple, non-shale gas in red and yellow.*

We then convert digitized vector maps to 1-m resolution raster land cover grids, and calculate land cover metrics within 100-m, 500-m, and 1-km buffers around individual point count locations, as well as across each 2-km radius landscape. We are currently exploring statistical models relating avian guild species richness, focal species abundances and spatial distributions versus land cover and fragmentation metrics, focal well pad and pipeline characteristics, field-based habitat variables, forest type and eco-region. Preliminary results based on a small subset of our data suggest that some species have a unique response to shale gas disturbance of forested landscapes (e.g., brown-headed cowbirds), while other species appear to respond similarly to both shale gas development and other types of non-gas related human disturbance. Our results should be finalized by May 2017.

LOUISIANA WATERTHRUSH RESPONSE TO MARCELLUS SHALE GAS DEVELOPMENT IN WEST VIRGINIA

Student Investigator: Mack Frantz
Principal Investigator: Petra B. Wood
Cooperators: Amy Welsh, Steve Latta, George Merovich
Years Ongoing: 2013–2016
Degree Program: PhD
Expected Completion: May 2017
Funding Sources: US Geological Survey, WV Division of Natural Resources, US Department of Energy, Pittsburgh National Aviary, Eastern Bird Banding Association, FCSL Association, West Virginia University

Objectives:
1. Determine how gas well development activities influence Louisiana Waterthrush (*Parkesia motacilla*) territory density and size, benthic macroinvertebrate community structure at foraging & nest locations, nest survival and productivity, riparian habitat quality, and site fidelity.

2. Determine how gas well and infrastructure development influence Louisiana Waterthrush epigenetic (DNA methylation) variation between areas of disturbed and undisturbed streams, and to determine how DNA methylation varies among individuals.

Progress:
Aquatic monitoring of core forested regions is needed, particularly where headwater streams occur, due to increasing shale gas development in these areas. Gas wells by surface waters may affect aquatic systems by potentially exposing them to increased sedimentation run-off, streamflow alterations from water withdrawal, and surface water contamination from gas well wastewater. Our research is comparing disturbed and undisturbed streams on the Lewis Wetzel Wildlife Management Area (LWWMA) on data collected during 2009–2015.

Nest monitoring occurred March 28 to 31 July 2009–2015 by searching 58.2 km of 14 headwater streams. A nest was considered successful if it produced at least one fledgling. Nest survival of 280 nests calculated with Program MARK varied annually and generally declined over the study (Table 1). Of 11 *a priori* candidate models containing temporal, gas, and habitat covariates, four that had temporal and shale gas covariates were the most supported ($\Delta AICc < 2$) and two of these models accounted for most of the variation in daily survival rate (DSR) ($w_i = 0.28$ and 0.27). Once accounting for temporal effects (rainfall and year), shale gas development had weak negative effects on nest survival (Fig. 1). Annual population productivity (# fledglings/successful nest/male) declined over time (Table 1). Individual annual productivity was lower in disturbed areas (1.4 ± 0.004 fledglings) than it was in undisturbed areas (1.6 ± 0.005).

We banded 435 individual waterthrush and mapped 400 territories during 2009–2015. Annual first-year return rates based on resightings of banded adults have declined during the study (Table 1), and natal fidelity of banded nestlings was low (0–3.8% annually). Territory density (#terr/km) per stream declined annually while territory size increased (Fig. 2); they were significantly correlated (Rho = -0.49, $Z = -9.66$, $p = <0.01$).

Table 1. Annual demographic parameters for Louisiana Waterthrush at Lewis Wetzel WMA.

Year	# Adults banded prev. year	% Adult fidelity	Daily Survival Rate ± SE	Nest Survival (DSR[29]) ± SE	# of Nests Monitored	% Nests Parasitized	Population Productivity (# fledglings / km stream) ± SE
2009	NA	NA	96.4 ± 0.9	34.3 ± 8.9	41	0.0	2.32 ± 0.003
2010	11	63.6	98.2 ± 0.5	59.8 ± 9.3	39	2.6	4.63 ± 0.002
2011	20	65	96.7 ± 0.7	38.0 ± 8.0	47	0.0	2.56 ± 0.001
2013	11	54.5	95.8 ± 0.7	28.5 ± 6.1	65	4.6	1.61 ± 0.001
2014	61	45.9	95.4 ± 0.7	25.7 ± 5.8	54	5.6	1.26 ± 0.001
2015	24	37.5	96.1 ± 0.9	31.9 ± 8.4	34	3.0	1.34 ± 0.002

