



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

FY 2015

ANNUAL REPORT

1 October 2014 - 30 September 2015



COOPERATING AGENCIES:

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

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

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

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

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

Graduate education is also an important mission of the Coop Unit. Cooperating with West Virginia University, the Coop Unit contributes to the quality education and training of graduate students in fisheries and wildlife at West Virginia University. Coop Unit scientists achieve educational goals by chairing graduate committees, serving on graduate committees, teaching graduate level courses and delivering guest lectures and seminars. As of September 30, 2015, 108 students have completed their degree requirements: 87 Masters and 21 Ph. D. The Unit scientists are currently supervising 3 Master's students and 15 Ph.D. students.



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UNIT STAFF

UNIT LEADER

Patricia M. Mazik, Adjunct Professor of Fisheries
Ph.D., Memphis State University, 1989. Stress physiology, Toxicology.

ASSISTANT LEADERS

Petra Bohall 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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Lara Hedrick, Research Assistant, Division of Forestry and Natural Resources
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PROJECT COOPERATORS

UNIVERSITY

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Kyle Hartman, Professor, Division of Forestry and Natural Resources
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Amy Welsh, Associate Professor, Division of Forestry and Natural Resources

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Todd Fearer, Appalachian Mountains Joint Venture
Christopher Good, Freshwater Institute
Joe Hankins, Freshwater Institute
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Steve Latta, Pittsburgh National Aviary
Zac Loughman, West Liberty University
Pat Rakes, Conservation Fisheries, Inc.
Michael Schwartz, Freshwater Institute
J.R. Shute, Conservation Fisheries, Inc.
Steve Summerfelt, Freshwater Institute
T. Bentley Wigley, NCASI

STUDENTS

<u>STUDENT</u>	<u>DEGREE</u>	<u>GRADUATION DATE</u>	<u>ADVISOR</u>
Joni Aldinger	M.S.	Completed Aug 2015	Stuart Welsh
Kyle Aldinger	Ph.D.	Expected May 2016	Petra Wood
Ryan Braham	Ph.D.	Expected Dec 2016	Pat Mazik
Sheila Eyler (NCTC)	Ph.D.	Completed Dec 2014	Stuart Welsh
Laura Farwell	Ph.D.	Expected Dec 2016	Petra Wood
Steve Foster (NCTC)	Ph.D.	Expected Dec 2017	Stuart Welsh
Mack Frantz	Ph. D.	Expected Aug 2016	Petra Wood
Cassidy Hahn	Ph.D.	Expected May 2016	Pat Mazik
Corbin Hilling	M.S.	Completed Aug 2015	Stuart Welsh
Carlos Martinez (NCTC)	M.S.	Expected Dec 2016	Pat Mazik
Eric Margenau	Ph. D.	Expected May 2020	Petra Wood
Gretchen Nareff	Ph. D.	Expected May 2017	Petra Wood
Christi Raines	M.S.	Completed May 2015	Amy Welsh
Austin Rizzo	Ph. D.	Expected Dec 2017	Stuart Welsh
Brian Rolek	Ph. D.	Expected Dec 2016	Petra Wood
Edward Olesh	M.S.	Expected Sept 2016	Kyle Hartman
Jim Sheehan	Ph.D.	Expected May 2016	Petra Wood
Dustin Smith	Ph.D.	Expected Dec 2015	Stuart Welsh
Daniel Sparks (NCTC)	Ph.D.	Expected Dec 2016	Pat Mazik
Jeff Thomas (NCTC)	Ph.D.	Expected Dec 2018	Stuart Welsh
Patricia Thompson	M.S.	Expected May 2016	Stuart Welsh
Thomas Waldrop (NCTC)	M.S.	Expected Dec 2016	Pat Mazik
Heather Walsh	Ph. D.	Expected Dec 2017	Pat Mazik

UNIT STAFF COURSES TAUGHT

Patricia M. Mazik , Adjunct Professor of Fisheries		
Aquatic Toxicology	Fall 2014	3 credits
Stuart A. Welsh , Adjunct Professor of Fisheries		
Advanced Ichthyology	Fall 2014	3 credits
Petra B. Wood , Adjunct Professor of Wildlife		
Wildlife and Fisheries Graduate Seminar	Spring 2015	1 credit

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

RELATIONSHIPS BETWEEN BODY SIZE, TIMING OF PASSAGE, AND ENVIRONMENTAL VARIABLES FOR UPSTREAM MIGRANT AMERICAN EELS AT THE MILLVILLE DAM EEL LADDER, SHENANDOAH RIVER

Student Investigator: Joni Aldinger

Principal Investigator: Stuart A. Welsh

Years Ongoing: 2013-2015

Degree Program: MS

Completion: August 2015

Funding Source: WVDNR, PE Hydro Generation LLC

Objectives:

1. Examine diel periodicity of upstream migrants using time-series spectral analysis
2. Describe the distribution of passage counts during diel periods (day, twilight, and night) among seasons (spring, summer, and fall)
3. Examine size of upstream migrants relative to diel and seasonal periods.

Results:

Passage count data were collected during 2011–2014 at the Millville Dam eel ladder on the lower Shenandoah River, West Virginia. A camera on the eel ladder has been used for monitoring and allows for data collection of passage counts, eel lengths, and timing of passage. The eel ladder camera photographs each eel that passes through the ladder, and each photograph has a time and date stamp. Using photogrammetric methods, total lengths of eels using the ladder are estimated from photographs (Figure 1). A total passage count of 9,042 individuals was recorded at the Millville eel ladder during 2011–2014, including annual counts of 1255 (6 May–9 November 2011), 4263 (28 June–7 November 2012), 2470 (1 July–12 October 2013), and 1054 (22 July–5 November 2014).

The digital photographs from the eel ladder camera were used to obtain data on passage counts, including photogrammetric measurements of American Eel lengths as well as date and time of passage. River flow, lunar phase, and astronomical data were also collected for the passage events. We reduced variation associated with temporal changes in lengths of twilight, night, and day periods by analyzing six short-term passage events, based on time periods that had high passage counts within a short time frame, which ranged in length from 7 to 18 days and represented 75.2% ($n=6,803$) of the total number of individuals that used the eel ladder during 2011–2014. These six passage events were categorized as four seasonal periods: spring (17–28 May 2012, $n=1,848$), summer (12–21 July 2013, $n=1,617$, and 22 July–3 August 2014, $n=285$), late summer/early fall (13 September–1 October 2011, $n=666$, and 18–27 September 2012, $n=1,868$), and fall (14–21 October 2014, $n=519$). Total lengths were photogrammetrically measured for 5,989 of the 6,803 individuals, with a mean TL of $305.2 \text{ mm} \pm 0.55 \text{ SE}$, range = 185–609 mm. Upstream movements were separated by periods of twilight, night, and day:



Figure 1. Photograph of an American Eel passing through the Millville Dam eel ladder

- Vespertine = movements during dusk period between sunset and evening astronomical twilight (i.e., when the sun is 0–18° below the horizon);
- Matutinal = movements during dawn period between sunrise and morning astronomical twilight (i.e., when the sun is 0–18° below the horizon);
- Diurnal = movements during the daytime period between sunrise and sunset; or
- Nocturnal = movements during the nighttime period after astronomical twilight ends in the evening and before astronomical twilight begins the next morning.

We quantified diel periodicity of upstream migration of yellow-phase American Eels using time series analysis. Time series data of passage events separated into 10-min intervals depicted diel periodicity of upstream movements, where most individuals moved during periods of twilight and night, and few individuals moved during the day (Figures 2, 3). Peaks in movement for the multi-day passage events were associated with an increase in river discharge (Figure 2). For diel periods, the counts of vespertine, nocturnal, matutinal, and diurnal movements were 506 (7.4%), 5,625 (82.7%), 588 (8.6%), and 84 (1.2 %), respectively. For each passage event, our time-series spectral analysis found that diel periodicity of movements closely follows a 24-h cycle of activity (Figure 2).

Few studies have addressed diel chronology and timing of upstream movements of yellow-phase American Eels during periods of twilight and night. To examine movement patterns and describe multiple modes or peaks of passage counts for vespertine, nocturnal, and matutinal movements for each passage event, we created histograms of the number of individuals passing through the ladder pooled into a 14-h

period (18:00–08:00 hours) using 10-min bins. We fit a normal model to the histogram data for each passage event representing a hypothesis of a unimodal peak as well as eight normal mixture models (2–9 mixtures) representing hypotheses of multimodal distributions and used Bayesian information criterion (BIC) to select the best approximating model. Multimodal models were supported by the data and differed across the six passage events, although there were some similarities in timing of modal peaks. Our results support that upstream migrants primarily undergo nocturnal movements. The majority of movements in this study were nocturnal, and all six events had peaks of movement occurring during the middle of the nighttime period (0:00–02:00, Figure 3), which is likely the darkest part of the night. Five of the passage events had movement peaks occurring at or near the interfaces of twilight (vespertine and matutinal movements) and night, which supports previous studies finding that eels exhibit nocturnal and crepuscular behavior. Based on our observational data, we were not able to explain why distribution patterns of diel chronology and timing of upstream migration were relatively complex (three to eight modal peaks) or why the most complex distribution patterns occurred during the two passage events of late summer/early fall (Figure 3).

A mixed-model methodology was used to examine relationships among total length (TL), diel period, and season. We fit four models to TL data: (1) an intercept model, (2) a diel period model, (3) a season model, and (4) an additive-effects model of diel period + season. We used BIC as a model selection criterion. To aid interpretation of model selection, we plotted descriptive statistics of mean \pm SE of TL for categories of movements during diel periods (vespertine, nocturnal, matutinal, diurnal) and seasons (spring, summer, late summer/early fall, fall). Given that passage events were selected from time periods of high counts associated with increased river discharge and often with new moon periods, we did not expect to find relationships between TL, river discharge, and lunar illumination and therefore did not fit models with covariates of river discharge and lunar illumination. The additive-effects model of diel period + season was selected as the best approximating model and was visually supported and interpreted with use of descriptive statistics, where mean TL depicted a decreasing trend across diel periods (vespertine = 311.5 mm \pm 1.7 SE, n = 235; nocturnal = 305.8 mm \pm 0.59 SE, n = 5182; matutinal = 298.3 mm \pm 1.9 SE, n = 514; and day = 282.8 mm \pm 5.5 SE, n = 58). Also, mean TL was highest during fall (330.3 mm \pm 1.9 SE) relative to similar mean values of TL for spring (304.1 mm \pm 1.0 SE), summer (301.2 mm \pm 1.1 SE) and late summer/early fall (303.4 mm \pm 0.87 SE). To our knowledge, no other studies have documented a difference in TL of upstream migrants among diel periods of day, twilight, and night.

In summary, this study quantified the pattern of diel periodicity of upstream migration. Based on the time-series spectral analysis of six multi-day passage events, periodicity of movements closely followed a 24-h cycle of activity with the majority of movements were nocturnal with a few peaks occurring near the interfaces of twilight (vespertine and matutinal periods) and night. The distribution patterns and timing of upstream migration were complex and variable. The TL of individuals passing the dam followed a chronological trend with time, where the longest individuals passed through the ladder during the vespertine period and the shortest individuals used the ladder during the day (diurnal movements). The mean TL of individuals passing was greater during the fall period than that of individuals using the ladder during the spring, summer, and late summer/early fall periods. This study increased our understanding of upstream migration ecology of yellow-phase American Eels and dam passage at the Millville Dam eel ladder.

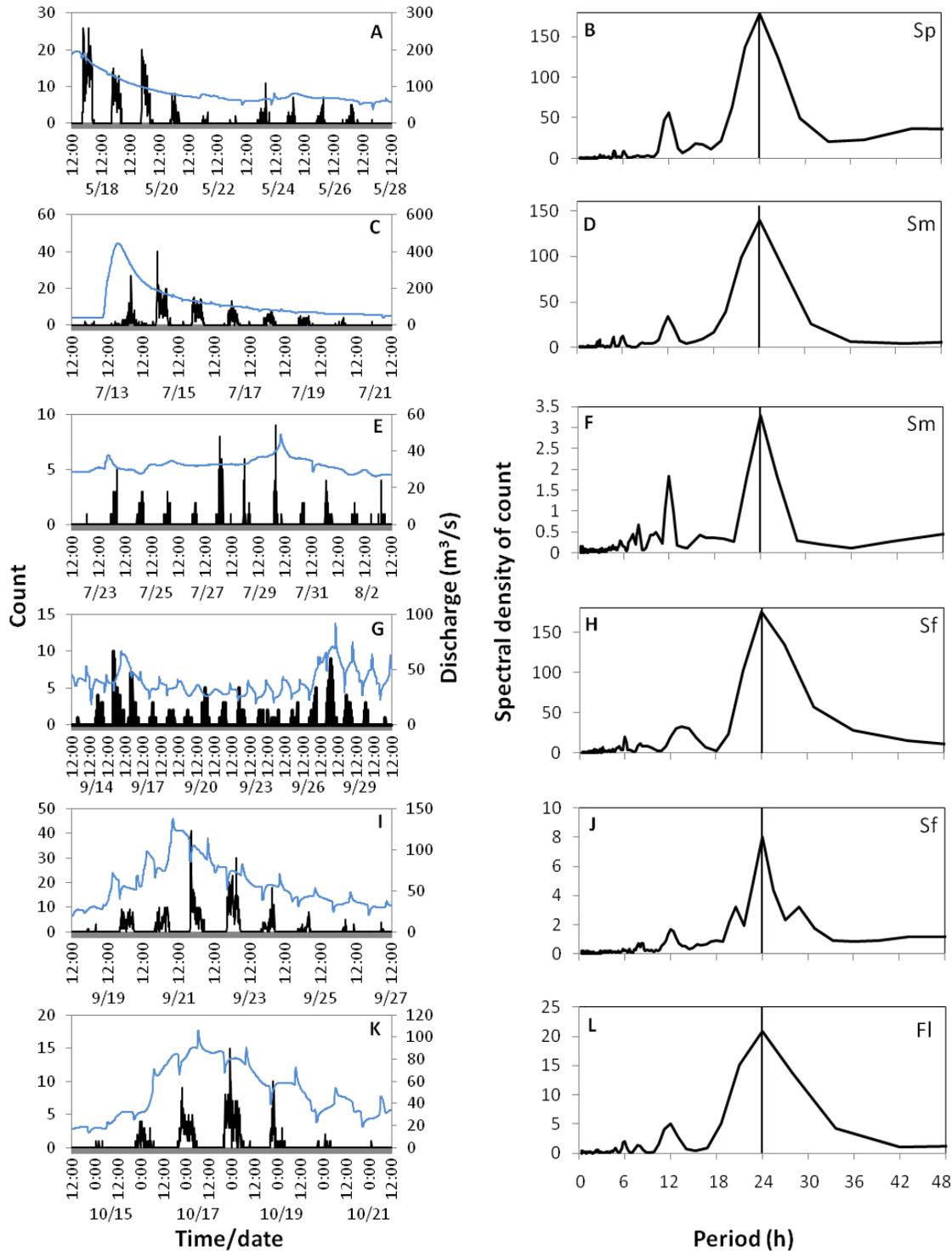


Figure 2. Frequency of counts of American Eels passing through the eel ladder and river discharge (m^3/s) at the Millville Dam using 10-min bins (starting at 12:00 on the first day of the period) for six periods: 17–28 May 2012 (A, B), 12–21 July 2013 (C, D), 22 July–3 August 2014 (E, F), 13 September–1 October 2011 (G, H), 18–27 September 2012 (I, J), and 14–21 October 2014 (K, L). Seasons are labeled as spring (SP), summer (SM), late summer/early fall (SF), and fall (FL). A reference line indicates the dominant frequency.

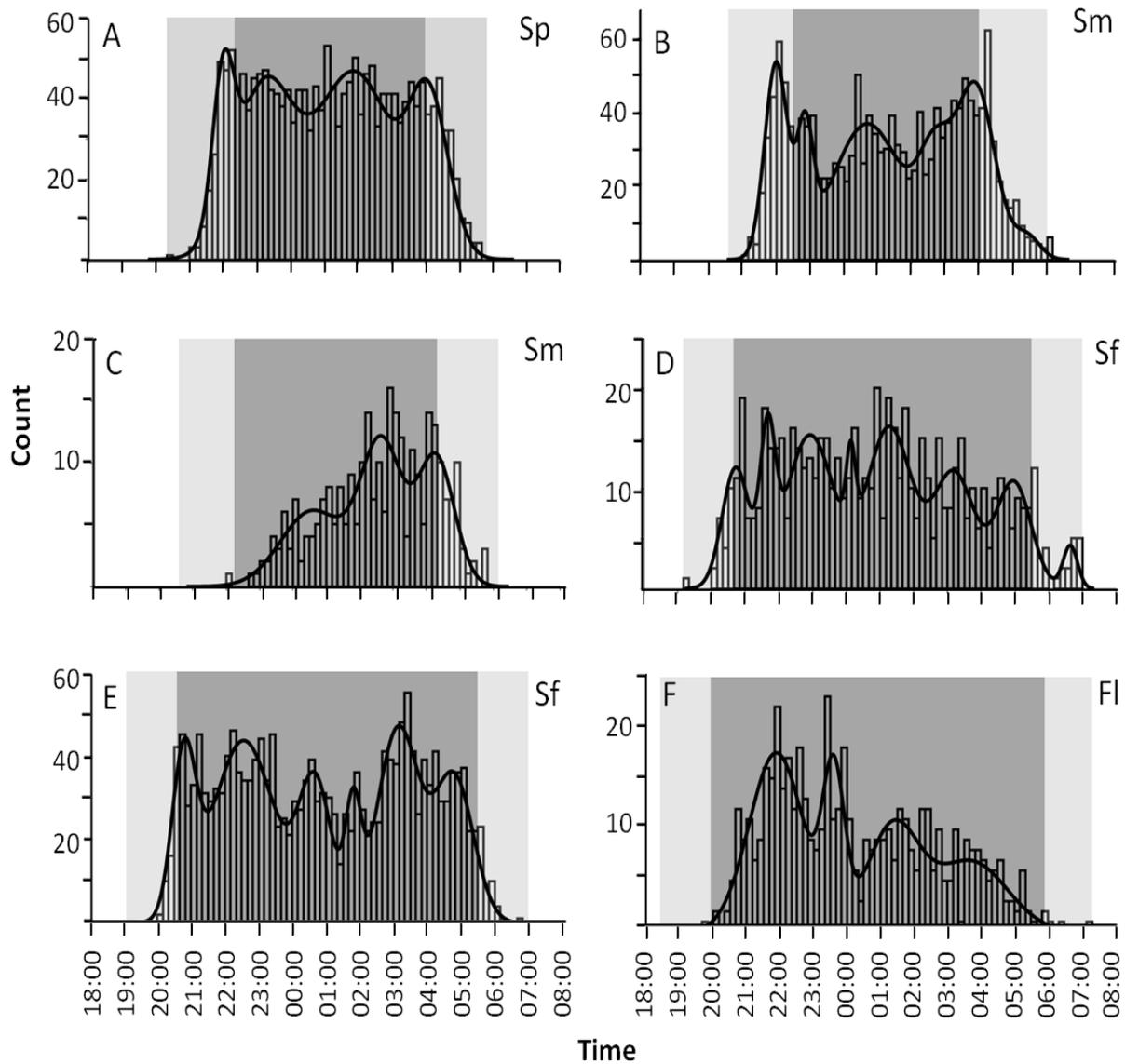


Figure 3. Frequency of counts (histogram bars) and modal peaks (solid lines) of American Eels passing through the Millville Dam eel ladder on the Shenandoah River. Count data are pooled into a 14 h period (starting at 18:00) using 10-min bins for six periods: 17–28 May 2012 (A), 12–21 July 2013 (B), 22 July–3 August 2014 (C), 13 September–1 October 2011 (D), 18–27 September 2012 (E), and 14–21 October 2014 (F). The lighter shaded areas represent twilight periods (vespertine and matutinal movements) and the dark shaded area represents night (nocturnal movements). Seasons are labeled as spring (SP), summer (SM), late summer/early fall (SF), and fall (FL).

DOWNSTREAM MIGRATION AND MORTALITY OF SILVER AMERICAN EELS ASSOCIATED WITH HYDROELECTRIC DAMS ON THE SHENANDOAH RIVER

Student Investigator: Sheila Eyler

Principal Investigators: Stuart A. Welsh and David R. Smith

Years Ongoing: 2007 – 2014

Degree Program: PhD

Completion: December 2014

Funding Source: WVDNR, PE Hydro Generation LLC

Objectives:

Examine out-migration of silver American eels relative to five hydroelectric dams on the lower Shenandoah River. Document eel passage relative to use of dam spillways versus canals and associated intakes to hydroelectric turbines, and document the timing and environmental cues of out-migration.