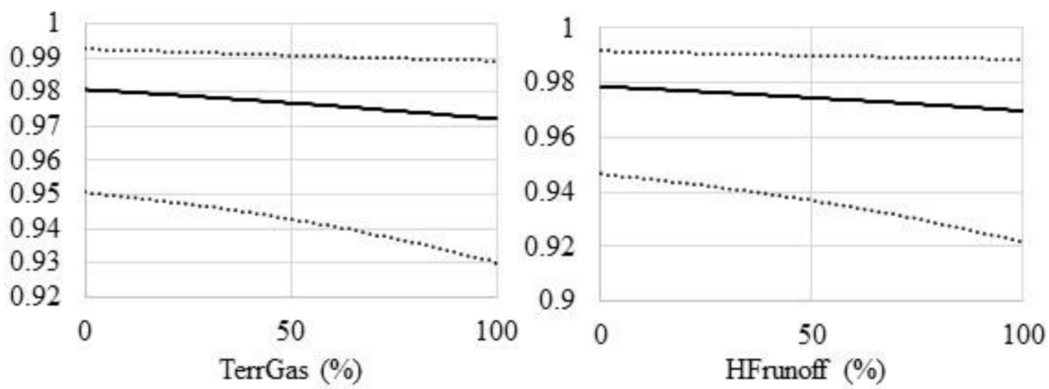


***Figure 1**. Nest survival declined with increasing shale gas disturbance within the territory (TerrGas) and subwatershed (via HFrunoff; potential surface runoff of contaminants if a territory was directly at or below a well pad or retention pond).*

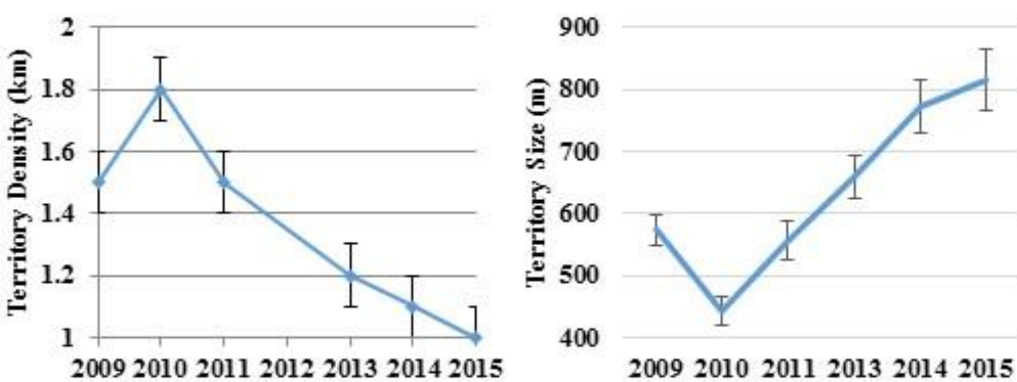


***Figure 2.** Average territory density (# terrs/km stream monitored) and territory size (m) plotted annually.*

During mist-netting, we collected 379 feather and 384 blood samples over three seasons that are being used for analysis of contaminants and epigenetic variation. Preliminary analyses of 108 blood samples suggest that DNA methylation varies between individuals, and that three DNA fragment sites out of 23 had differential methylation between individuals in disturbed and undisturbed areas. Individuals with high Barium and Strontium contaminant loads had a lower number of methylated DNA sites (n=47, Fig. 3). Barium and Strontium are associated with the fracking process and heavy metals are known to interfere with methyl transfer. Final statistical analyses that incorporate age and sex of individuals will occur after all samples are analyzed.

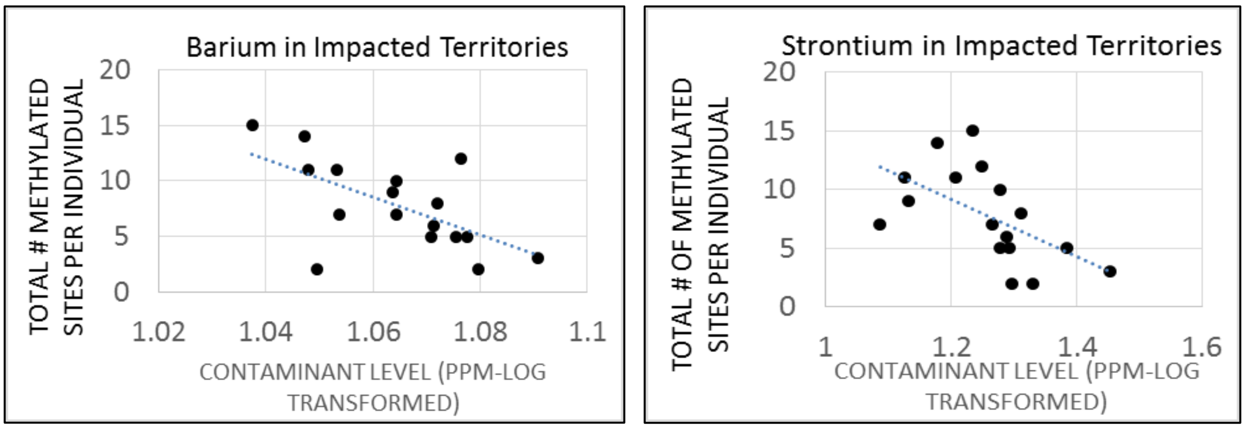


Figure 3*. High levels of Barium and Strontium were correlated with a lower number of methylated DNA sites from individuals in disturbed areas (n=47).*

We annually assessed stream and habitat quality at nest sites with the EPA Rapid Bioassessment protocol for high gradient streams and a Louisiana Waterthrush Habitat Suitability Index (HSI) in 2009–2014. Habitat suitability index (HSI) and EPA index scores were related to amount of shale gas territory disturbance ($\chi^2 = 65.342$, df = 1, p = <0.001; F= 14.54, p = 0.0002), where increased shale gas disturbance meant lower riparian quality scores. We also collected 314 macroinvertebrate samples in 2013 and 2014 at foraging and nesting locations that will be compared between disturbed and undisturbed areas, and will follow up from a 2011 benthic pilot study (Wood et al., *In Press*). Water chemistry was measured while collecting foraging and nest-site macroinvertebrate samples, during the EPA stream assessment, and systematically every 50m along the stream. Lastly, stream channel measurements were collected every 50m along the study streams that will allow us to test underlying spatial properties of the watershed that may affect waterthrush demographics and benthic macroinvertebrate communities. Further data summaries and analyses will be completed this fall and winter.

LONG-TERM SONGBIRD POPULATION RESPONSE TO SHALE GAS DEVELOPMENT

Student Investigators: Laura Farwell, Jim Sheehan, Greg George
Principal Investigator: Petra B. Wood
Years Ongoing: 2008-2016
Degree Program: PhD
Expected completion: May 2017
Funding Source: Department of Energy, US Fish and Wildlife Service, West Virginia Division of Natural Resources

Objectives:

1. Quantify the effects of shale gas development on forest cover at the study site, both in terms of forest loss and fragmentation.

2. Determine if changes in avian guild species richness, focal species abundance, and spatial distributions reflect sensitivity to forest disturbance associated with shale gas well pad and infrastructure 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 are quantifying effects of habitat impacts, both positive and negative, on avian populations and linking these effects with land cover changes to develop spatially explicit models that will aid land managers in mitigating habitat changes related to shale gas development.

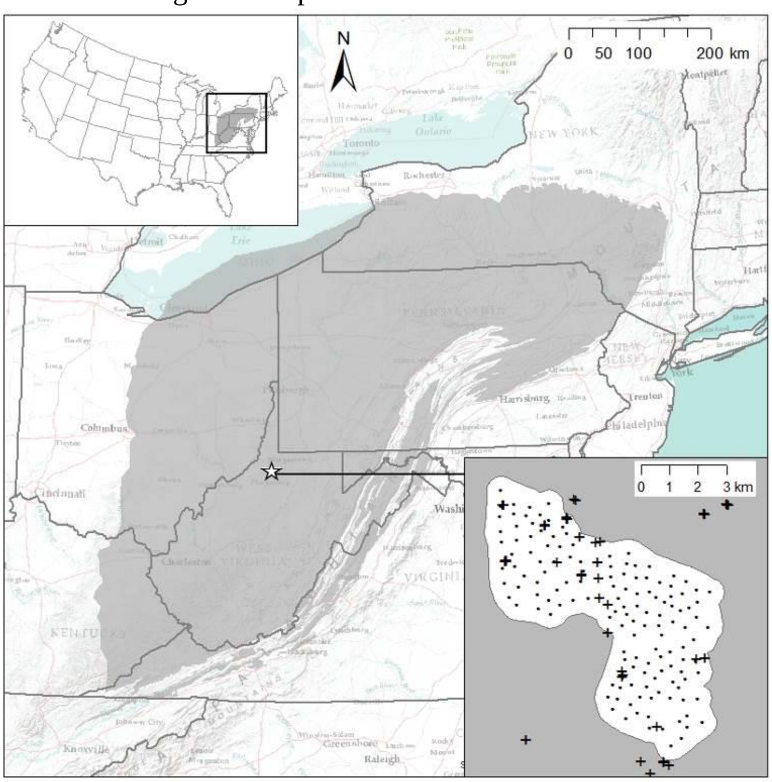


Figure 1. *Star represents location of study site (LWWMA) in relation to the Marcellus shale formation, shaded in gray. Lower inset shows locations of 142 point count locations (black dots) within the study area (shown in white). Black crosses show locations of completed unconventional shale gas wells on and near the study site.*

We established 142 point count locations throughout the ~4,326 ha study area (Fig. 1), in both ridge-top and riparian habitat. Points were surveyed during the 2008-2015 breeding seasons to determine abundance, diversity, and distribution of songbirds. We sampled areas with current Marcellus activity, areas with past O&G activity, and mature forest reference areas. We also mapped changes in forest cover from 2008–2015, specified which breeding seasons were affected, and categorized shale gas impacts as either well pads or linear impacts (roads and pipelines). We then calculated site-wide and point-level landscape metrics to assess changes in annual land cover and forest edge density. Shale gas development contributed to an overall 4.5% loss in forest cover across the site (Fig. 2a), a 12.4% loss in core forest habitat (Fig. 2b), and a 51.7% increase in forest edge density (Fig. 2c), primarily due to the construction of new access roads and pipelines (Fig. 2d).