Results:

Hydroelectric dams can impact downstream migrating American Eels (*Anguilla rostrata*) through migratory delays and turbine mortality. The cumulative impacts of multiple dams in a river can have significant impacts on American Eels migrating out of the upstream reaches of freshwater rivers. I used radio telemetry to determine the timing and survival of American Eels migrating downstream past five hydroelectric dams on the Shenandoah River in Virginia and West Virginia (Figure 1). The five hydroelectric dams operated a seasonal nighttime turbine shutdown period to protect downstream migrants. The shutdown period was conducted from September 15 to December 15 annually, operating from 18:00 to 06:00 daily. During the fall months from 2007 to 2009, large American Eels were collected primarily by electrofishing above the Luray, Newport, and Shenandoah dams. A total of 145 American Eels were radio-tagged and released near their capture location. All five hydroelectric dams were outfitted with telemetry monitoring equipment to determine the time of arrival to the dam, the time of passage at the dam, the method of passage at a dam, and immediate mortality of tagged fish. Telemetry equipment at the dams was deployed during the fall of 2007 and monitored continuously through the summer of 2010.

A total of 96 tagged American Eels migrated downstream past at least one dam during the study. Downstream passage events occurred during every month of the year except July, with peak migrations in the fall and spring months. A total of 67% of the downstream migration events occurred during the nighttime turbine shutdown period (September 15 to December 15) and 26% of downstream migration events occurred from February to May (Figure 2). Most (87%) downstream migration events occurred between 17:00-05:00 (Figure 3). Downstream migration for an individual American Eel usually occurred during one study year (August 1 to July 31) and multi-year downstream migratory activity for an individual was rarely observed.

Migration out of the Shenandoah River occurred relatively quickly. Median migration time was 18 days for American Eels to pass all five dams at a distance of 195km. The majority (81%) of migratory delay experienced at each dam was less than 24 hours in the Shenandoah River. Travel speed was similar between the Shenandoah River (with five hydroelectric dams) and the Potomac River (with only one low head non-hydroelectric dam), suggesting that hydroelectric dams on the Shenandoah River did not cause a substantial migratory delay.

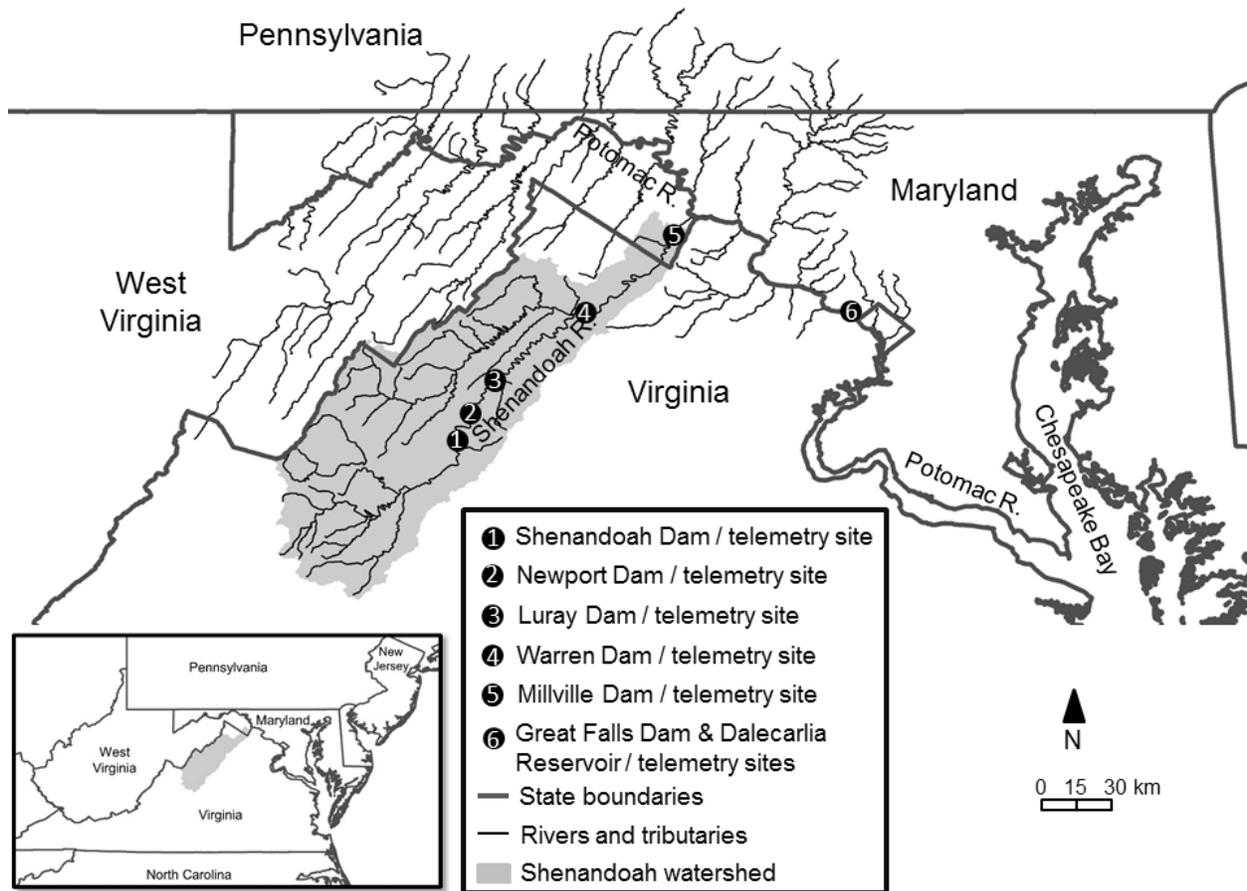


Figure 1. Potomac and Shenandoah river watersheds and location of five hydroelectric dams monitored with stationary radio telemetry for silver American Eel passage. All collection and tagging occurred upstream of the Luray Dam.

A total of 28 American Eels experienced immediate turbine mortality during the study. Turbine mortality occurred at all dams, with individual dam mortality rates ranging from 14-36% during hydroelectric generation. Turbine mortality rates were reduced to 0-6% during the turbine shutdown periods. Overall mortality rates at each dam, including the turbine shutdown periods, ranged from 3-14%, with a cumulative mortality for eels passing all five dams of 37%. The seasonal nighttime turbine shutdown period was effective in reducing mortality to downstream migrating eels.

Environmental variables were associated with downstream migration events of American Eel. River discharge, proportional increases in river discharge, and water temperature were significant factors in describing when downstream migration events occurred during the study. A logistic regression model was able to accurately describe when downstream migration events occurred 85% of the time. Lunar phase, time of year, and dam location were not significant variables in describing downstream migration events.



Shenandoah River dams likely have little impact on migration delay for American Eel, however, cumulative mortality for eels migrating while turbines are operating is significant. Turbine shutdowns are an effective method to reduce or eliminate mortality during downstream migration events. The current time period for implementation of nighttime turbine shutdowns (September 15-December 15) encompassed two-thirds of downstream migration events, however American Eel passage only occurred on a small portion of days during the shutdown period on any given year. On the Shenandoah River, the nighttime turbine shutdown period could be more protective for downstream migrating American Eel if it were implemented based on environmental variable triggers.

Stuart Welsh holding a large silver American eel from the upper Shenandoah River. The eel was surgically implanted with a radio tag prior to its release.

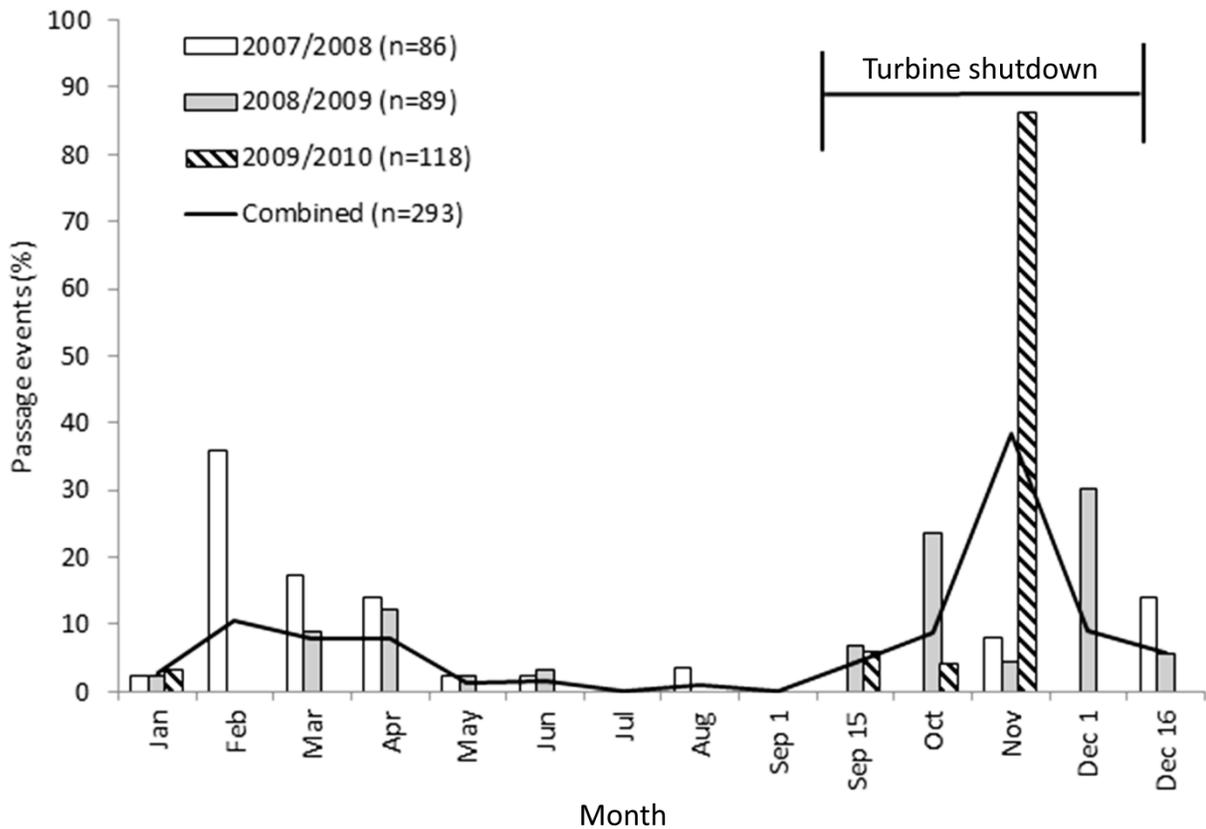


Figure 2. Percent of American Eel passage events by month during the three study years on the five hydroelectric dams on the Shenandoah River. Passage was monitored continuously from September 2007 through August 2010. Annual study periods began on 1 September and continued through 31 August of the following year.

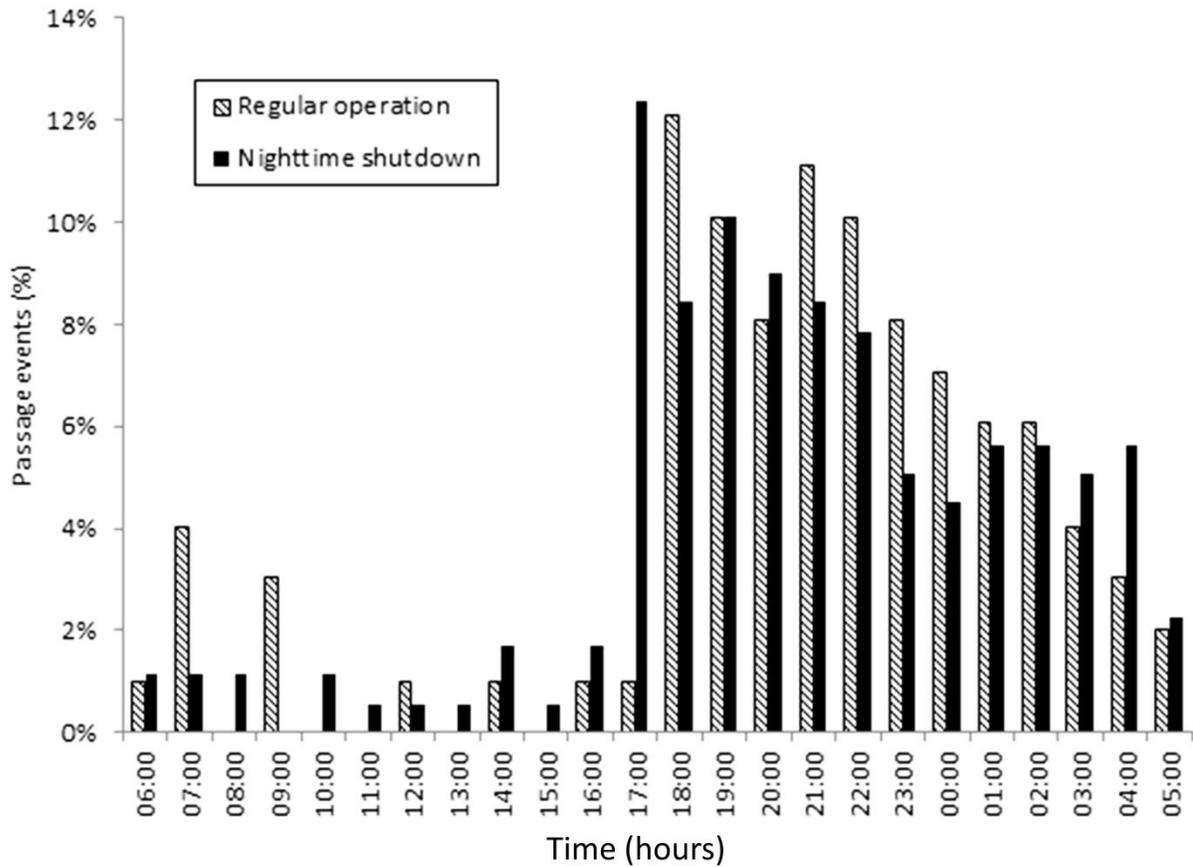


Figure 3. Percent of American Eel passage events by hour of day during the season of turbine shutdowns (15 September to 15 December) and during the rest of the year at five hydroelectric dams on the Shenandoah River from 2007–2010.



Shenandoah River (photo by Shelia Eyer)

**INVESTIGATION OF DIET, AGE, AND GROWTH OF CHANNEL CATFISH IN
CHEAT LAKE, WEST VIRGINIA**

Student Investigator: Corbin Hilling

Principal Investigator: Stuart A. Welsh

Cooperators: Frank Jernejcic and Dave Wellman

Years Ongoing: 2013-2015

Degree Program: MS

Completion: August 2015

Funding Sources: West Virginia Division of Natural Resources, FirstEnergy Corp.

Objectives:

Research objectives include examination of age/size structure, growth rates and food habits of Channel Catfish *Ictalurus punctatus* in Cheat Lake, WV.

Results:

Cheat Lake, a hydropower reservoir also known as Lake Lynn, was created in the lower Cheat River drainage in 1926. Acid mine drainage within the Cheat River watershed has been linked to decades of poor water quality, but water quality and fisheries within the reservoir have improved drastically owing to mitigation efforts and regulations controlling operation of the Lake Lynn dam. Hydropower reservoirs often experience water level fluctuations, and water level fluctuations have been regulated for Cheat Lake, in part, to manage fish populations. Cheat Lake contains a large population of Channel Catfish and provides anglers with an excellent Channel Catfish fishery. Analyzing the size structure, growth rates and food habits of Channel Catfish in Cheat Lake will provide useful information toward further management of this fishery.

A total of 155 Channel Catfish was collected during the sampling period, most of which were collected using gill nets ($n = 136$, Figure 1). A smaller proportion of fish were collected using boat electrofishing ($n = 17$) and baited hoop nets ($n = 2$). Sampling efforts of gill nets, boat electrofishing and hoop nets were 85 net-nights, 3.9 h and 4 net-nights, respectively. Mean CPUE estimates of gill nets, boat electrofishing and baited hoop nets were calculated as 1.6 fish net-night⁻¹, 4.4 fish h⁻¹ and 0.5 fish net-night⁻¹, respectively. The mean TL of the sample was 466 mm (SD = 117 mm) and the length frequency distribution shows a majority of fish collected were in excess of 400 mm (Figure 2).

Length and relative weight data were summarized using the Gabelhouse (1984) five category system. The number of individuals in the Gabelhouse (1984) categories of stock (S), quality (Q), preferred (P), memorable (M) and trophy (T) were 34, 94, 16, 1 and 0, respectively. A total of 10 fish were shorter than the minimum stock length (280 mm). Proportional size distributions with 95% confidence intervals were estimated as follows; PSD = 77 ± 8 , PSD-P = 12 ± 6 , PSD-M = 1 ± 2 and PSD-T = 0. Incremental PSDs with 95% confidence intervals were estimated as follows; PSD S-Q = 23 ± 8 , PSD Q-P = 65 ± 9 , PSD P-M = 11 ± 6 and PSD M-T = 1 ± 2 . Relative weight (W_r) was calculated for all Channel Catfish collected ($n = 155$) (Table 1). Mean W_r of the sub-stock, stock, quality and preferred categories were 98, 93, 93 and 91, respectively.



Figure 1. Corbin Hilling holding a Cheat Lake channel catfish during a night electrofishing survey.

Age was estimated for 148 Channel Catfish using lapilli. Independent readers agreed on 87% of assigned ages and agreement within 1 year was 96%. An age was agreed upon for all individuals after mutual examination. Estimated ages ranged from age 2 ($n = 5$) to age 19 ($n = 1$). Age-0 (young-of-the-year) and age-1 fish were absent from the sample. The age frequency plot showed the largest age classes in the sample were age-3,4 and 7 fish, while ages 5 and 6 were less frequent. A total of 143 fish from age 3 to age 19 were included in catch-curve analysis. Instantaneous mortality was estimated as $Z = -0.163 \pm 0.079$ (95% CI) using weighted linear regression. Annual total mortality ($A = 17.8\% \pm 9.4$ (95% CI)) was calculated from instantaneous mortality.

Four candidate growth models were fit to length-at-age data from 148 Channel Catfish (ages 2–19, length 154–721 mm). The VBGM was selected by AIC_c as the best approximating model of Cheat Lake Channel Catfish growth ($w_i = 0.39$), while the power ($w_i = 0.38$, $\Delta = 0.02$) and Gompertz ($w_i = 0.17$, $\Delta = 1.65$) models were also supported. The logistic model was the least supported model ($w_i = 0.06$, $\Delta = 3.74$). Using model averaging of the parameter estimates from the three asymptotic models, L_∞ was estimated as 589.8 mm (SE = 16.8).

Cheat Lake Channel Catfish growth was compared to growth standards produced from populations throughout the species range (Table 2). Using the Hubert (1999b) growth standards, Cheat Lake Channel Catfish mean lengths at age were at the 75th percentile or above for ages 3–8. Age-9 fish were between the 50th and 75th percentile, while age-10 fish were between the 25th and 50th percentile. Similar results were found using the Relative Growth Index. Fish of ages 3–9 exhibited a mean RGI value greater than 100, while the mean RGI value of age-10 fish was 94.9 (SD = 18.2).

Stomach contents were quantified for 55 Channel Catfish TL ranging from 274–606 mm (mean = 458.2, SD = 73.0). Channel Catfish stomachs contained food items 74.5% of the time. Of the stomachs containing food items, 31.7% contained detritus and 2.4% contained detritus only. Fall diets were numerically dominated by aquatic invertebrates. Chironomidae and Chaoboridae larvae were the most common prey items, while Ephemeroptera and Sialidae larvae were also frequently present. Piscivory was observed as fish remains were present in 9.8% of stomachs with food items.

Terrestrial food items were present in 39% of stomachs. A wide diversity of terrestrial invertebrate taxa were consumed, with Lepidoptera being the most common. Adult terrestrial insects were commonly identified in stomach contents. Mammalian remains were also present in Channel Catfish diets, as Rodentia were found in 4.9% of stomachs and were the only prey items present in those stomachs. Channel Catfish stomachs also contained seasonally available fruits and acorns 14.6 % of the time. Acorns (*Quercus* spp.), grapes (*Vitis* spp.) and paw-paw (*Asimina triloba*) seeds were identified in stomach contents.