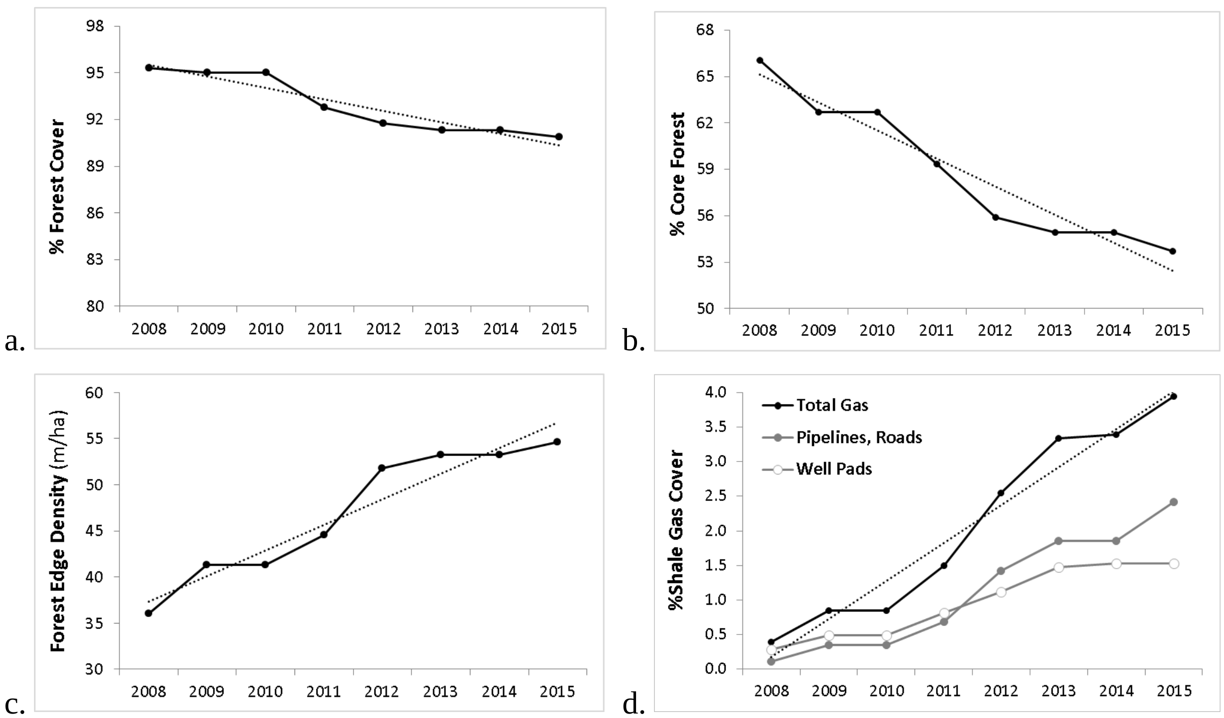


Figure 2. Site-wide changes in land cover at LWWMA during 2008–2015. Dotted lines are linear trends obtained from ordinary least squares regression.

We evaluated the relationship between these land cover metrics and species richness within three avian guilds: forest-interior, early-successional, and synanthropic, in addition to abundances of 21 focal species. We found distinct trends in long-term response among the three avian guilds. Forest-interior guild richness declined at all points across the site and at points impacted within 100 m by shale gas but did not change at unimpacted points (Fig. 3a). Early-successional and synanthropic guild richness increased at all points and at impacted points, but did not change at unimpacted points (Figs. 3b, 3c).

Model-averaged results of generalized linear mixed models directly relating point-level guild species richness to localized land cover metrics revealed similar trends, with all three guilds exhibiting distinct patterns of response to predictor variables (Table 1). For the forest interior guild, species richness was negatively related to shale gas cover within a local extent (100 m) and positively related to forest cover within a neighborhood extent (500 m). Conversely, early successional guild species richness showed a strong positive response to local edge density, and synanthropic guild species richness was positively related to edge density and shale gas cover at a local extent, and negatively related to forest cover at a neighborhood extent.

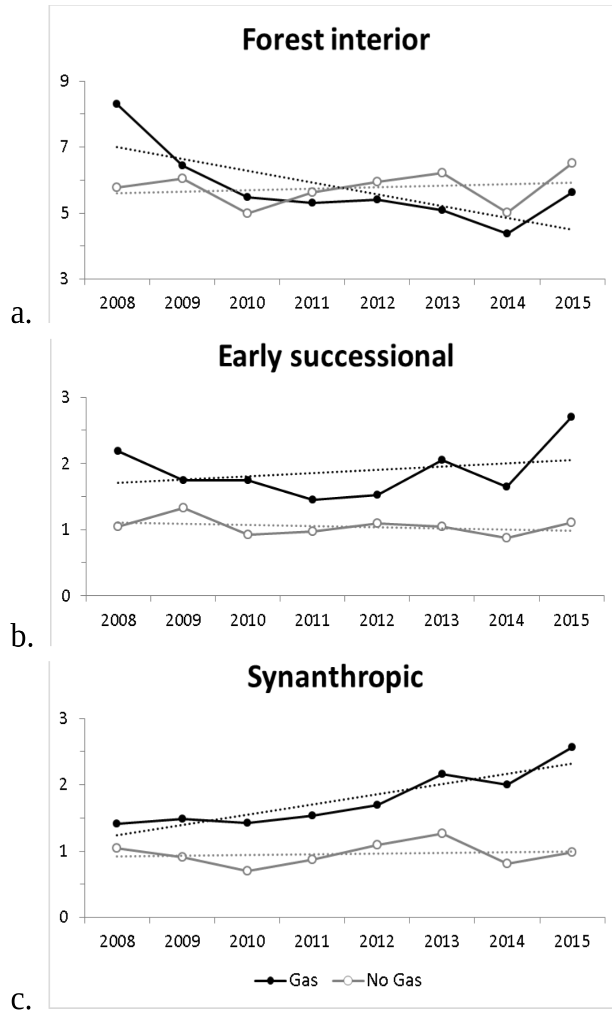


***Figure 3**. Line graphs illustrating average annual species richness from 2008–2015, at points impacted by shale gas development within 100 m ("Gas") and points unimpacted within 100 m ("No Gas"), for species in three habitat guilds (dotted lines indicate linear trends obtained from ordinary least squares regression).*