Given the lack of regional published data on Channel Catfish growth, the robust population of Channel Catfish in Cheat Lake provided an opportunity to gather data on age, growth, and diet within a West Virginia hydropower reservoir. Channel Catfish in Cheat Lake exhibited faster than average growth. Low exploitation may contribute to Cheat Lake Channel Catfish reaching relatively old ages compared to other populations. Fall diet of Channel Catfish was also comparable to other studies. However, the high incidence of terrestrial items possibly suggests increased availability related to constant small water level fluctuations in Cheat Lake. Results from this study provide valuable information for potential future management of the population and for comparison to other populations in the region.

Table 1. Mean relative weights (W_r) of Cheat Lake Channel Catfish ($n=155$). W_r is summarized by Gabelhouse (1984) length categories, where length was measured as total length (TL) in mm. Percentile values were determined from Brown et al. (1995) using distributions of W_r by length category. NA indicates an insufficient sample size for calculation.

Length Category	TL	n	Mean W_r	95% CI	Percentile
Sub-stock	<280	10	98	(88, 108)	50–75 th
Stock	280-409	34	93	(90, 96)	50–75 th
Quality	410-609	94	93	(91, 95)	50 th
Preferred	610-709	16	91	(84, 97)	10–25 th
Memorable	710-909	1	115	NA	75–90 th
Trophy	≥910	0	NA	NA	NA

Table 2. Description of Channel Catfish growth from Cheat Lake, West Virginia ($n = 148$). Mean RGI values and Hubert (1999) percentiles are provided to compare Cheat Lake Channel Catfish growth to populations throughout the species range.

Age	n	Mean TL	Mean RGI	SD	Percentile
3	22	309	123.18	19.84	75-90
4	21	394	129.10	14.34	90-95
5	6	416	117.49	23.23	75-90
6	11	505	126.61	12.25	90-95
7	39	503	114.33	14.06	75-90
8	4	549	115.15	16.78	75-90
9	7	543	106.41	15.59	50-75
10	7	533	94.85	18.17	25-50

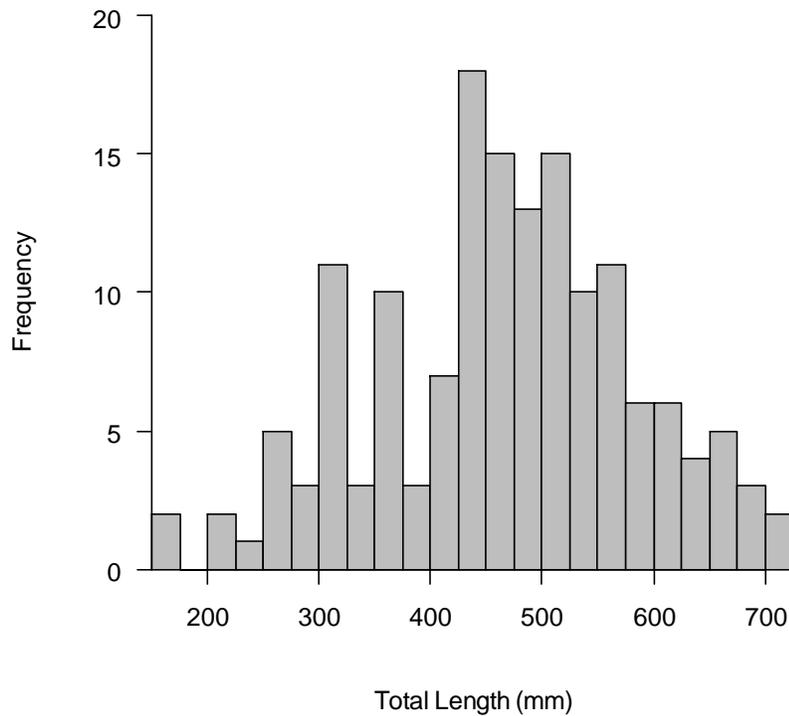


Figure 2. Length frequency distribution of Channel Catfish from Cheat Lake, West Virginia ($n = 155$) using 25 mm bins.

SOURCE POPULATION ASSIGNMENT OF LAKE SUPERIOR LAKE STURGEON

Student Investigator: Christi Raines
Principle Investigator: Dr. Amy B. Welsh, WVU
Years of Project: 2013 - 2015
Degree Program: M.S.
Completion Date: May 2015
Funding Source: U.S. Fish and Wildlife Service (RWO 63)

Objective:

The objective of this project is to determine the source spawning population of juvenile lake sturgeon (*Acipenser fulvescens*) captured throughout Lake Superior.

Progress as of August 2015:

Knowledge of lake sturgeon movements and habitat use during non-spawning times is limited. Available information suggests that lake sturgeon are capable of long-distance migrations and tend to densely aggregate in relatively small areas. Movement patterns can vary depending on life-stage. Traditional mark-recapture methods or radio telemetry studies of tagged fish provide limited data about lake sturgeon movement because relatively few individuals from any breeding population are tagged or marked. Genetic techniques can overcome these obstacles and can identify which spawning populations are represented in non-spawning groups of lake sturgeon.

Genetic analyses were conducted on samples from 690 sampled juveniles. Estimates of proportional contributions of sturgeon from all potentially represented spawning populations to samples collected in all assessments during non-spawning periods was made based on established statistical methods. The relative contribution of each potential spawning lake sturgeon population was estimated by comparing the distribution of expected genotype frequencies of each spawning population with genotype frequencies observed from lake sturgeon of unknown origin captured during non-breeding time periods. Mixed stock analysis (MSA) uses the relative differences in genotype frequencies between baseline spawning populations to estimate the composition of a mixed sample. The power of the genetic baseline of spawning populations was also tested in order to better understand the level of confidence associated with the assignment of the unknown individuals.

Results indicate that most juvenile lake sturgeon stay close to their spawning site of origin. The large proportion of genetic assignments was to the spawning site closest the site of capture. However, there were several individual migrants detected as well, indicating some long distance movement away from the spawning site. Individuals from the Wolf River in Lake Michigan were stocked into the St. Louis River in Lake Superior. Some of these stocked individuals were detected at spawning sites other than the St. Louis River, indicating straying of stocked lake sturgeon. A manuscript is currently being prepared for submission to a peer-reviewed journal.

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 – 2015
Degree Program: PhD
Expected completion: Dec 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.

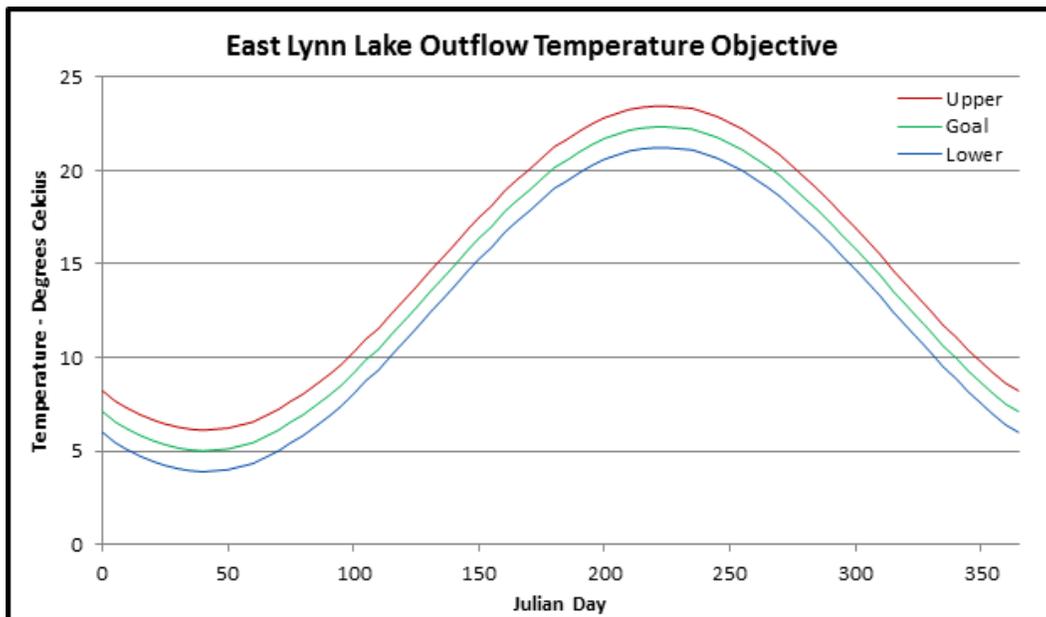


Figure 1. East Lynn Lake outflow temperature guideline curve.

FISH USE OF MISSISSIPPI RIVER DREDGE AND DISPOSAL SITES

Student Investigator: Edward Olesh
Principal Investigator: Kyle Hartman
Years Ongoing: 2015-2016
Degree Program: M.S.
Expected Completion Date: September 30, 2016
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 during fall-winter 2015-16. The M.S. student on the project (Ed Olesh) is onboard and has been busy preparing the research vessel and research equipment for use on this project. River stages prevent the safe operation of research vessels during the late spring and summer months. During this time we have performed maintenance on the R/V Anchoa and constructed a benthic sled for sampling benthic communities at dredge, disposal and reference locations. We anticipate conducting survey work beginning in September or October 2015 using a before/after and reference/treatment design. We will select 4 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: 2014-2015
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 potential Diamond Darter habitat in the lower Elk River.
5. 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). The Diamond Darter was originally distributed throughout the Ohio River Basin; however, it is 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.



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

Prior to 2011, researchers struggled to locate 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 spotlifter 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, nighttime searches of glide habitat have occurred during the spring and summer 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 searches during spring and summer and nighttime searches during summer 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 Variegated darters) are being used to test the methodology. Preliminary analysis shows a strong correlation between direct measurements and those made via the photogrammetry technique ($r > 0.95$). I have also begun using this technique to take measurements of Diamond Darters during night surveys (Figure 2).

The third research objective involves evaluation of seasonal and diel variation of Diamond Darter occupancy and abundance within glide habitats. Specifically, this study will examine temporal variation in occupancy and detection probability during summer and during night hours. In addition to these temporal covariates, other sampling covariates will also be investigated. One outcome of this study is to document the best times during the year and night to sample in order to maximize the effectiveness of monitoring efforts for Diamond Darter presence/absence and abundance. We will focus our efforts for this study on three glide areas (sites) associated with gas pipeline crossings within the lower Elk River.

Sampling during 2015 occurred during May and August, but high river discharges prevented sampling during June and July. Totals of 121 and 44 Diamond Darters were detected during May and August, respectively. The study will continue through 2016.

A fourth research objective includes mapping potential Diamond Darter habitat in the lower Elk River. This study aims to develop a method that will allow for mapping a relatively large stretch of river with minimal expenses and time invested. The focus is on mapping benthic habitat in enough detail to be able to detect landscape level changes that may explain Diamond Darter distribution. This study will be initiated in 2016.

A final research objective is to develop an occupancy model for Diamond Darters in the lower 50 km of the Elk River. One of the biggest obstacles when assessing the status of the Diamond Darter is that the species is difficult to detect during surveys, which can result in underestimating the proportion of sites occupied, and likely the full extent of its range. Fortunately, through the use of occupancy modeling, adjustments can be made in the occupancy estimates to account for incomplete detection. By using both site and sampling covariates in the occupancy model, we will be able to determine variables which influence site occupancy and detectability. Specific outcomes of this study will include (1) documenting site covariates which influence occupancy at a glide, (2) documenting sampling covariates that influence detectability, (3) estimates of detection probabilities for our sampling method, and (4) current status information for the Diamond Darter in the lower 50 km of the Elk River. This project is scheduled to begin during the summer of 2016.



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

**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

Expected completion: Dec 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) is a continuation of previous work by West Virginia Division of Natural Resources. Three tasks of the proposed work focus on Cheat River and Cheat Lake tailwaters (Tasks 1 – 3), and 6 tasks focus on Cheat Lake (main lake and embayments). Field data collection for all objectives has been completed. Data analysis and final report preparation are currently ongoing. Quarterly progress reports are provided to the funding agencies.

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 has been 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 has consistently maintained water quality that is 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 $\mu\text{s}/\text{cm}$. In contrast, water quality in Grassy Run, an acidic tributary to the Cheat River, as expected has had poor water quality with an average pH of 3.1 and conductivity of 1422 $\mu\text{s}/\text{cm}$. In general, Cheat River water quality downstream of Grassy Run reflects the impacts of acid mine drainage (AMD) from Grassy Run with an average pH of 5.5 and an average conductivity of 220 $\mu\text{s}/\text{cm}$.

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 when the combined effects of AMD and acidic snowmelt impact the lake. This trend of early spring pH depression has occurred since the initiation of lake profiles by WVDNR in 2005. However, in 2012, 2013 and 2014 Cheat Lake did not experience pH depressions below 6.0, possibly due to increases in AMD remediation upstream in the Cheat River watershed. Otherwise, lake pH was satisfactory, remaining above 6.0 the majority of the time. Stratification of water temperature and dissolved oxygen has historically occurred in lower Cheat Lake from approximately June-September. In general, the upper 6-8 meters of the water column is characterized by warmer water with suitable dissolved oxygen levels (above 5.0 mg/L), while the lower portion of the water column is characterized by much colder water with increasingly less dissolved oxygen (less than 5.0 mg/L). This phenomenon occurs primarily in the lower portion of Cheat Lake which is characterized by much greater depths. However, given the increases in precipitation and cooler air temperatures in 2013 and 2014, Cheat Lake did not experience the severity of stratification during these years that normally occurs during summer months.

Night boat electrofishing and gill netting were conducted during May and October 2011-2014 in Cheat Lake. The primary focus of these surveys was to monitor the health of the fish communities of Cheat Lake. In total, 839 fishes were captured with gill nets, while 5,683 were collected using electrofishing. The upper lake, which retains many riverine characteristics, consistently produced a greater abundance of fish compared to both the lower lake and embayment areas. The embayment areas produced the lowest fish abundances. 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 area. 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.



Figure 1. Dustin Smith with a walleye captured during walleye population surveys.

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. The tailwater area just below the dam has a relatively low abundance of macroinvertebrates, likely stemming from the variation in outflow from the upstream dam. The family Chironomidae (midges) accounts 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 support a much greater abundance of macroinvertebrates. However, the macroinvertebrate community at these sites has low diversity mainly comprised of tolerant taxa. Macroinvertebrates from the families Chironomidae and Hydropsychidae (net-spinning caddisflies) account for most of the macroinvertebrates at these downstream stations.

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 in order to reach spawning habitat. By early March (2012) to early April (2013-2015), most tagged fish moved to the head of 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 increases during summer when main lake water temperature increases and dissolved oxygen decreases. Tagged walleyes can potentially alleviate stressful temperature and oxygen conditions by using the cooler, more oxygenated riverine area.

Walleye population monitoring and stocking assessment surveys were conducted in Cheat Lake during March/April and November of 2012, 2013, and 2014. 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 most likely that the walleye population was greatly underrepresented during our surveys. Most adult walleye were likely near the head of the lake, thus 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, and 2014 was much higher with 1.3-2.8 fish per net night.

Currently, an aquatic vegetation map of Cheat Lake is being created and is near completion. Areas of Cheat Lake that harbor aquatic vegetation have been visually assessed to determine species composition and relative abundance. This information is currently being incorporated into a lakewide map using GIS, and will depict species presence/abundance information. Areas of highest aquatic vegetation abundance are embayment habitats such as the Morgans Run and Rubles Run embayments. Dominant vegetation taxa found in Cheat Lake include *Potamogeton* spp., *Vallisneria americana*, and *Najas* spp. This

information will help determine areas that likely represent import nursery habitat for larval/juvenile fishes of Cheat Lake.

A bathymetric map of Cheat Lake was created in 2011 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.

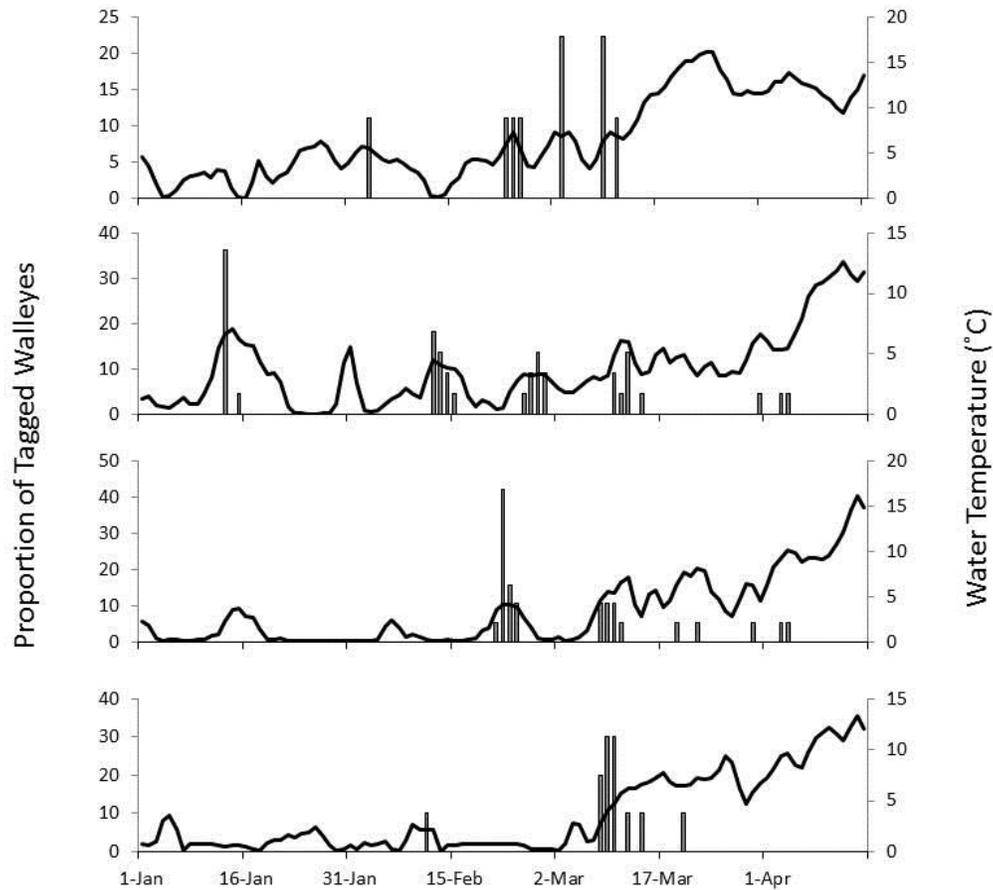


Figure 2. Daily proportion of tagged walleyes migrating toward spawning areas (gray bars) during the pre-spawn period and associated water temperature (black line) data for 2012-2015.

DISTRIBUTION 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: July 2016

Funding Sources: Currently not funded

Objectives:

1. Create a distribution map of Eastern and Western sand darters within the Elk River.
2. Determine microhabitat use for the Western Sand Darter.
3. Examine if microhabitat use differs between the two sand darter species.

Progress:

Eastern and Western sand darters (subgenus *Ammocrypta*) are the only sympatric sister species of the genus *Ammocrypta*. The Elk River of West Virginia is one of the few rivers where these species are sympatric. These sand darters generally inhabit medium sized rivers with loose sand and gravel substrates, a moderate current, and spend the majority of the time buried in sandy streambeds. They were once broadly distributed, but have since undergone range-wide population declines, due to siltation of their preferred substrate, impoundment of rivers, and the pollution of waterways. The Eastern Sand Darter is distributed in the lower Great Lakes (Lakes Huron, Erie, and Ontario), the St. Lawrence River and Lake Champlain drainages, with fragmented populations also in Illinois, Kentucky and West Virginia. In contrast, the Western Sand Darter which is concentrated along the Mississippi River system and the Lake Michigan drainage system, with fragmented populations in the Ohio and Tennessee river drainages. Published literature on the Eastern and Western sand darters is sparse, and both distributions are not well defined in the Elk River. Understanding a species distribution and habitat use is essential for identifying areas for protection, inventory, and restoration.

Thus far, we sampled 55 sites throughout the lower 200 rkm of the Elk (Fig. 1). We detected Eastern Sand Darters at 39 sites from Mink Shoals (7.8 rkm) to Frametown (133 rkm). The Western Sand Darter was detected at only 4 sites near the town of Clendenin (36 rkm), and at 3 sites both species were present. There are some areas in the upper and lower Elk River that will be sampled later this year. The distribution of the Western Sand Darter in the Elk River is limited compared to the Eastern Sand Darter, and through the use presence-absence data along with habitat information, and a laboratory study we aspire to elucidate what is driving the differences between their distributions.

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. 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.

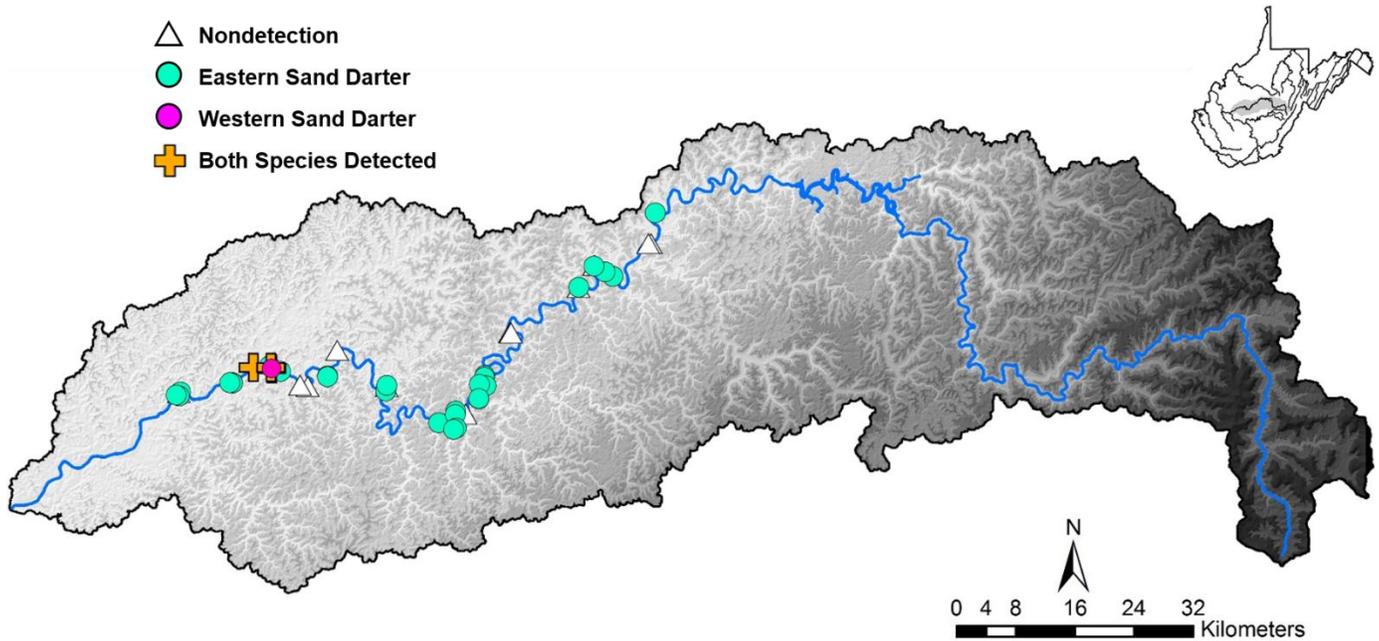


Figure 1. Map of the Elk River watershed (shaded by elevation) with sampling site locations.

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

Principal Investigator: Stuart A. Welsh
Years Ongoing: 2009 – 2015
Expected Completion: 2015
Funding Source: WVDNR, PE Hydro Generation, LLC

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.

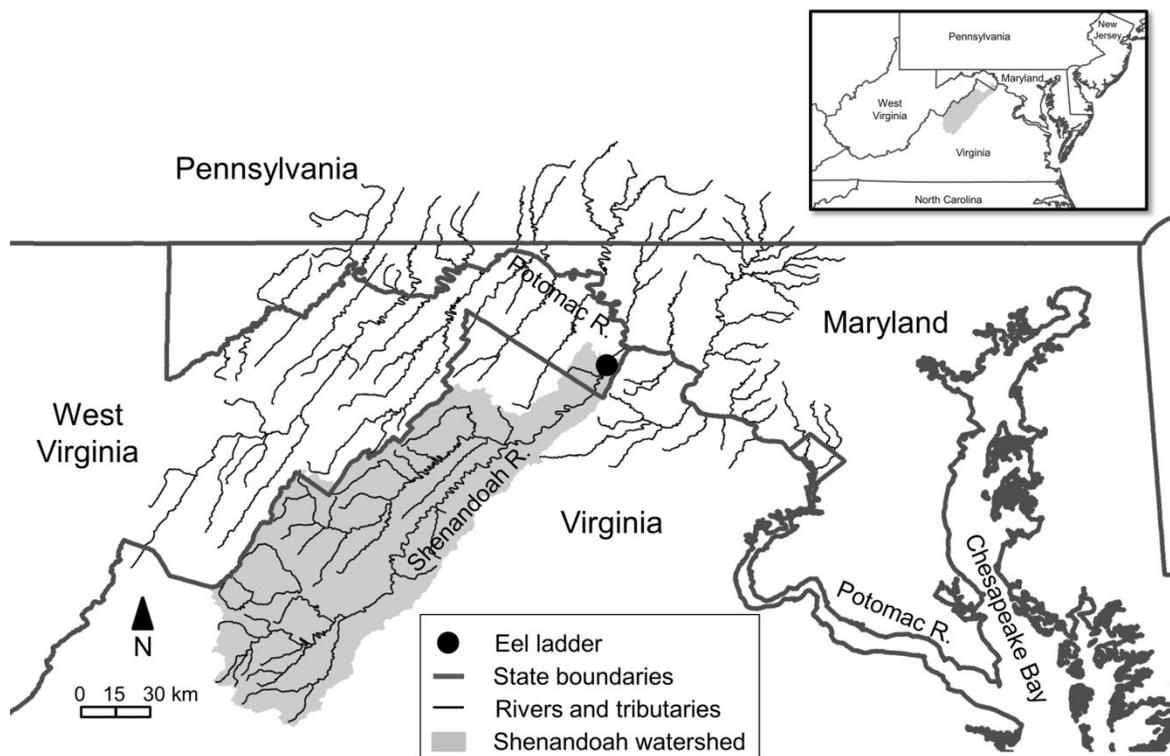


Figure 1. Map of the Potomac River drainage and the location of the Millville Dam eel ladder on the Shenandoah River.



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

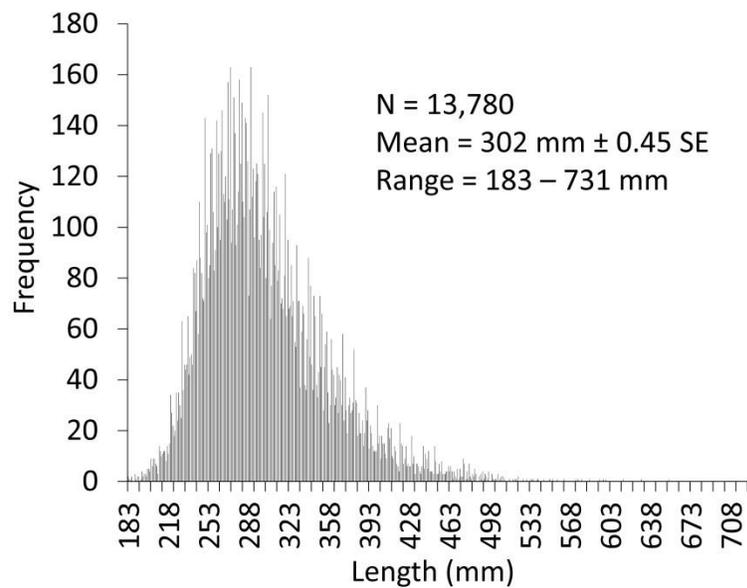


Figure 3. Length-frequency histogram of 13,780 American Eels from the Millville Dam eel ladder on the Shenandoah River. The mean length of American Eels using the ladder is 302 mm with a range of 183–731 mm, although individuals >500 mm rarely use the ladder.

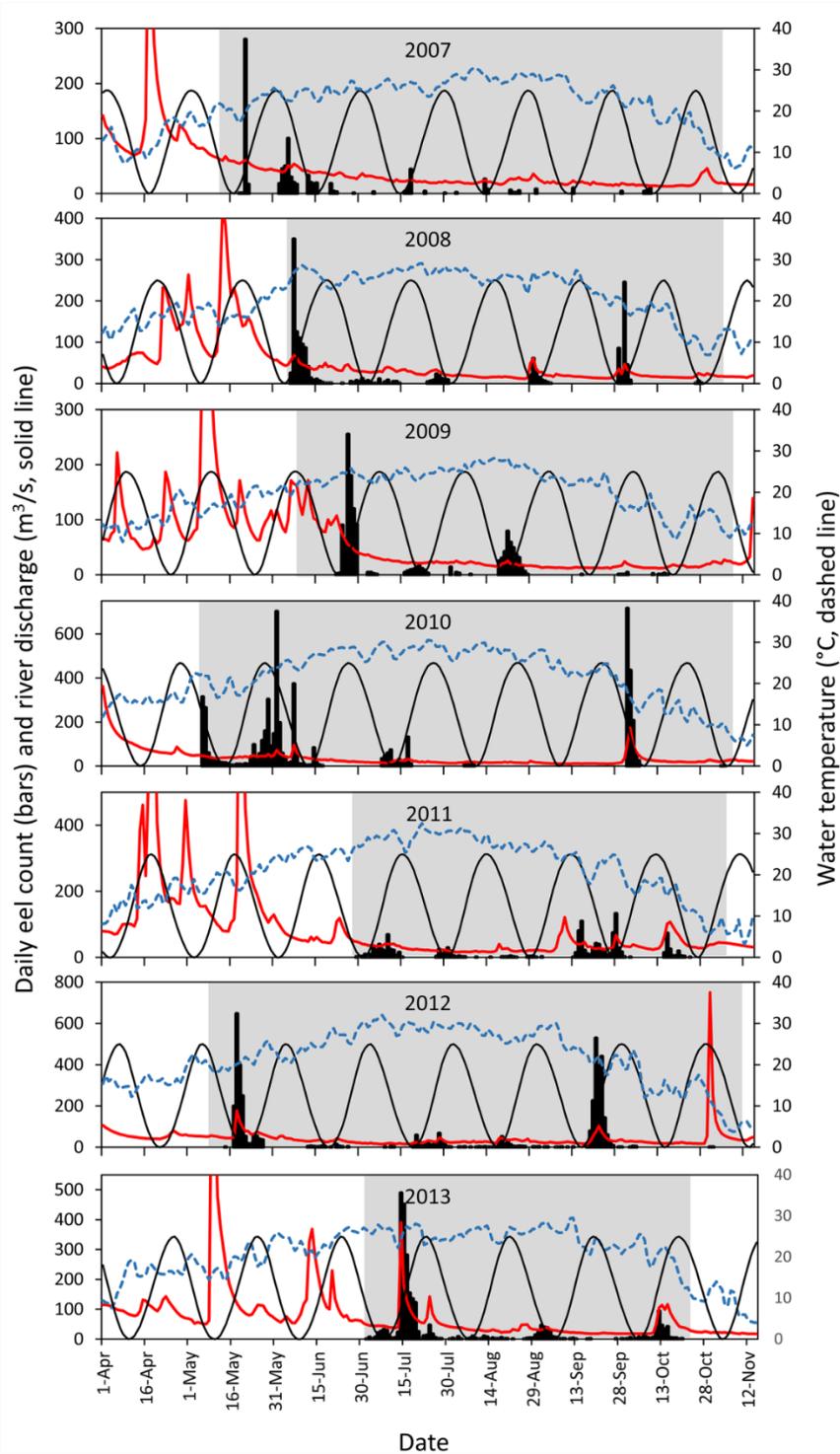


Figure 4. Daily counts of American eels at the Millville Dam eel pass and associated environmental variables for seven annual sampling periods from 2007 to 2013. Mean daily river discharge values (m^3/s) are from the Shenandoah River at Millville, West Virginia. Daily values of lunar illumination range from 0 to 100%. Shaded area represents the time period of eel pass operation.

AQUATIC FISH HEALTH PROJECTS

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

Student Investigator: Ryan Braham, M. S.

Principal Investigators: Dr. Patricia Mazik

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

Years Ongoing: 2013 - 2016

Degree Program: PhD

Expected Completion: December 2016

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) and total estrogenicity in the pelagic and periphyton community at selected sites in West Virginia, Maryland, and Virginia. Investigate spatial and temporal distribution of microcystin toxins and total estrogenicity present in the water column, as well as the potential toxin and estrogenicity available in both the water column and the periphyton communities.
2. 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.
3. 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.
4. Quantify changes in relative immune function of smallmouth bass (*Micropterus dolomieu*) and golden redhorse suckers (*Moxostoma erythrurum*) at selected sites in West Virginia, Maryland. Examine molecular endpoints (identified in objective 3) which may indicate exposure to biologically relevant concentration of 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 and total estrogenicity present in the filtered water column, unfiltered water column and periphyton. Preliminary analysis has shown the presence of microcystin toxins and total estrogenicity present in both the filtered and unfiltered water fractions at relatively low levels. Analysis is ongoing. During FY 2015, 3 sites were identified to have filtered water, unfiltered water, and periphyton samples and collected bimonthly for total microcystin and total

estrogenicity quantification. 93 samples were collected between June 24, 2014 and August 7, 2015 and analyzed for total microcystin toxin and total estrogenicity present in the filtered water column, unfiltered water column and periphyton. Collection will continue bimonthly through November, 2015. Analysis is ongoing. In addition, 87 samples have been collected between June 24, 2014 and August 7, 2015 and analyzed for total nitrogen, total phosphorous, and total dissolved phosphorous in the water column and total nitrogen and total phosphorous in the sediment at these sites. Collection will be continued bimonthly through November, 2015. Analysis is ongoing.

Objective 2: A total of 240 smallmouth bass were collected among 4 sites between March 29, 2013 and May 5, 2015. A total of 254 golden redhorse suckers were collected among 2 sites between April 4, 2013 and April 7, 2015. Samples of liver were retained for quantification of microcystin present. Analysis is ongoing.

Objective 3: A total of 18 smallmouth bass were collected on April 7, 2015 and May 28, 2015. Anterior kidneys were removed and exposed to microcystin-LR *in vitro*. RNA was extracted and prepared for quantification of the transcriptome. Analysis is ongoing.

Objective 4: A total of 240 smallmouth bass were collected among 4 sites between March 29, 2013 and May 5, 2015. A total of 254 golden redhorse suckers were collected among 2 sites between April 4, 2013 and April 7, 2015. Samples of anterior kidney were retained for quantification immune function genes identified in objective #3. These were the same individuals collected for objective #2 allowing for comparison across biological endpoints. Analysis is ongoing.



Ryan Braham with a small mouth bass on the South Branch River, WV

**EVALUATION OF MOLECULAR BIOMARKERS FOR USE IN THE ASSESSMENT OF FISH HEALTH IN
GREAT LAKES AREAS OF CONCERN**

Student Investigator: Cassidy Hahn

Principal Investigators: Dr. Patricia Mazik

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

Years Ongoing: 2011 – 2015

Degree Program: PhD

Expected Completion: May 2016

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

Objectives:

The primary objectives of this study are the creation and evaluation of molecular endpoints to assist in the assessment of wild fish health from Areas of Concern (AOCs) throughout the Great Lakes region. These AOCs are known to contain both legacy and emerging environmental contaminants for which the biological effects are unknown. To address this issue, a suite of biological endpoints from the molecular to organismal level were collected and analyzed. At each site samples were collected from 20 mature fish of two species, brown bullhead (*Ameiurus nebulosus*) or white sucker (*Catostomus commersoni*) and largemouth bass (*Micropterus salmoides*) or smallmouth bass (*Micropterus dolomieu*). Whole transcriptome databases for each species of interest were created using pooled tissue samples. Liver samples from these collections were then used to design and evaluate gene expression biomarkers using the Nanostring Technologies nCounter Assay.

Progress:

Sample Collection and Processing

Sampling for this project is complete. Samples were collected in the fall of 2010, spring of 2011, and spring of 2012 from a total of 8 AOCs throughout the Great Lakes region. Whole transcriptome databases were created for the three non-model species, smallmouth bass, brown bullhead and white sucker. The initial database for brown bullhead and smallmouth bass was created using wild caught and laboratory fish which had been exposed to estradiol. Liver, gonad, anterior kidney and spleen tissue was pooled. Total RNA was extracted using an E.N.Z.A. ® Total RNA Kit (Omega Biotek, Norcross, GA) pooled and enriched for non-ribosomal RNA using a RiboZero rRNA removal kit (Epicentre, Madison, WI). Total RNA was extracted and sent to Cofactor Genomics (St. Louis, MO) for sequencing on an Illumina (San Diego, CA), BLASTx gene annotation and non-mapping reads assembly. An additional smallmouth bass run was later performed at Duke Institute for Genome Science and Policy (Durham, NC) using 454 sequencing on a GS Junior (Roche, Branford, CT). Hybrid assembly of these databases was carried out using CLC workbench. White sucker transcriptome sequencing was performed using pooled samples of male and female wild fish collected throughout the Great Lakes region. Total RNA was isolated from skin and liver samples using an E.N.Z.A. ® RNA Kit (Omega Biotek, Norcross, GA) from nine individuals, pooled and enriched for non-ribosomal RNA using a RiboZero rRNA removal kit (Epicentre, Madison, WI). An RNA sequencing library was prepared and paired-end (2 x 100) ultra-deep sequencing was carried out at the Institute for Genome Sciences (Baltimore, MD) on an Illumina HiSeq2000 (San Diego, CA). Paired-end libraries were then *de novo* assembled into putative contigs (>200bp) using CLC workbench and identified through BLASTn and BLASTx searches on a local database.

The Blast2GO plugin for CLC Genomics Workbench was used for functional annotation of sequence data. First, sequences were used as queries in a blastx searches against the uniprot databases for medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*) applying the BLOSUM62 matrix and an e-value of 1×10^{-6} . We kept the top 10 alignments for each sequence. Next, functional information for all blast hits was retrieved from the Gene Ontology database and annotated using default parameters in Blast2GO. To reduce the amount of functional information obtained, GO-Slim reduction was then performed using default parameters in Blast2GO.

Transcriptome databases were used to identify genes of interest. In each species 50 genes including, 5-7 housekeeping genes were chosen for analysis (Table 1). Liver samples collected for gene expression analysis were processed using NanoString Technologies nCounter system. Approximately 17 – 23 mg of liver tissue was combined with 400 μ l of TRK lysis buffer (Omega Biotech, Norcross, GA). The tissue was then homogenized on a tissue lyser (Qiagen, Valencia, CA) with 5 mm stainless steel balls (Boca Bearings, Boynton Beach, FL). Samples were then centrifuged for 10 minutes at maximum speed and the supernatant was removed. Samples were stored at -80°C until shipment. Tissue lysate was sent to the University of Pittsburgh Genomics and Proteomics Core Laboratory (Pittsburgh, PA) for mRNA expression analysis on the NanoString Technologies platform. This system relies on multiplexed, digital counting of individual RNA molecules and does not require amplification therefore limiting bias of results. The assay allows for simultaneous measurement of genes and thus is ideal in the study of contaminant exposure where specific pathway effects are largely unknown. Sequence data for the genes of interest in this assay must be supplied by the user, which allows more specificity than microarray analysis while providing quantitative data at a similar sensitivity as real time PCR. To date, samples for smallmouth bass (n=192), largemouth bass (n=384), brown bullhead (n=413) and white sucker (n=288) have been processed using this method.

Cross-species hybridization of target mRNA probes between *Micropterus* species was tested. On a single day in the spring of 2012, both species of bass were collected from a site at the Raisin River (Monroe, MI). These collected individuals allowed for the assessment of the utility of the *M. dolomieu* codeset for the use in *M. salmoides* without sampling collection bias. Six LMB bass, 3 males and 3 females, were added to the initial NanoString run for SMB. Data from these individuals was used for regression analysis of all probes between species in SigmaPlot (Systat Software, San Jose, CA).

To verify the sensitivity and accuracy of the nCounter system, we compared it to real-time PCR. Total RNA was isolated from tissue lysates for smallmouth bass and largemouth bass, which had been previously sent for nCounter analysis, using the E.N.Z.A. [®] Total RNA Kit (Omega Biotek, Norcross, GA). Extracted RNA was converted into cDNA using a high capacity cDNA reverse transcription kit (Life Technologies, Grand Island, NY). Primers were designed using Integrated DNA Technologies Real-Time qPCR Assay design tool for the vitellogenin gene and a housekeeper. SYBR Green real-time PCR was used to analyze cDNA samples on a Rotor-Gene Q (QIAGEN, Valencia, CA) in duplicate. For both assays, the data were normalized to elongation initiation factor 3D. Correlation between relative expression patterns for nCounter and real-PCR data were tested using SigmaPlot (Systat Software, San Jose, CA).