Although we observed variability in species-specific responses, relative abundance of avian species dependent on mature forest habitats has generally declined from 2008-2015 across the study area. For example, Cerulean Warblers, a species of conservation concern, declined by 34.8% over the study period (Fig. 4a). Conversely, early successional and edge-associated species have increased in abundance and distribution, particularly in association with expanding shale gas impacts on the study area. Brown-headed Cowbirds, a brood parasite implicated in declines of other songbird species, increased sharply over the study period (Fig. 4b).

a. b.

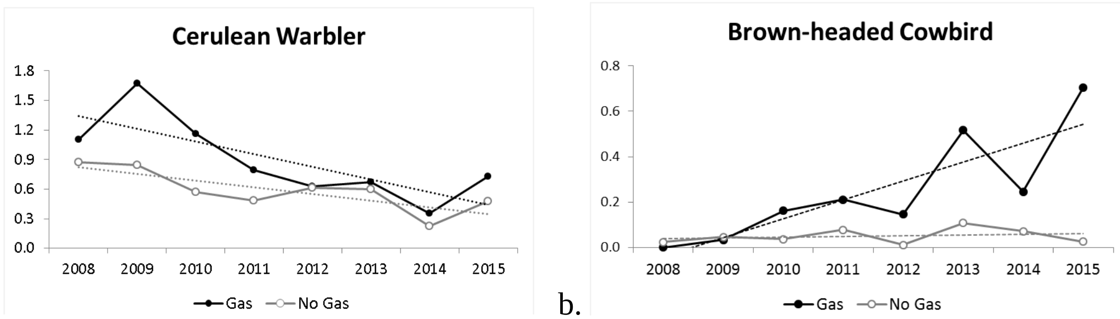


Figure 4. *Average annual relative abundance at points impacted by shale gas development within 100 m ("Gas") and points unimpacted within 100 m ("No Gas"). Dotted lines are linear trends obtained from ordinary least squares regression.*

Our results suggest that shale gas development has the potential to fragment regional forests and alter avian communities, and that efforts to minimize new development in core forests will reduce negative impacts to forest dependent species. We are currently working on spatial analyses of changing distributions of focal species in response to particular forms of shale gas disturbance at LWWMA (i.e., well pads versus pipelines), which should be finalized this winter.

Pipeline crew on a bird survey (photo by Laura Farwell)

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

SCIENTIFIC PUBLICATIONS

Blazer, Vicki S., Heather L. Walsh, Ryan P. Braham, Cassidy M. Hahn, Patricia Mazik, and Peter McIntyre. 2016. Tumors in white sucker from Lake Michigan tributaries: pathology and prevalence. Journal of Fish Diseases In Press.

Cincotta, D.A., D.P. Wegmen, T.E. Oldham, S.A. Welsh, and L.B. Hedrick. 2015. Fishes of the Blackwater River drainage, Tucker County, West Virginia. *Southeastern Naturalist* 14(Special issue 7):297–313.

Eyler, S.M., S.A. Welsh, D.R. Smith and M.M. Rockey. In press. Downstream passage and impact of turbine shutdowns on survival of silver American eels at five hydroelectric dams on the Shenandoah River. *Transactions of the American Fisheries Society*.

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

Frantz, M.W., K.R. Aldinger, P.B. Wood, J. Duchamp, T. Nuttle, A. Vitz, and J. Larkin. 2016. Space and habitat use by breeding golden-winged warblers in the central Appalachian Mountains. Studies in Avian Biology 49:81-94.

Hahn, C.M., L.R. Iwanowicz, V.S. Blazer, R.S. Cornman. 2015. Characterization of a Novel Hepadnavirus in the White Sucker (*Catostomus commersonii*) from the Great Lakes Region of the USA. Journal of Virology. 89:11801-11811. DOI:10:1128/JVI.01278-15

Hahn, C.M., L.R. Iwanowicz, R.S. Cornman, P.M. Mazik and V.S. Blazer. 2016. Whole transcriptome sequencing for the development of transcript abundance biomarkers in non-model fish species. Journal of Comparative Biochemistry and Physiology: Part D, 20:27-40.

Hilling, C. D., S. A. Welsh, and D. M. Smith. In press. Age, growth and fall diet of channel catfish in Cheat Lake, West Virginia. *Journal of Fish and Wildlife Management*.

Iwanowicz, L.R., V.S. Blazer, A.E., Pinkney, C.P. Guy, A.M. Major, K. Munney, S. Mierzykowski, S. Lingenfelser, A. Secord, K. Patnode, T.J. Kubiak, C. Stern, C.M. Hahn, D.D. Iwanowicz, H.L. Walsh, and A. Sperry. 2016. Evidence of Estrogenic Endocrine Disruption in Smallmouth and Largemouth Bass Inhabiting Northeast U.S. National Wildlife Refuge Waters: A Reconnaissance Study. Ecotoxicology and Environmental Safety 124: 50-59.