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

Micropterus species	<i>Catostomus commersonii</i>	<i>Ameiurus nebulosus</i>
03 β Hydroxysteroid Dehydrogenase (3 β HD)	11 β Hydroxysteroid Dehydrogenase (11BHD)	03 β Hydroxysteroid Dehydrogenase (3 β HD)
17 β Hydroxysteroid Dehydrogenase (17 β HD)	17 β Hydroxysteroid Dehydrogenase (17 β HD)	17 β Hydroxysteroid Dehydrogenase (17 β HD)
Actin β (β ACT)	Androgen Receptor (AR)	Androgen Receptor (AR)
Androgen Receptor (AR)	Apolipoprotein A1 (APA1)	Apolipoprotein A1 (APA1)
Apolipoprotein A1 (APA1)	Aryl Hydrocarbon Receptor (AHR)	Arginase (ARG)
Arginase (ARG)	Catalase (CAT)	Aryl Hydrocarbon Receptor (AHR)
Aryl Hydrocarbon Receptor (AHR)	CTNNB1	Catalase (CAT)
Catalase (CAT)	CYP11C1	Catenin β (β CAT)
Catenin β (β CAT)	CYP1B2	CYP17
Choriogenin (CGR)	CYP3A	CYP19A1A (Aromatase)
CYP17	Elongation Factor 1 α (EF1 α)*	CYP1A
CYP19A1A (Aromatase)	Epidermal Growth Factor Receptor (EGFR)	CYP3A
CYP1A	Epoxide Hydrolase (EH)	Cytochrome C Oxidase (COX)
CYP3A	Estrogen Receptors α (ER α)	Elongation Factor 1 α (EF1 α)*
Cystenin-Rich Protein (CRP)	Estrogen Receptors β 1 (ER β 1)	Epidermal Growth Factor Receptor (EGFR)
Elongation Factor 1 α (EF1 α)*	Eukaryotic Translation Initiation Factor 3D (ETIF3D)*	Epoxide Hydrolase (EH)
Epidermal Growth Factor Receptor (EGFR)	Ferritin (FRT)	Estrogen Receptors α (ER α)
Epoxide Hydrolase 1 (EH1)	Fibroblast Growth Factor (FGF)	Estrogen Receptors β 1 (ER β)
Estrogen Receptors α (ER α)	Follicle Stimulating Hormone Receptor (FSHR)	Eukaryotic Translation Initiation Factor 3D (ETIF3D)*
Estrogen Receptors β 1 (ER β 1)	Glucocorticoid Receptor (GR)	Ferritin (FRT)
Estrogen Receptors β 2 (ER β 2)	Glutathione Peroxidase 1 (GP)	Fibroblast Growth Factor (FGF)
Eukaryotic Translation Initiation 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 90 α (HSP90 α)	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 β 1 (TGF β 1)
		Thyroid Hormone Receptor α (THR α)
		Thyroid Hormone Receptor β (THR β)
		Tumor Protein p73 (TP73)
		Tumor protein p53 (TP53)
		Superoxide Dismutase (SOD)
		v-K-ras
		Vitellogenin (VTG)
		Warm Temperature Acclimation Protein 65 (WAP65)

Statistical Analysis

NanoString nSolver software (V2.0) was used for quality control and normalization of gene expression data. To normalize, negative controls with high average counts (values ranging from 7-37) were removed before calculating and subtracting the geometric mean from each sample to account for background noise. Next, the average geometric mean of positive spike-in RNA control samples across all lanes was used to calculate a scaling factor which was applied to all samples. Lastly, reference (housekeeper) gene normalization was performed. Housekeeping genes varied between species as necessary to account for variation in each custom codeset. Housekeeping genes were chosen to span average counts and %CV values of all data collected. Potential housekeeping genes included RBMX2, RPL8, PEPCK, TBP, ET1F3D, BACT, EF1A, MUS81, HPRT1. Specific genes used for each species are denoted in table 1. The geometric mean of the housekeeping genes was used to calculate a scaling factor which was applied to all data. To eliminate genes with low count data the assay's reporting limit was calculated. The mean and standard deviation for the negative controls in each lane was calculated. The mean plus three standard deviations was then subtracted from all gene expression data. Genes with expression data falling below this limit were eliminated from subsequent analysis.

The Bioconductor edgeR package (Robinson et al. 2010) was used to assess differential expression of genes. This package is typically used in the analysis of RNA-seq data, but can be applied to other digital gene expression profiles when normalization procedures are omitted. edgeR uses empirical Bayes estimation and exact tests based on the negative binomial distribution which is more appropriate for NanoString data than the traditional t-test. The test allows for higher estimates of variance when expression levels are low and should therefore reduce the number of false positives relative to the t-test. In order to test for differential expression of genes between sexes, data for each species was grouped according to season of collection for analysis. Genes determined to be differentially expressed between sexes were then removed in order to analyze for genes with differential expression between seasons. Data for each species was then analyzed to assess differential gene expression between seasons. Genes analyzed under this method were considered statistically significant when log₂ fold changes were ≥ 2 and p-values and false discovery rates were reported at <0.05 .

Results

Hybrid assemble of the transcriptome sequencing runs for SMB samples yielded 591,960 contigs (>200 bp). The mean length of reads produced was 348bp with a median length of 290bp. Transcriptome sequencing of BBH samples produced 340,943 contigs (>200 bp) with a mean length of 300bp and a median length of 257bp (BioProject PRJNA167114). The WHS transcriptome database produced contained 74,548 contigs (>200 bp) with a mean length of 856bp and a median length of 533bp (Table 2). A high amount of functional diversity was identified in each of the databases. The smallmouth bass transcriptome contained 42 GO-slim terms with top hits in cell, cellular process, and binding. The brown bullhead transcriptome contained 31 GO-slim terms with top hits in cell, cellular processes and organelle. The white sucker transcriptome contained 31 GO-slim terms with top hits in cell, cellular process and single-organism process.

Table 2. Transcriptome sequencing results by species.

Species	Platform	SRA Experiment #	Total # Contigs (>200 bp)	Mean length of read
<i>Micropterus dolomieu</i>	Illumina	SRR199239	591960	348
	454	SRX156704		
<i>Catostomus commersonii</i>	Illumina		74548	856
<i>Ameiurus nebulosus</i>	Illumina	SRX199312	340943	300
	454	SRX148685		

Regression analysis of probes between *Micropterus* species was performed separately for males and females. In males, probes were highly correlated (adj. $R^2 = 0.90$) between LMB and SMB with 3 falling outside of the 95% prediction band (Fig. 1). In females, probes also showed high correlation (adj. $R^2 = 0.87$) between LMB and SMB with 3 probes falling outside of the 95% prediction band (Fig. 1).

Regression analysis of the relative expression of the vitellogenin gene between nCounter and real-time PCR data was able to identify a correlation. The correlation was consistent for genes that are expressed at both low and high levels (Fig. 2).

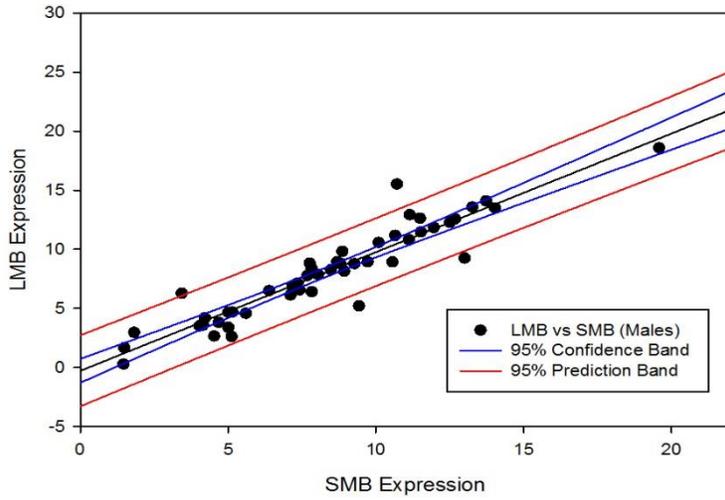


Figure 1. Regression analysis of male (A) and female (B) LMB and SMB with 95% confidence and 95% prediction bands. In males, adj. Rsqr = 0.90. In females, adj. Rsqr = 0.87.

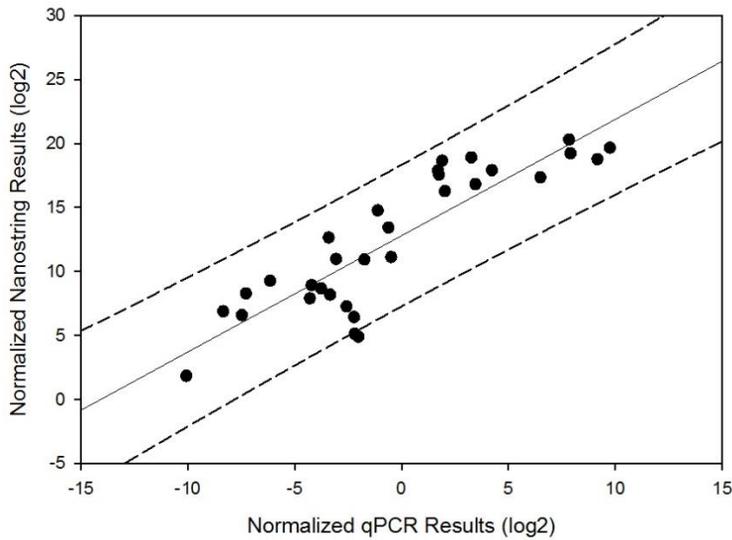
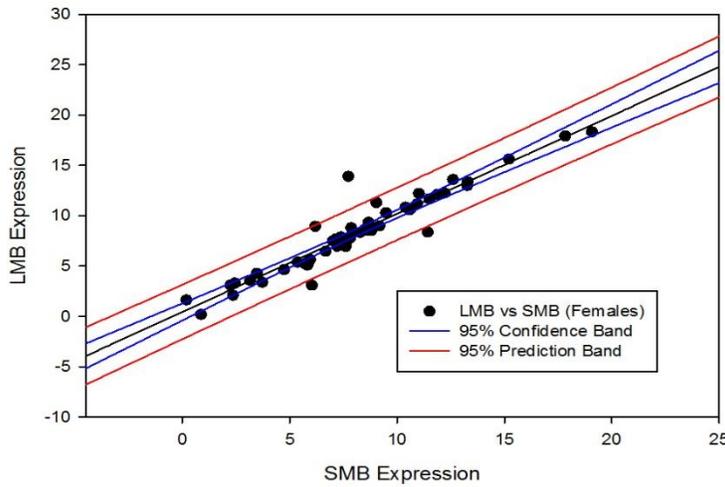


Figure 2. Regression analysis Nanostring Results vs qPCR results.

Genes reported with differential expression between males and females varied between species (Fig. 3). The largest number of differentially expressed genes between males and females was reported in the *Micropterus* species. In spring collections, 5 genes in SMB (VTG, ER α , CGR, WAP65, MT), 2 genes in LMB (VTG, CGR) and 4 genes (ER α , CAT, PCNA, WAP65) in BBH were reported as differentially expressed. In fall collections, 5 genes in SMB (VTG, CGR, ER α , HEP2, TFGb), 2 genes (CGR, VTG) in LMB and one gene (ER α) in BBH were reported as differentially expressed. In WHS, seasonality had no effect with only one gene in WHS (VTG) found to be different between males and females in both seasons.

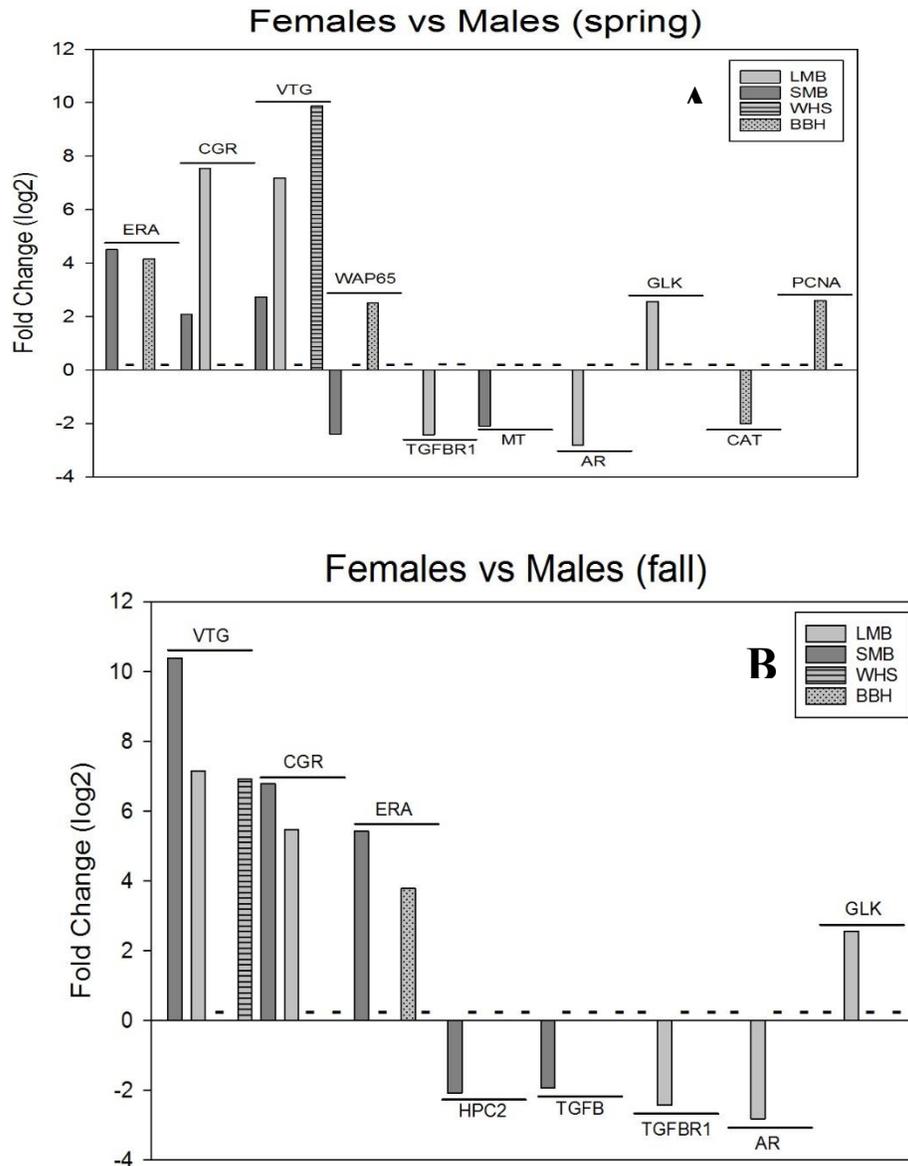


Figure 3. Genes reported with differential expression between males and females during each season. A=spring, B=fall.

Genes reported with differential expression between spring and fall varied between species (Fig. 4). In males, six genes (HEP2, VTG, CGR, MT, WAP65, ERA) in SMB, four genes (DOI1, CGR, GLK, VTG) in LMB, eight genes in BBH (COX, GRNB, 17BHD, CAT, GP, MYXO, WAP65, ERB) and three genes (11BHD, VTG, PCNA) in WHS were reported with differential expression between seasons. In females, one gene (HEP2) in SMB, four genes (ARG, DOI1, AR, VTG) in LMB, two genes (3BHD, MYXO) in BBH and one gene (GPX) in WHS were reported with differential expression between seasons.

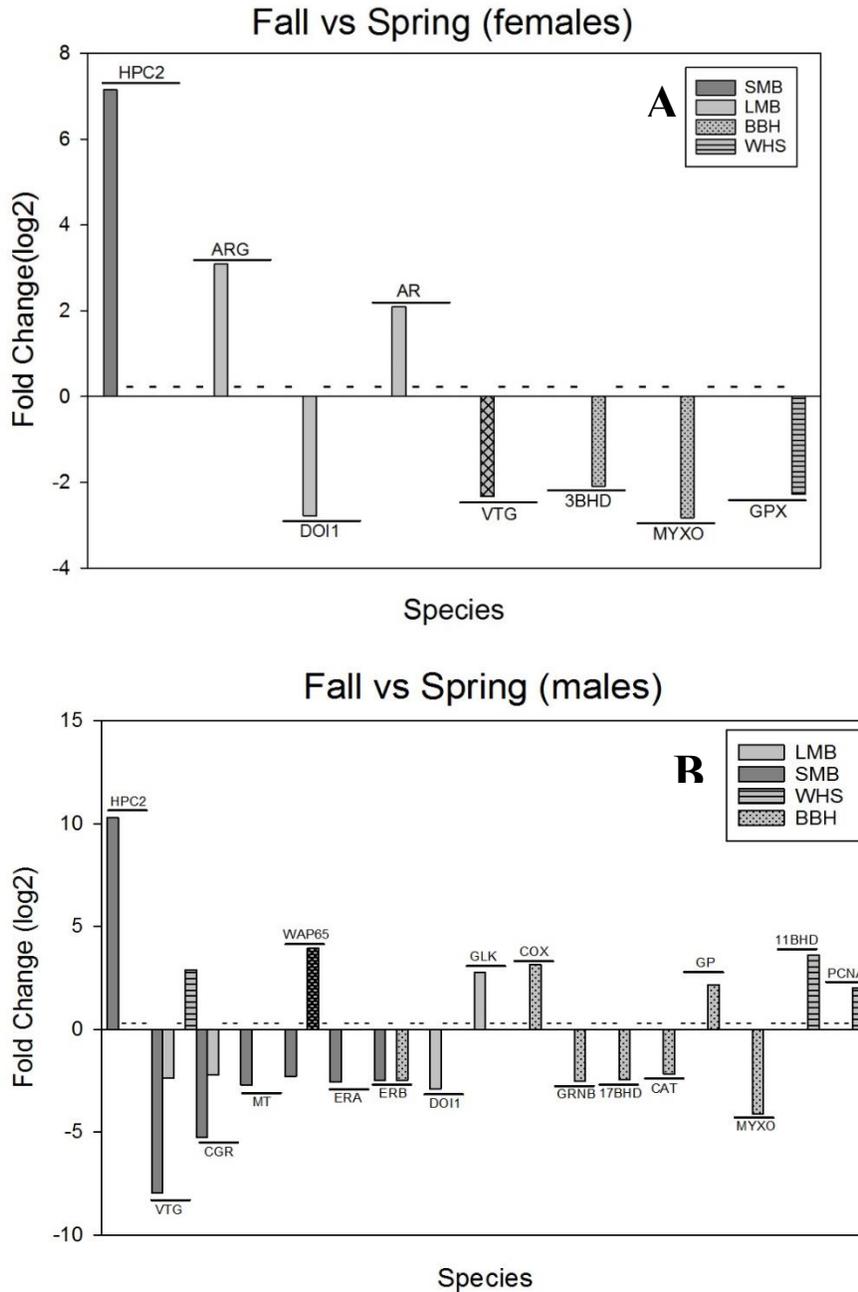


Figure 4. Genes reported with differential expression between each season in (A) females and (B) males.

Additional Products

Access to transcriptome data for non-model species has also allowed for the identification of unknown risks factors. Transcriptome sequencing for the white sucker was used to identify the full genome of a hepatitis B-like virus (Fig. 5). Prior to the transcriptome sequencing presented here, hepadnaviruses had only been identified in birds and mammals. Following the identification of this virus, prevalence was assessed using Nanostring, which found transcription in 9.5% (16 of 169) of white suckers evaluated (Fig. 6). Hepatitis B viruses have been associated with acute and chronic liver disease including fibrosis, cirrhosis, cholangiocarcinoma and hepatocellular carcinoma in mammals (Di Bisceglie, 2009; Li et al., 2012) and less commonly in birds (Funk et al., 2007). It is currently unknown whether this virus could be an additional risk factor for liver tumors of white sucker. Interestingly, it was not found in brown bullheads which display similar hepatic tumors. Identification of viruses through transcriptome sequencing adds additional pathways to the AOP framework as such viruses may represent initiation events which were previously unaccounted for.

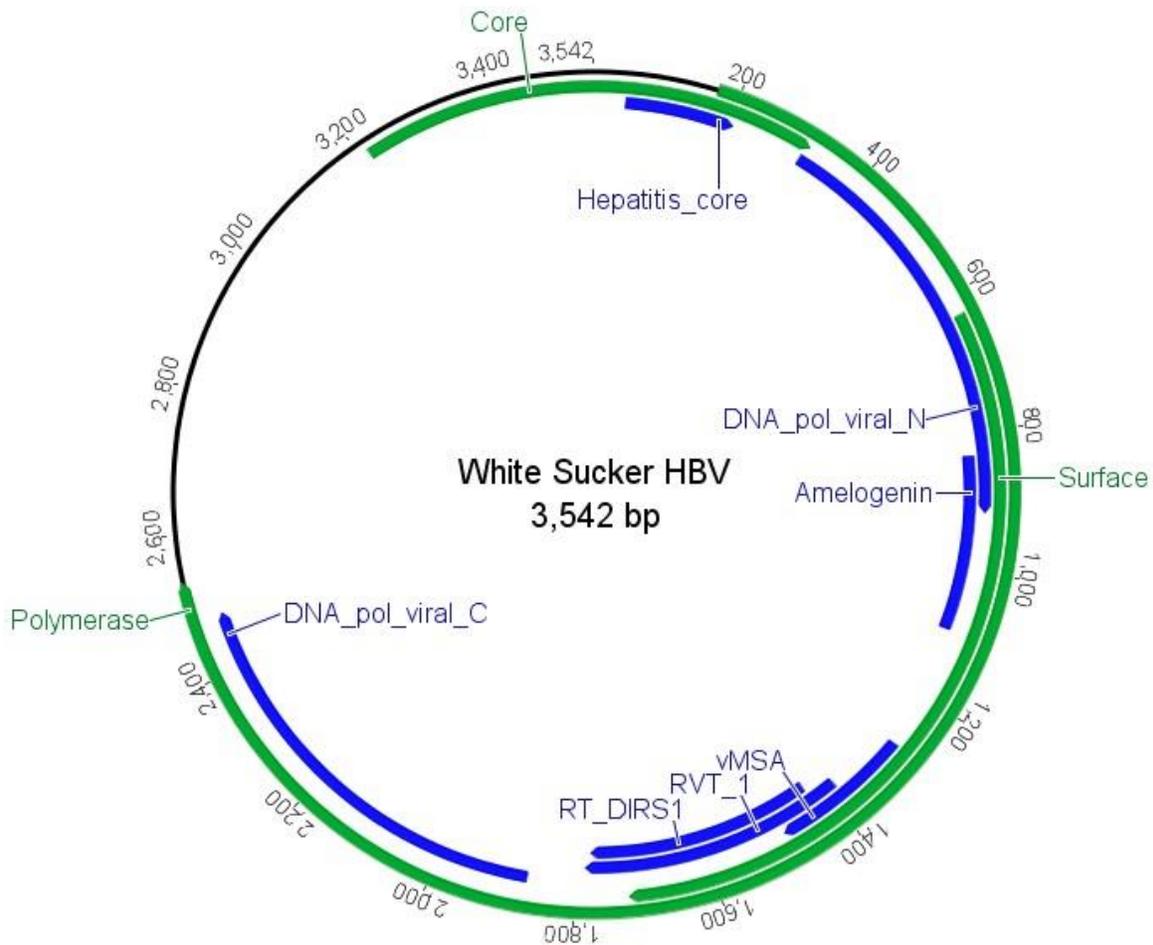


Figure 5. Genome organization of the white sucker hepatitis B virus. The complete genome consists of 3542 nucleotides of double-stranded DNA that encode three partially overlapping ORFs (RF +1, +2, +3).

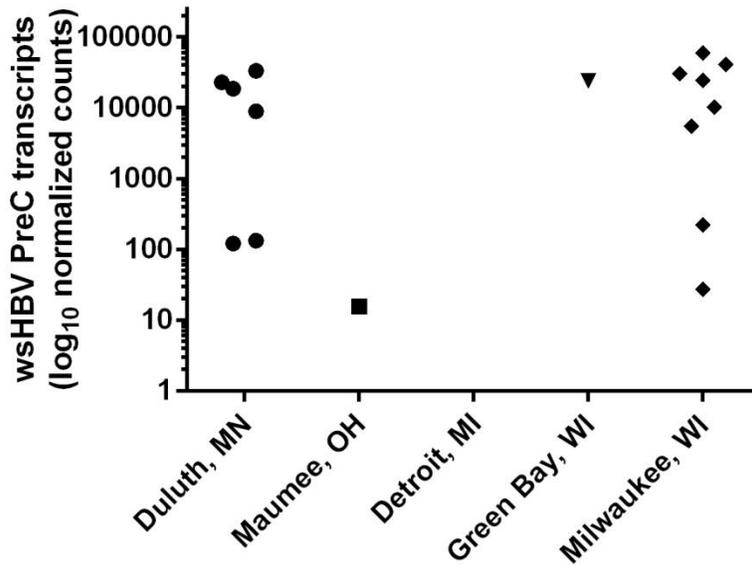
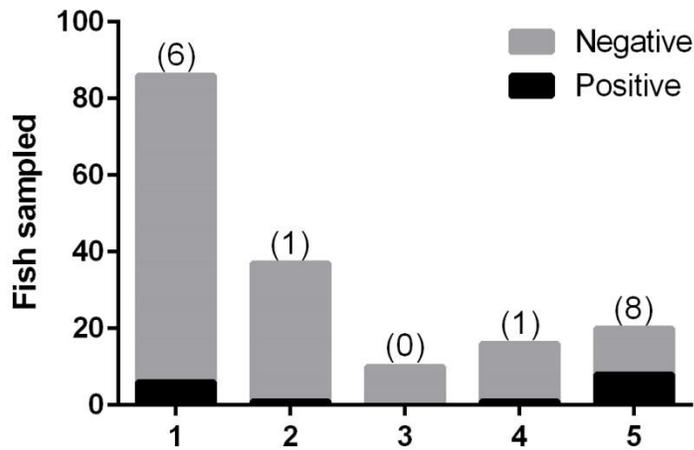


Figure 6. Prevalence of PreC mRNA transcription in liver tissue of white sucker collected in the Great Lakes (USA) region. (A) absolute number of fish with detectable transcripts (B) normalized number of transcripts detected.

Future Work

Future analysis will evaluate the link between gene expression methods presented here and concurrent collections of water and sediment contaminant concentrations and biological endpoints, including gonadosomatic and hepatosomatic indices, plasma hormones, vitellogenin and histopathology to provide insight on the adverse outcome pathways associated with biological effects observed in wild resident fish at Great Lakes AOCs and elsewhere.

**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 – 2015

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:

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 for 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 (YOY) smallmouth bass 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 of the histology samples, observations of areas of inflammation are only observed and ISH will be 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 a bacterial co-infection.

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 lingering question, the testis and ovary transcriptomes will be sequenced and biomarker genes will be used to determine which genes are present in intersex males that are absent in normal males. Subsequently, ISH will be used to visualize where in the fish these intersex genes are turned on, which will be very beneficial in young fish whose gonads are too small to be extracted for histology.

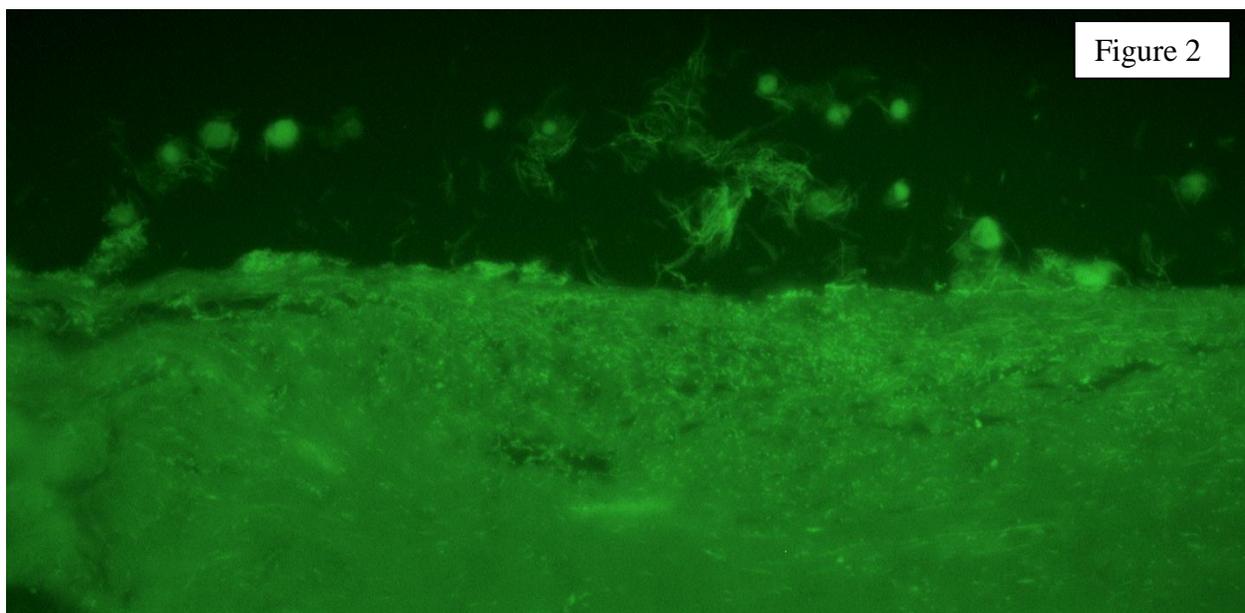
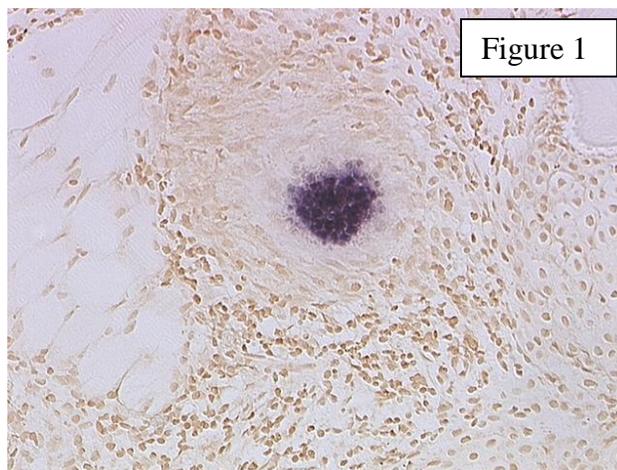
Finally, many smallmouth bass sampled from the Susquehanna River drainage have exhibited melanistic pigmentation in their skin in the form of small to large dark patches. It remains unclear as to what is causing this abnormality. So far, these patches have been ruled out as melanomas and seem to remain non-neoplastic. Skin samples have been taken this year in PAXgene and Z-fix in order to observe these patches histologically and with ISH. Prior to conducting ISH assays, the transcriptome will be analyzed and compared to skin from bass where melanistic spots have not been observed (Potomac River drainage). Biomarker genes can then be used to make probes for ISH to visualize when and where the genes are expressed.

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 decalcified 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 decalcified 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 (Figure 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 (Figure 2; note bright green bacteria in the dermis and rods above the epidermis).

Progress on the testis transcriptome so far includes RNA extractions and nanodrop nucleic acid quantification on testes sampled from adult smallmouth bass collected this spring (approximately 165 samples).



AQUATIC OTHER PROJECTS

CONSERVATION ASSESSMENT OF WEST VIRGINIA CRAYFISHES

Principle Investigators: Zac Loughman and Stuart Welsh

Years Ongoing: 2007-2015

Expected completion: 2016

Funding Source: WVDNR

Objectives:

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

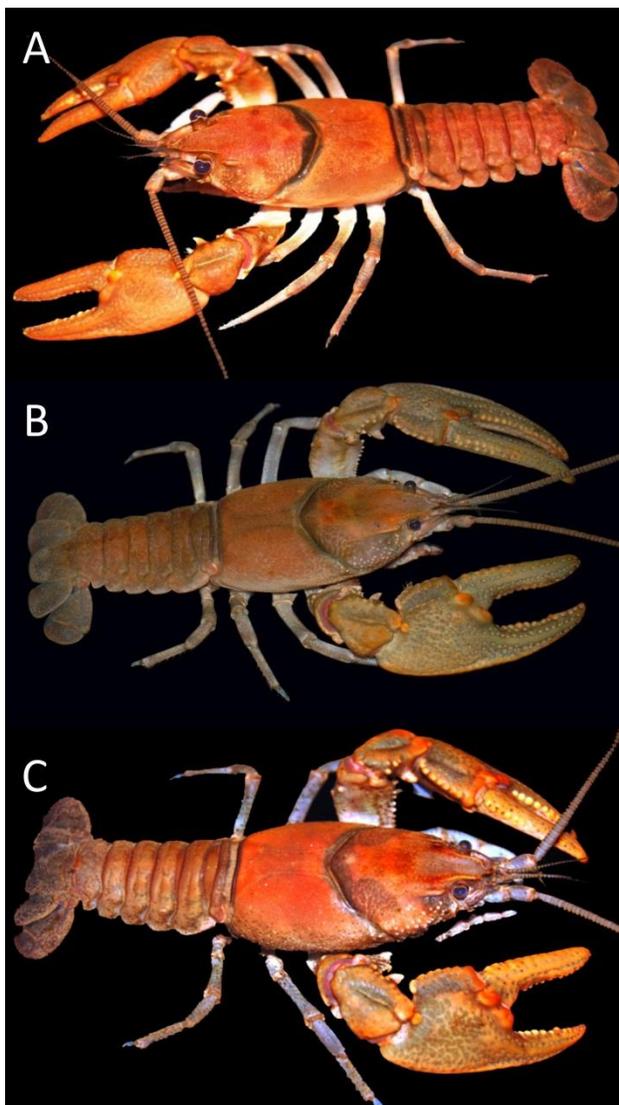
Efforts within the state to identify conservation threats, potential state records, and conservation concerns since the publication of Jezerinac et al. have been disjunct in nature. Key species (*Cambarus (H.) elkensis*, *Cambarus (P.) veteranus*), have received attention since Jezerinac’s effort, while 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: 2002-2015

Expected Completion: May 2016

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.

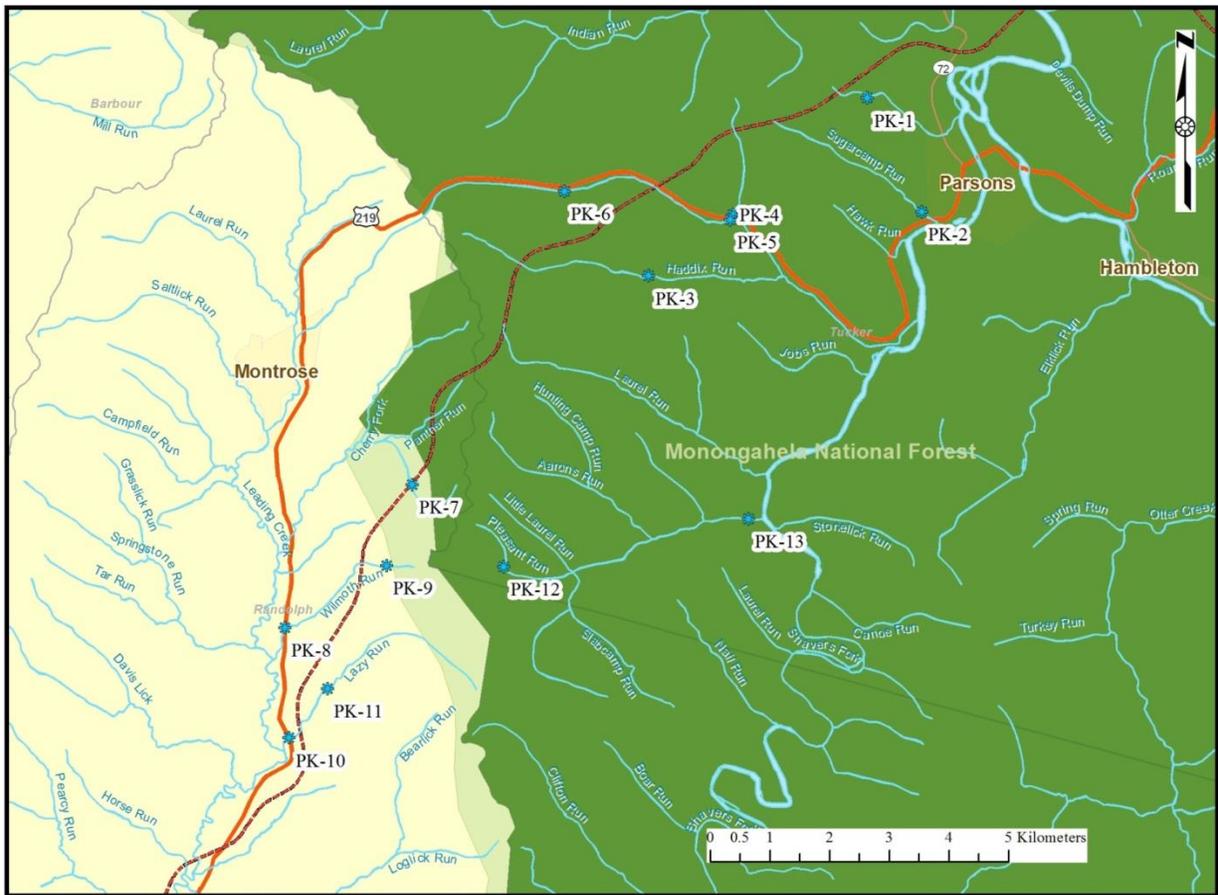


Figure 1. Location of sampling sites along the Parsons to Kerens, WV proposed Corridor H alignment.

In fall 2014 and spring 2015, 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 2012-2013 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 2015-2016 includes identification of benthic macroinvertebrate samples collected during 2013-2014. Benthic macroinvertebrate collections will be made in the fall of 2015 and the spring of 2016 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, Rob Tallman

Years Ongoing: 2008-Present

Degree Program: PhD

Expected Completion: May 2016

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:

The study is examining

1. response of Golden-winged Warblers to habitat management.
2. site-fidelity, survival, and movements of banded males, females, and nestlings.
3. correlates of Golden-winged Warbler and other bird species abundance at multiple spatial scales.

Progress:

Golden-winged Warbler (*Vermivora chrysoptera*, GWWA) populations declined by 8.8% year⁻¹ in West Virginia during 1966-2013 based on Breeding Bird Survey data. Declines throughout their range prompted the U.S. Fish and Wildlife Service to review their status for Endangered Species Act listing. Main threats to GWWA include breeding and wintering habitat availability, Brown-headed Cowbird (*Molothrus ater*) brood parasitism, climate change, and hybridization with the Blue-winged Warbler (*V. cyanoptera*, BWWA). Our research has focused on potential breeding habitat throughout the West Virginia highlands and is part of two larger range-wide investigations of GWWA breeding ecology. GWWA breeding territories typically contain a mix of herbaceous vegetation, blackberry (*Rubus* spp.), shrubs, saplings, and a few scattered trees embedded within a forested landscape.

We discovered 196 *Vermivora* spp. nests (Fig 1; 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. Daily survival rate (DSR) of nests did not differ by species (Fig. 2) or year, but decreased sharply about 30 days into the nesting season (Fig. 3), which started 12 May (± 1.4 days [SE]) each year. Complete clutch and fledged brood size for *Vermivora* spp. nests were 4.6 ± 0.1 eggs and 3.9 ± 0.2 fledglings, respectively. Only three nests were parasitized by Brown-headed Cowbirds.



Figure 1. Golden-winged Warbler nest with nestlings (photo by Kyle Aldinger).

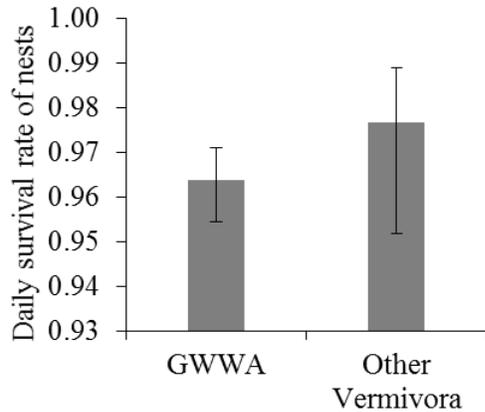


Figure 2 (left). Daily survival rate (\pm 95% CI) of *Vermivora* spp. nests.

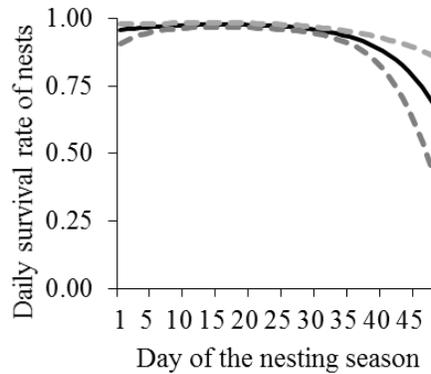


Figure 3 (right). Daily survival rate (\pm 95% CI) of *Vermivora* spp. nests over time.