Latta, S. C., L. C. Marshall, M. W. Frantz, and Judith Toms. 2015. Evidence from two shale regions that a riparian songbird accumulates metals associated with hydraulic fracturing. Ecosphere 6:144.

Loughman, Z.J., S.A. Welsh, J.W. Fetzner, and R.F. Thoma. 2015. Conservation of imperiled crayfish species - *Cambarus veteranus* (Decapoda: Cambaridae). *Journal of Crustacean Biology* 35:850–860.

Rizzo, A.A., S.A. Welsh, and P.A. Thompson. In press. A photogrammetric method for in situ length measurement of benthic fishes. *North American Journal of Fisheries Management*.

Smith, B.W., A.N. Tri, C.A. Dobony, J.W. Edwards, and P.B. Wood. 2015. Behavior and nesting ecology of Appalachian ruffed grouse. Canadian Field Naturalist 129:245-253.

Smith, J., S.A. Welsh, J.T. Anderson, and R. Fortney. 2015. Water quality trends in the Blackwater River watershed, West Virginia. *Southeastern Naturalist* 14(Special issue 7):103–111.

Terhune, T.M., K.R. Aldinger, D.A. Buehler, D.J. Flaspohler, J.L. Larkin, J.P. Loegering, K.L. Percy, A.M. Roth, C.G. Smalling, and P.B. Wood. 2016. Golden-winged warbler nest-site habitat selection. Studies in Avian Biology 49:109-145.

Welsh, S.A. and Z.J. Loughman. 2015. Upstream dispersal of an invasive crayfish aided by a fish passage facility. *Management of Biological Invasi*ons 6:287–294.

Wood, P., M. Frantz, and D. Becker. 2016. Louisiana Waterthrush and Benthic Macroinvertebrate Response to Shale Gas Development. Journal of Fish & Wildlife Management. 7(2). *Online at* http://dx.doi.org/10.3996/092015-JFWM-084

PRESENTATIONS

Bakermans, M., D. McNeil, K. Aldinger, P.B. Wood, … and J. Larkin. 2016. Breeding bird communities in sites created by Natural Resources Conservation Service (NRCS) conservation practices targeted for Golden-winged Warbler. North American Ornithological Congress. Washington, DC. August 16–20, 2016

Bakermans, M., D. McNeil, K. Aldinger, P.B. Wood… J. Larkin. Breeding bird communities across conservation practices associated with NRCS's Working Lands for Wildlife targeting golden-winged warbler nesting habitat. Northeast Association of Fish and Wildlife Agencies Annual Conf. Annapolis, MD. April 3–5, 2016.

Blazer, Vicki S., Luke R. Iwanowicz, Heather L. Walsh, Adam Sperry, Ryan Braham, and Megan K. Schall. Biological Effects Monitoring to Identify Consequences of Exposure to Endocrine Disruptors. National Conference on Ecosystem Restoration, Coral Springs, Florida. April 18-22, 2016.

Braham, Ryan P., Vicki S. Blazer, Jim D. Hedrick, and Patricia M. Mazik. Temporal Variations in Microcystin Toxins within the Potomac River Drainage. Southern Division of the American Fisheries Society Annual Meeting, Wheeling, West Virginia. February 17-21, 2016.

Brittingham, M.C., L.S. Farwell, and M.W. Frantz. 2016. Effects of Marcellus shale gas development on forest birds, and management implications. Appalachian Mountains Joint Venture Energy Webinar Series. September 7, 2016. (invited)

Farwell, L.S., P.B. Wood, J. Sheehan, and G.A. George. 2016. Avian response to shale gas: a long-term case study from West Virginia. Northeast Association of Fish and Wildlife Agencies Annual Conference, Annapolis, MD. April 3–5, 2016.

Farwell, L.S., P.B. Wood, J. Sheehan, and G.A. George. 2016. Breeding songbird response to Marcellus-Utica shale gas well and infrastructure development. North American Ornithological Conference, Washington D.C. August 16–20, 2016.

Farwell, L.S. and P.B. Wood. 2016. Breeding songbird response to shale gas development. Mountaineer Audubon Chapter Meeting. September 13, 2016. (invited)

Farwell, L.S., P.B. Wood, J. Sheehan, and G.A. George. 2016. Avian response to shale gas development in the Marcellus region. West Virginia Cooperative Fish and Wildlife Research Unit Annual Coordinating Committee and Cooperators Meeting. September 21, 2016.

Frantz, M. W., and P. B. Wood. Site-specific and region-wide Louisiana Waterthrush and benthic macroinvertebrate response to shale gas development. Mountaineer Audubon. Morgantown, WV, 13 Sept. 2016.

Frantz, M. W., P. B. Wood, J. Sheehan, D. Becker, and G. George. Louisiana Waterthrush & Benthic Macroinvertebrate Response to Shale Gas Development. North American Ornithological Conference. Washington, D.C., 14-19 Aug. 2016.

Frantz, M. W., and P. B. Wood. Site-specific and region-wide Louisiana Waterthrush and benthic macroinvertebrate response to shale gas development. Brooks Bird Club (BBC) Annual Foray. Parsons, WV, 16 June 2016.

Frantz, M. W., A. Welsh, S. Latta, and P. Wood. Epigenetic (DNA methylation) variation in the Louisiana Waterthrush (*Parkesia motacilla*) associated to shale gas development in West Virginia. Eastern Bird Banding Association Annual Meeting. Davis, WV, 8-10 April 2016.

Frantz, M. W., P. B. Wood, J. Sheehan, D. Becker, and G. George. Louisiana Waterthrush & Benthic Macroinvertebrate Response to Shale Gas Development. Northeast Association of Fish and Wildlife Agencies 72nd Conference. Annapolis, MD, 3-5 April 2016.

Hahn, C.M., L.R. Iwanowicz, R.S. Cornmna, P.M. Mazik and V.S. Blazer. 2016. Transcriptome discovery in non-model species for the development of quantitative gene expression assays: A case study of the Rochester Embayment Area of Concern. American Fisheries Society Southern Division Meeting. February 19 – 21, 2016. Wheeling, WV.

Hahn, C.M., L.R. Iwanowicz, R.S. Cornmna, P.M. Mazik and V.S. Blazer. 2016. Transcriptomics for use in the assessment of non-model, wild-caught fish health. American Fisheries Society Fish Health Section. June 6 – 9. Jackson Hole, WY.

Hilling, C, S. Welsh, and D. Smith. 2016. Population characteristics of Channel Catfish in Cheat Lake, West Virginia. Annual Meeting of the Southern Division of the American Fisheries Society, Wheeling, WV, 20 February 2016

Iwanowicz, L.R., C.M. Hahn, V.S. Blazer, R.S. Cornman, and L. Sanders. 2015. The First Report of a Hepadnavirus from Fishes: Molecular Evidence for a Novel Genus of Hepatitis B Virus in White Sucker (*Catostomus commersonii*) that inhibit the Great Lakes region. American Fisheries Society Fish Health Section. July 2015. Ithaca, NY.

Kramer, G.R., D.E. Andersen, D.A. Buehler, P.B. Wood, S.M. Peterson, J.A. Lehman, K.R. Aldinger, L.P Bulluck, B. Gray, S. Harding, J.A. Jones, D.I. King, J.L. Larkin, J.P Loegering, D.J. McNeil, D.B. Miles, C. Smalling, R. Vallender, and H.M. Streby. 2016. Range-wide assessment of wintering distributions and migration routes of golden-winged warblers using geolocators. North American Ornithological Conference. Washington, D.C., 14-19 Aug. 2016.

Kramer, G.R., H.M. Streby, S.M. Peterson, J.A. Lehman, D.A. Buehler, P.B. Wood, D.J. McNeil, J.L. Larkin, and D.E. Andersen. 2016. Wintering grounds and migratory routes of golden-winged warblers discovered using light-level geolocators: implications for full life-cycle management. 76th Midwest Fish and Wildlife Conference, Grand Rapids, Michigan, USA.

Loman, Z. G., W. D. Deluca, D. Harrison, C. S. Loftin, B. Rolek, and P. B. Wood. 2016. Evaluation of a fine-grained landscape conservation planning tool for songbird conservation in the Northeastern United States. North American Ornithological Conference 2016. Washington, DC, August 16-20.

Loman, Z., D. Harrison, C. S. Loftin, and P. B. Wood. 2016. Validating predictions of upland gamebird space use in multiple management contexts. Northeast Association of Fish and Wildlife Agencies Conference. Annapolis, MD, April 3-6.

Loughman, Z.L. and S.A. Welsh. 2016. Physical Habitat and Water Quality Correlates for *Cambarus veteranus* in the Upper Guyandotte Basin of West Virginia. Annual Meeting of the Southern Division American Fisheries Society, Wheeling, WV, 20 February 2016

Margenau, E.L., P.B. Wood, C.A. Weakland, and D.J. Brown. Avian community shift in a reclaimed mine landscape in southern West Virginia. North American Ornithological Conference. 16-20 Aug 2016.

McNeil, D., K. Aldinger, M. Bakermans, P.B. Wood, … J. Larkin. 2016. Golden-winged Warbler Breeding Season Demographics Across Conservation Practices Associated with NRCS: Working Lands For Wildlife. Northeast Association of Fish and Wildlife Agencies Annual Conf. Annapolis, MD. April 3-6

Nareff, G.E., P.B. Wood, T. Fearer, K. Parker, W.M. Ford, J. Larkin, and S. Stoleson. Cerulean warbler and associated species response to silvicultural prescriptions in the Central Appalachian Region. 6^{th} North American Ornithological Conference. Washington, D.C. 16-20 August 2016.

Nareff, G.E., and P.B. Wood. Cerulean warbler and associated species response to silvicultural prescriptions in the Central Appalachian Region. West Virginia Division of Forestry Managers Training Workshop. Jackson's Mill, WV. 19 Nov 2015 (invited)

Phillips, P. J., D. W. Kolpin, K. L. Smalling, V. Blazer, L. Iwanowicz, M. Schall, R. Braham, C. Ladino. Emerging Chemical Contaminants in the Chesapeake Bay Watershed - Where are we going? Where should we go? National Conference on Ecosystem Restoration, Coral Springs, Florida. April 18-22, 2016.