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. 4-5).

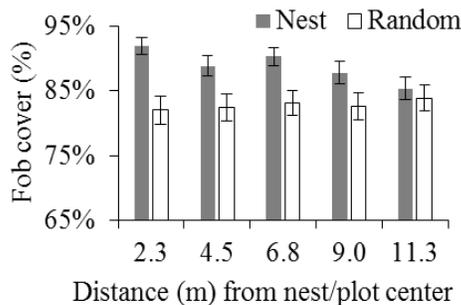


Figure 4. Forb cover (\pm SE) as a function of distance from GWWA nest or random plot center.

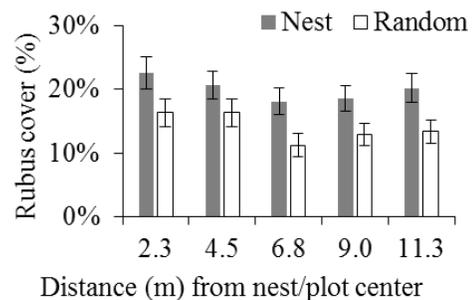


Figure 5. *Rubus* cover (\pm SE) as a function of distance from GWWA nest or random plot center.

We mapped 268 male *Vermivora* spp. territories (88% GWWA, 8% BWWA, and 4% hybrids) during 2008-2014. Randolph County GWWA sites ($n = 4$) experienced declines and an increase in BWWA, while Pocahontas County GWWA sites ($n = 4$) were relatively stable during our study (Fig. 6, left panel). Based on point counts ($n = 1096$ point counts at 273 point count locations on 122 sites) during May-June 2008-2015, GWWA territory density was associated with 5-km and 1.5-km radii elevation, 100-m radius shrubland cover, and 11.3-m radius shrub height and cover (Figure 6, right panel).

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 in 2015, we fitted 21 GWWA, 1 BWWA, and 1 hybrid with light-level geolocators to track their year-round movements.

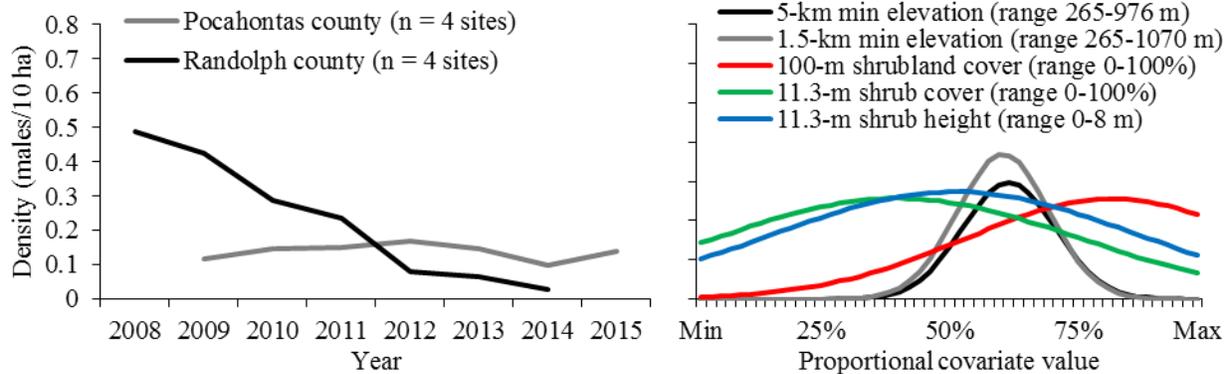


Figure 6. Annual GWWA breeding territory density based on spot-mapping of intensive study sites (left) and variables associated with density based on point counts on a larger set of 122 sites (right panel).

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 in 2015, we fitted 21 GWWA, 1 BWVA, and 1 hybrid with light-level geolocators to track their year-round movements.

WILDLIFE RESPONSE TO CREATION OF EARLY SUCCESSIONAL HABITAT

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

Objective:
 Evaluate the response of various wildlife species to creation of early successional habitat on wildlife management areas throughout West Virginia.

Progress:
 West Virginia is 80% forested and that forest is predominantly in the saw-timber (15" d.b.h.) age class. On state owned wildlife management areas (WMA), this forested percentage and median age is even greater. Aging forest condition has had a negative impact on over 70 species of wildlife in the northeast region of the U.S. that require early successional habitat. Many of these species reside on state-owned WMA's. Routinely recommended habitat management techniques call for a creation of forest ecotone, thus providing additional habitat to these early successional specialists. Creating and developing early successional habitat in a more structured manner, over a spectrum of areas, and with an experimental research approach will allow for a better understanding of wildlife species response and will allow refinement of management recommendations. Thus, we are implementing a new project focused on early successional habitat and wildlife.

Forest edge is a strong concern among wildlife managers because habitat edges provide beneficial habitat for wildlife. However, hard edges between two disparate habitat types are of less value to wildlife. Land

managers began to develop ways to transition between two habitat core areas by “softening” or “feathering” of these distinct forest edges.

We are interested in these border or edge areas and how we can increase their usefulness to various wildlife species. Through timber harvest at varied border widths and varied basal area retention along field openings, oil pipeline openings, and right-of-way openings, we will determine the best management strategy to provide beneficial habitat for bird communities (upland game birds and songbirds) and other fauna.

This project is currently in the planning stages. Field work will begin in the summer of 2016, with pre-treatment data being collected. In subsequent years, we will return to the same areas to quantify any changes in the bird community through fixed radius point counts (songbirds) and call back surveys (upland game birds). Game bird surveys will be completed during April, while songbird surveys will be done during May-June of each year. This project will span four field seasons (2016-2019), with one year of pre-treatment data and three years of post-treatment data. We will sample a set of control sites annually as well. This before-after-control-impact study design allows for rigorous statistical analyses. This study will take place throughout the state of West Virginia on Wildlife Management Areas. Through this research, we attempt to aid in the management of lands to create suitable habitat for wildlife and provide a guide for future studies looking to evaluate the optimum size and characteristics of feathered edges on wildlife communities.

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

Student Investigator: Gretchen E. Nareff

Principal Investigator: Petra Wood

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

Years Ongoing: 2013-Present

Expected Completion: May 2017

Funding Sources: U.S. Geological Survey (WV RWO 62), WV Division of Natural Resources, Pennsylvania Game Commission

Objectives:

1. Quantify and compare broad-scale influence of timber harvests on relative avian abundance and community composition pre- and post-harvest across four cooperating states and territory density at two sites in West Virginia, pre- and post-harvest.
2. Examine how silvicultural practices impact habitat use of an interior-forest passerine, the Cerulean Warbler, in West Virginia, pre- and post-harvest.
3. Examine the response of focal indicator species (Eastern towhee, hooded warbler, indigo bunting, worm-eating warbler, and wood thrush) 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) is a declining, late successional songbird species whose core breeding range is in the hardwood forests of the Appalachian Mountains. It is considered a focal species of management concern by the U.S. Fish and Wildlife Service (USFWS), based on a range-wide population decline of ~3% per year on the breeding grounds. This is one of the steepest declines in population size for any warbler species in North America. Understanding habitat selection on

multiple scales and managing for cerulean warbler preferences is critical in reversing this trend and conserving the species. Because the cerulean uses heavily forested landscapes with heterogeneous vegetation structure, timber harvesting can be used to enhance its habitat.

Our study will expand on the recently published *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. This study is a region-wide, cooperative project with study sites in Kentucky, Pennsylvania, Virginia, and West Virginia. Fieldwork will be conducted within the host states by local teams and data management and analysis will be a cooperative effort. The West Virginia team will complete global analyses. Ideally, we will recommend harvest regimes that benefit the cerulean and associated Species of Greatest Conservation Need (SGCN) that can be integrated into existing harvest regimes. We will quantify cerulean warbler and SGCN selection of various vegetative characteristics (e.g. residual basal area, tree species composition) and landscape characteristics (e.g. slope position, aspect, landform) at the point count and territory levels. We hope to explain how ceruleans select territories on a landscape-scale within an implemented harvest mosaic that offers structural diversity to the birds and how other songbird species may be managed under the umbrella of cerulean breeding habitat management. Our results will be used to refine the existing habitat management guidelines.

Each state implemented timber harvests incorporating the recommendations of the Guidelines. The five West Virginia study sites are at Coopers Rock State Forest (Scott's Run, 77.2 ha, and Pisgah, 151.8 ha) in Monongalia and Preston counties, Stonewall Jackson Wildlife Management Area (WMA; 32.0 ha) in Lewis County, and two sites (Dynamite, 39.8 ha and Wolf Creek, 26.6 ha) at the Elk River WMA in Braxton County. Harvests occur as a mosaic at each West Virginia site (Fig. 1), and may include shelterwood harvests, clearcuts, singletree selection, or group selection, with unharvested areas throughout.

We quantified songbird response to a range of forest management treatments in the four states during the 2013-2015 breeding seasons. We are using point counts to evaluate changes in songbird abundance pre- and post-harvest with 10-minute counts conducted three times at each point. In West Virginia, we used spot mapping techniques 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

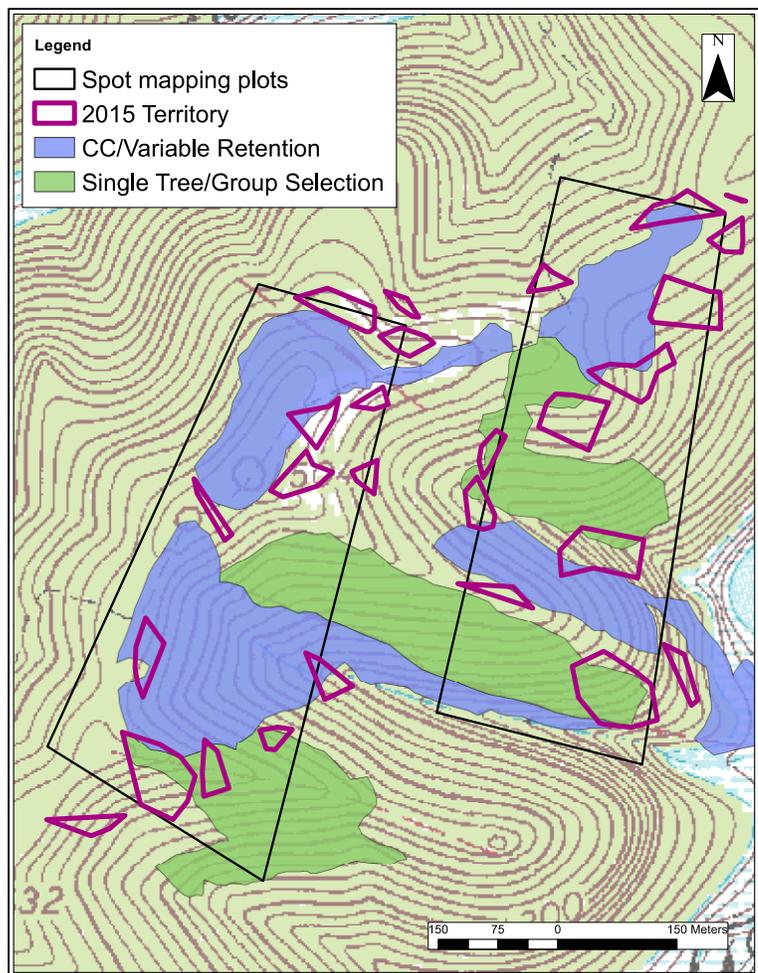


Figure 1. Cerulean Warbler territories from 2015 at the Wolf Creek harvest mosaic.

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 and visited 6-8 times per season to determine territory locations of the six focal species.

Spot-mapping in West Virginia revealed greater cerulean territory density at post-harvest than pre-harvest sites (Fig. 2 and 3). Territories often incorporate or lie near harvest borders (Fig. 1).

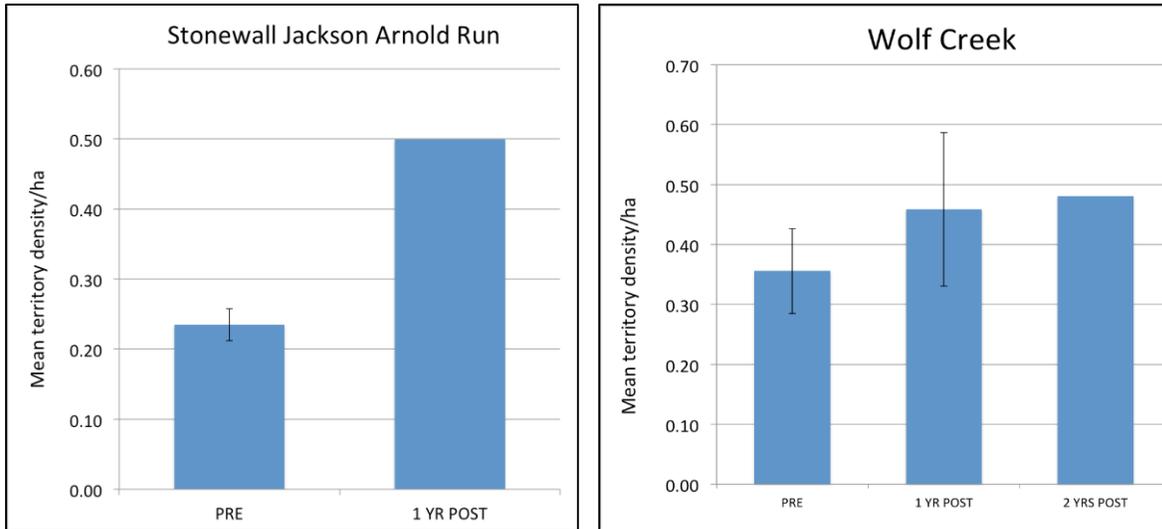


Figure 2 (left). Cerulean Warbler territory density pre- and post-harvest at the Stonewall Jackson Lake Wildlife Management Area Arnold Run Harvest Mosaic in Lewis County, WV. Territories were mapped for 2 seasons pre-harvest and 1 season post-harvest.

Figure 3 (right). Cerulean Warbler territory density pre-harvest, 1 year post-harvest, and 2 years post-harvest at the Wolf Creek Harvest Mosaic within the Elk River Wildlife Management Area in Braxton County, WV.

Results presented here summarize data from Kentucky and West Virginia where we have pre- and post-harvest data for comparison. Across the two states, ceruleans were detected at 76.5% of point count stations at harvest interior points, 64.7% of point count stations at harvest edge points, and 60.0% of point count stations in unharvested reference stands. Cerulean abundance generally increased after harvesting (Table 1). Species richness increased at all sites post-harvest. Detailed analyses of the regional data will be completed after the 2016 field season and will account for detection probability and time removal methods.

Table 1. Cerulean Warbler detections (mean ± SE) within 75m of point count stations pre- and post-harvest in KY and at two sites in WV.

	Pre-harvest		
	Harvest	Edge	Reference
Kentucky	0.86±0.34	0.86±0.46	0
West Virginia	0.94±0.26	0.95±0.22	0.93±0.11
	Post-harvest		
Kentucky	0.79±0.19	0.57±0.23	0.29±0.13
West Virginia	1.55±0.34	1.29±0.27	1.33±0.23

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

Student Investigator: Brian Rolek

Principal Investigators: Petra Wood, Daniel Harrison, Cynthia Loftin

Years Ongoing: 2012-2015

Degree Program: PhD

Expected Completion: Dec 2016

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 changes in mature forest structure?
- 2) How does forest management influence mature forest structure?

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 on focal bird species that use spruce-fir habitat and related mature forest structure to forest management treatments. Focal bird species included Bay-breasted Warbler (*Setophaga castanea*), Cape May Warbler (*Setophaga tigrina*), Blackburnian Warbler (*Setophaga fusca*), Canada Warbler (*Cardellina canadensis*), Yellow-bellied Flycatcher (*Empidonax flaviventris*), and Boreal Chickadee (*Poecile hudsonicus*).

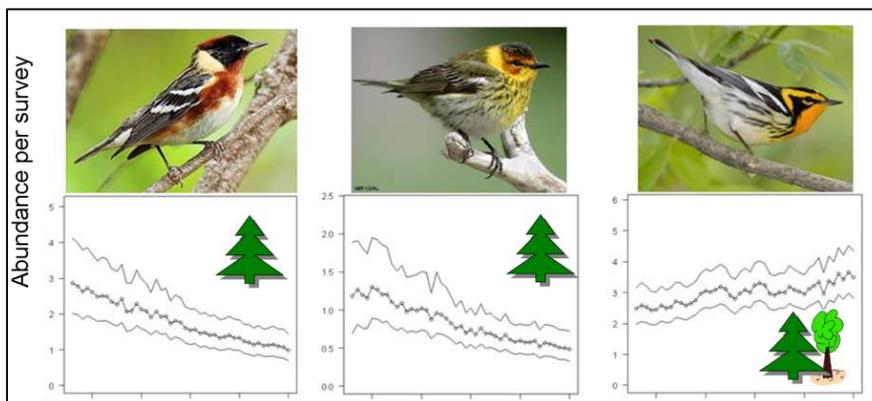


Figure 1. Softwood bird trends, 1970-2010, in Bird Conservation Region 14 from Breeding Bird Survey data. Left to right are Bay-breasted, Cape May, and Blackburnian warblers.

We sampled five forest treatments including clearcut, pre-commercially thinned, shelterwood, overstory removal, and selection harvests and 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, 2014, and 2015 at 657 point count locations 3 times per year. Physiognomic and compositional vegetation characteristics were measured at each point count location in 2014.

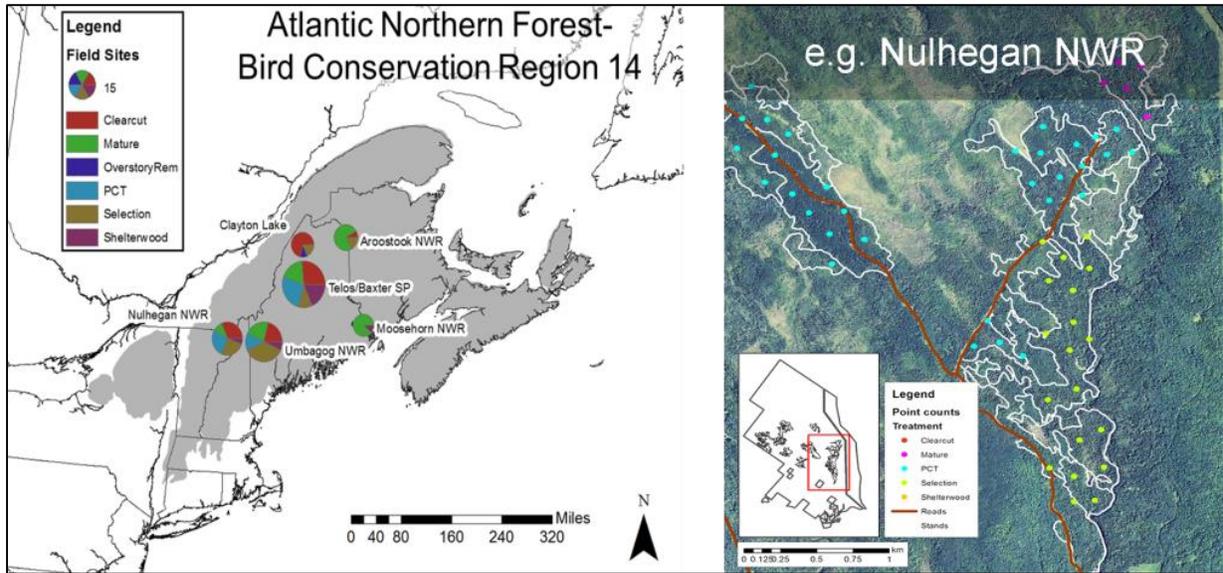


Figure 2. Study stands were located throughout northern New England that were ≥ 30 acres and contained primarily softwood trees. Size of pie charts is proportional to the number of stands at each site.

To model the effects of forest structure on abundance of focal species, we used a Bayesian regression with a Poisson log-normal distribution. Our response variable was the maximum abundance of birds detected during any survey across all years and quadratic mean diameter was used as an explanatory variable. Quadratic mean diameter is similar to basal area but this metric emphasizes mature trees by attributing larger values to trees with large diameters.