Rizzo, A., and S. Welsh. 2016. Photogrammetric analysis of fish lengths. Annual Meeting of the Southern Division of the American Fisheries Society, Wheeling, WV, 20 February 2016

Rizzo, A., S. Welsh, P. Thompson. 2016. Photogrammetric analysis of fish lengths. Annual Meeting of the West Virginia Academy of Science, Huntington, WV, 9 April 2016

Rolek, B.W., D. Harrison, C.S. Loftin, P.B. Wood. 8 October 2015. Workshop on spruce-fir birds and forest management in Northern New England at U.S. Fish and Wildlife Service Migratory Bird Division. Hadley, MA, USA.

Rolek, B.W., D. Harrison, C.S. Loftin, P.B. Wood. 23 March 2016. Softwood forest birds and forestry in New England. Cooperating Committee Meeting for Maine Cooperative Fish and Wildlife Research Unit, Orono, Maine, USA.

Rolek, B.W., D. Harrison, C.S. Loftin, P.B. Wood. 17-20 July 2016. Opportunities to enhance habitat for spruce-fir passerines using commercial forest management. North American Congress for Conservation Biology, Society for Conservation Biology, Madison, Wisconsin, USA.

Rolek, B.W., D. Harrison, M. Hartley, C.S. Loftin, P.B. Wood. 16-20 August 2016. Opportunities to enhance habitat for spruce-fir passerines using commercial forest management. North American Ornithological Conference, Washington D.C.

Paulson, M.D., P.B Wood, T.E. Katzner, and C.L. McIntyre. 6 Nov 2015. Using Brownian Bridge Movement Models to describe winter space use of migratory juvenile golden eagles from interior Alaska. Raptor Research Foundation Annual Meeting, Sacramento, CA.

Smith, D.M., S.A. Welsh, C.D. Hilling, and N.D. Taylor. 2016. Environmental and biological correlates of seasonal movement patterns of Walleyes in a hydropower reservoir. Presented at the Southern Division American Fisheries Society meeting, Wheeling, WV, 20 February 2016.

Thompson, P., S. Welsh, A. Rizzo. 2016. A tale of two darters: Substrate selection and preference of sympatric sand darters from the Elk River, WV. Annual Meeting of the Southern Division of the American Fisheries Society, Wheeling, WV, 20 February 2016

Thompson, P., S. Welsh, A. Rizzo. 2016. Substrate selection of sympatric sand darters from the Elk River, WV. Annual Meeting of the West Virginia Academy of Science, Huntington, WV, 9 April 2016

Walsh, H.L. 2016. Chemicals of Emerging Concern and Fish Health. Intro to Fish Health Class, Shepherdstown, WV, February 1-5.

Walsh, H.L. 2016. Chemicals of Emerging Concern and Fish Health. 2nd Annual Mason Water Research Symposium, Fairfax, VA, March 18.

Welsh, S.A. and J.L. Aldinger. 2016. Patterns and periodicity of upstream passage of American Eels at an eel ladder. Annual Meeting of the Southern Division American Fisheries Society, Wheeling, WV, 20 February 2016

Welsh, S.A. and Z.J. Loughman. 2016. Use of an eel pass by virile crayfish on the lower Shenandoah River. Annual Meeting of the West Virginia Academy of Science, Huntington, WV, 9 April 2016

Wood, P. B., M. W. Frantz, and D. A. Becker. Louisiana Waterthrush and Benthic Macroinvertebrate Response to Shale Gas Development. Southeastern Association of Fish and Wildlife Agencies (SEAFWA) 69th Conference. Asheville, NC, 1-4 Nov. 2015.

Wood, P.B. Cerulean warbler partnerships, atlas project, and habitat management guidelines. West Virginia Division of Forestry Stewardship Workshop. Jackson's Mill, WV. 19 Nov 2015 (invited)

Wood, P.B. Mature Forest Birds and Response to Forest Management. Appalachian Mountains Joint Venture and US Forest Service Land Managers Workshop. Lewisburg, WV 14-16 Oct 2015. (invited)

AWARDS

Laura Farwell, Mack Frantz, Eric Margenau, and Gretchen Nareff (PhD students), were awarded travel grants from the WVU Davis College and the Division of Forestry and Natural Resources to attend and present their research results at the North American Ornithological Conference, Washington DC, 16-20 August 2016. Laura, Mack, and Gretchen also received a student travel award from the NAOC for this conference.

Laura Farwell (PhD student) received the following awards:
- Southern Regional Education Board Dissertation Fellowship, 2016–2017.
- American Ornithologists' Union Robert B. Berry Student Presentation Award for best oral paper pertaining to avian conservation at the 2016 North American Ornithological Conference
- West Virginia University 3–Minute Thesis Final Competition, 1st place award
- Davis College 3-Minute Thesis Competition, 1st place PhD award

Mack Frantz (PhD student) received the following awards:
- Outstanding PhD Student in the WVU Wildlife and Fisheries Program in 2015-16
- WVU STEM Mountains of Excellence Scholarship
- Davis College A. D. Hopkins Graduate Student Research Enhancement Award
    - WVU Provost Summer Graduate Fellowship
    - Slovak League of America Scholarship Participant Stipend

Pat Mazik received the Outstanding Achievement Award at the Southern Division of the American Fisheries Society Annual Meeting, Wheeling, West Virginia. February 17-21, 2016.