Forest management treatments reflect a range of vegetative structural conditions from mature reference stands with large diameter trees and relatively low shrub cover to clearcut stands with few large trees and high shrub cover (Fig. 3). Mature forest structure measured as quadratic mean diameter showed a gradient among forest treatment types from least to most mature forest structure: clearcut, pre-commercially thinned, selection, shelterwood, mature.

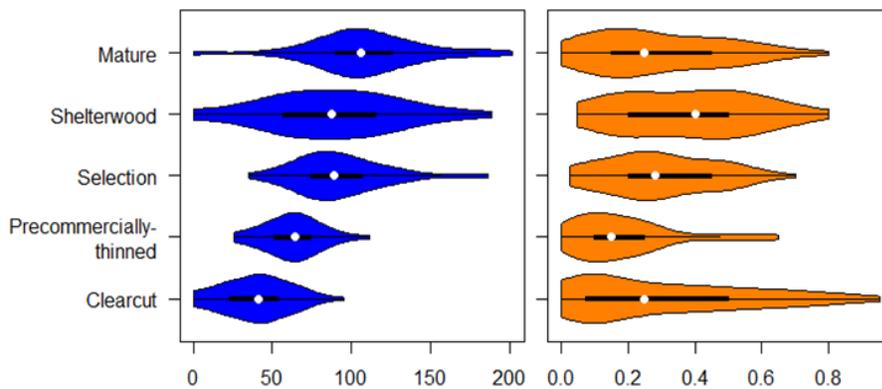


Figure 3. Vegetation characteristics summarized by silvicultural treatment. Vertical width of plots are proportional to kernel density estimates.

We found quadratic relationships between abundance and quadratic mean diameter for Bay-breasted Warbler, Cape May Warbler, and Boreal Chickadee and these species show selection for specific mature forest structure. Blackburnian Warbler had a positive association with quadratic mean diameter, while

Canada Warbler and Yellow-bellied Flycatcher had negative associations (Fig. 4).

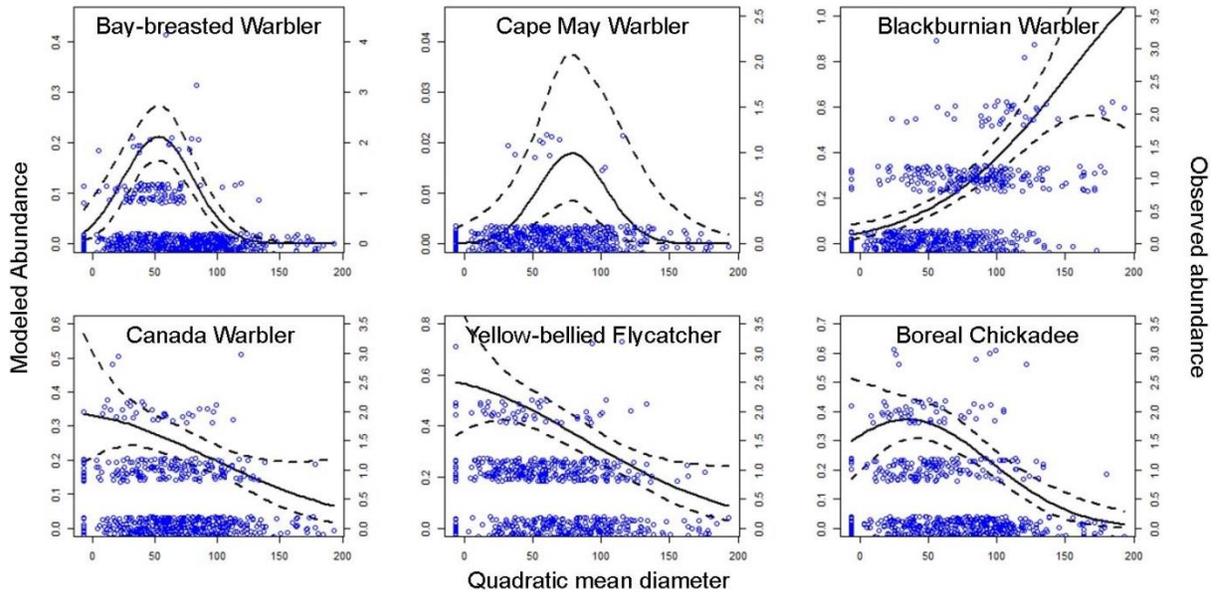


Figure 4. Focal species relationship with quadratic mean diameter. Model estimates (solid lines), 95% prediction intervals (dashed lines), and data (blue points).

Future work will incorporate detection probability into estimated abundance, use spatially-explicit models including land cover, and incorporate vegetation composition.

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: Ph.D.
Expected Completion: May 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, Ovenbirds, and Hooded Warblers.
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) were found to predict 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 also found to be 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 habitat for a number of bird species dependent on structurally heterogeneous forests.

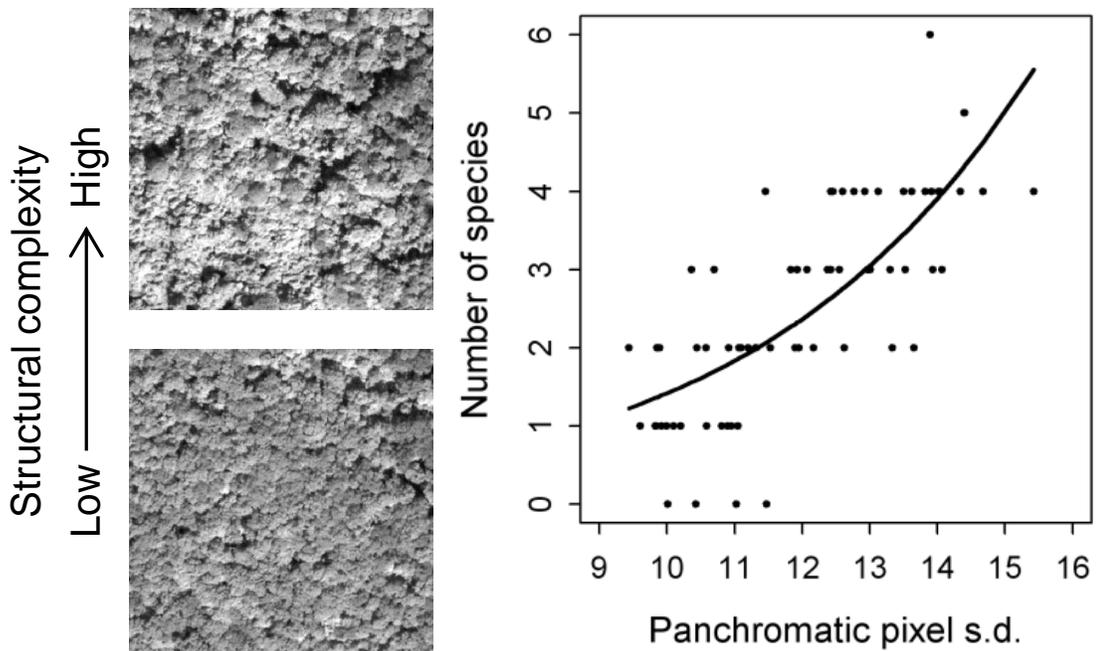


Figure 1. A gradient in forest structural complexity and the richness of six forest gap understory-associated bird species in relation to a Quickbird satellite image texture measure of this gradient.

For the second objective, the remote sensing analysis was refined to focus on the distribution patterns of three territorial songbirds obtained from 2010-11 focal ridgetop transect surveys. Ovenbirds, Hooded Warblers, and Cerulean Warblers (Fig. 2) are widespread and abundant at the site, and differ in the forest habitat components they require for breeding.



Figure 2. Three focal ridgetop songbirds: the ground nesting Ovenbird (left), shrub nesting Hooded Warbler (center), and upper canopy nesting Cerulean Warbler (right).

A multivariate analysis technique, redundancy analysis (RDA), was used to relate field-collected habitat data to satellite data and DEM-derived topographic information, and 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 also 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 (Fig. 3; right). Predictions based on these results will be tested using data that was collected on additional focal ridgetops in 2011.

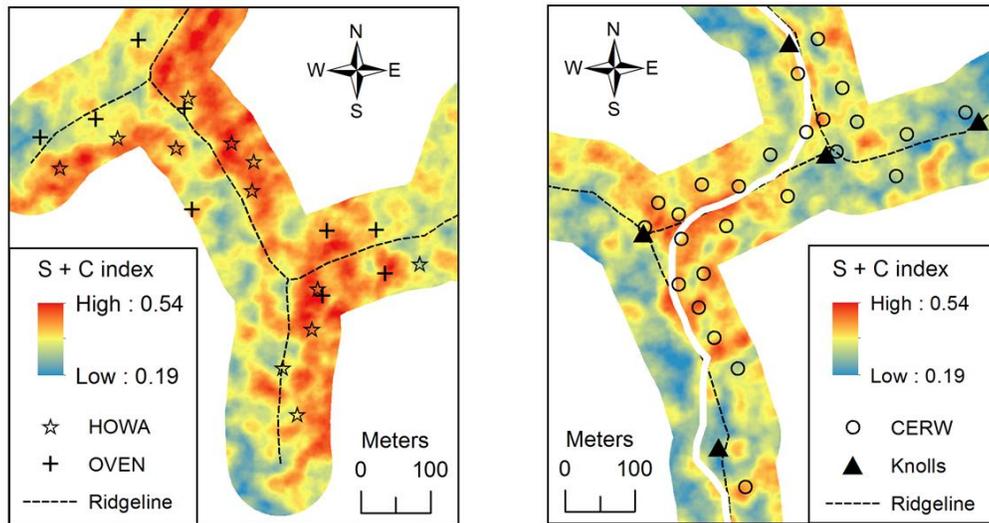


Figure 3. Examples of the ridgetop RDA index of forest structure and composition ($S + C$ index) and the species’ territory distributions. Left: Hooded Warbler (HOWA) and Ovenbird (OVEN) territories. Right: Cerulean Warbler (CERW) territories, topographic knolls, and a narrow ridgetop pipeline (white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

To achieve the third objective, spatial point pattern analysis is being used to quantify the distribution of Cerulean Warbler territories in relation to each other, and the remote sensing data. Understanding how habitat, topography, and potentially sociality are responsible for their observed distribution patterns may be of particular value for the management of this high conservation priority species. Finally, results from objectives two and three will be used to suggest potential remote sensing-based approaches for assessing how populations of these species may intersect with energy industry activities throughout the region.

**WILDLIFE
MARCELLUS SHALE PROJECTS**

**SONGBIRD RESPONSE TO GAS WELL AND INFRASTRUCTURE DEVELOPMENT IN THE
MARCELLUS SHALE 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: Dec 2016
Funding Source: US Fish and Wildlife Service

Objectives:

1. Quantify how the size, shape, age and placement of gas well pads and pipelines in the landscape impact abundance and diversity of forest songbirds through the following analyses:
 - a. Compare relative abundance and diversity of breeding bird populations at Marcellus well pad sites and infrastructure with that of comparable reference sites not impacted by Marcellus development.
 - b. Quantify effects of well pad site size, age and density on abundance and diversity of breeding bird population and identify thresholds for percent of landscape impacted by well pads and infrastructure beyond which birds are negatively impacted.
 - c. Determine distance that edge effects on avian populations extend into adjacent forest from well pad sites.
2. Compare the results of this project with recent Pennsylvania land cover change analyses (USGS) and model projections of hot spots for shale gas development (TNC), to potentially identify forest areas of high conservation concern that stand to be heavily impacted, and which should be excluded from further shale gas development.
3. Describe management recommendations regarding pad shape, size, age and placement that minimize impacts to forest interior birds and restoration of pipelines to maximize habitat improvement for early successional species.

Progress:

Extraction of natural gas from Marcellus shale has increased exponentially in the central Appalachians. Our goal is to quantify how the size, shape, age and placement of gas well pads and pipelines in the landscape impact abundance and diversity of forest songbirds across the Marcellus shale region. We seek to identify thresholds of habitat and landscape metrics beyond which birds are negatively impacted and quantify how far edge effects from well pads extend into the surrounding forest.

We used stratified sampling to select well sites from landscapes across a range of forest cover and with high and low density shale gas well development. We selected reference sites using a similar stratification of forest cover and high and low density development from sources other than shale gas. We used standard protocols to quantify avian, vegetation, and landscape metrics and are currently developing statistical models to relate bird abundance and diversity to habitat and landscape metrics. We hope these results will inform conservation professionals as well as industry regarding effects of Marcellus development on forest birds and will provide baseline data that can be used to monitor bird populations and assess effects over a longer period of time. The results will also inform management recommendations regarding pad shape, size, and placement that minimize impacts to forest interior birds

and will inform restoration of pipelines to maximize habitat improvement for the early successional suite of species.

In 2015, we surveyed 88 field sites (1 km radius circular plots) in five states: WV, OH, PA, MD and NY. In 2014 we surveyed 103 field sites in WV, PA, and OH. Of these 191 sites surveyed across two field seasons, 120 contained Marcellus gas development and 71 did not (37% reference sites). Of the same 191 sites, 154 were located on public lands and 37 were on private lands (20% privately owned). We conducted a total of 2664 point count and vegetation surveys, averaging 14 survey points per field site.

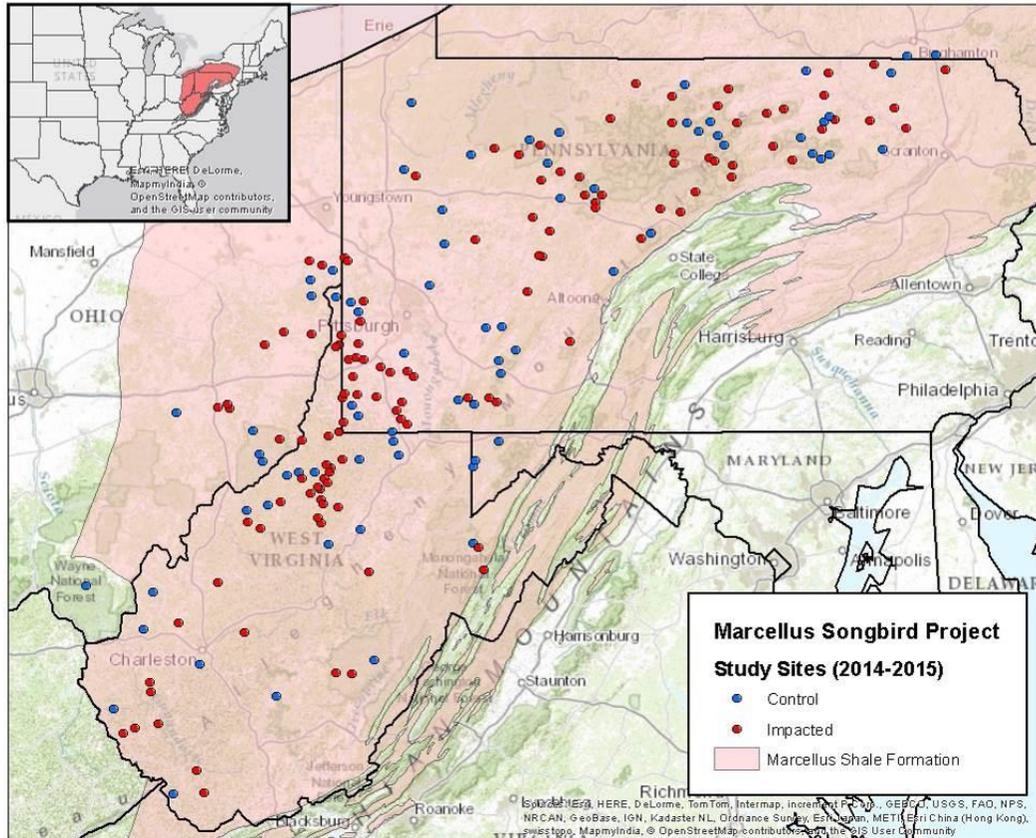


Figure 1. Field site locations from 2014-2015, in WV, OH, PA, MD, NY: red dots represent shale gas impacted sites ($n = 120$), blue dots are reference sites ($n = 71$).

We are currently focusing on digitization of shale gas impacts and land cover classes within a 2 km buffer around each of our field sites, using Google Earth and ArcGIS, based on the most recent aerial and satellite imagery available as well as personal knowledge of the sites (Fig. 2). We are categorizing different classes of shale gas development (e.g., well pads, ponds, roads, pipelines), in addition to other types of non-gas development and land cover classes of interest (e.g., forests and farmlands). The goal is to complete digitization of all 191 sites by the end of 2015. Landcover metrics and field sampled metrics (Fig. 3) will then be related to avian metrics.

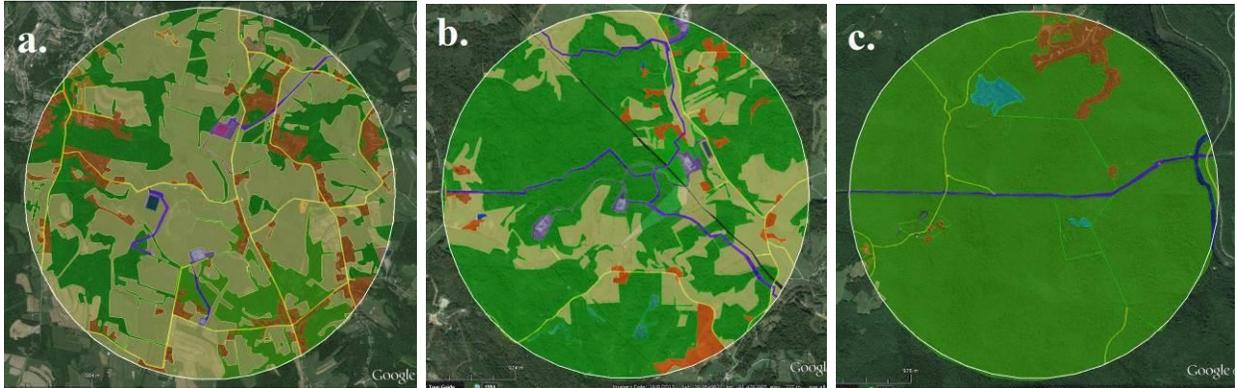


Figure 2. Examples of digitization of land cover types within a 2 km radius, at three field sites representing a gradient of forest cover: (a) a site with low percent-forest cover and complex land-use mosaic (Washington County, PA); (b) a site with moderate levels of forest cover (Noble County, OH); and (c) a site with high percent-forest cover, bisected by a shale gas pipeline (Fayette County, PA). Forests are represented in green, shale gas disturbance in purple.



Figure 3. Top: Taking digital sound level meter readings prior to an avian point count survey (Lycoming County, PA). Bottom: Using prism sampling and Biltmore sticks to estimate basal area (Wetzel County, WV). Photos by Laura Farwell.

**SPATIAL ASSESSMENT AND EPIGENETIC VARIATION IN THE LOUISIANA WATERTHRUSH
MARCELLUS SHALE GAS DEVELOPMENT IN WEST VIRGINIA**

Student Investigator: Mack Frantz

Principal Investigator: Petra B. Wood

Cooperators: Amy Welsh, Steve Latta

Years Ongoing: 2013–2015

Degree Program: PhD

Expected Completion: August 2016

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 Provost Fellowship

Objectives:

1. Determine how gas well development activities influence Louisiana Waterthrush territory density, benthic macroinvertebrate community structure at foraging & nest locations, nest survival, and site fidelity.
2. Determine how gas well and infrastructure development influence Louisiana Waterthrush epigenetic (DNA methylation) variation between areas of impacted and unimpacted 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 and terrestrial systems by potentially exposing them to increased sedimentation run-off, streamflow alterations from water withdraw, and surface water contamination from gas well wastewater. This research is examining how gas development activity and possible environmental stressors influence Louisiana Waterthrush (*Parkesia motacilla*) DNA methylation between individuals nesting adjacent to impacted and unimpacted streams on the Lewis Wetzel Wildlife Management Area (LWWMA). In addition, stream and nesting features were assessed to determine if gas development activity influences waterthrush demography. In this long-term study, a variety of data were collected during 2009-2015.

Nest monitoring took place during March 28 – 31 July 2009–2015 by searching 50 km of headwater streams on LWWMA (n=12). Of the 280 nests with known fates, 120 were in territories impacted by gas activity or infrastructure. Nest survival calculated with Program MARK varied annually and in four of the six years was highest in unimpacted territories (Fig. 1). In general, nest survival was 6% higher in unimpacted territories ($37.3\% \pm 4.3$) than in impacted territories ($30.7\% \pm 4.5$). Preliminary AIC modeling suggests that increasing impacts from shale gas development lower daily survival rate of nests (Fig. 2). Additional AIC modeling is planned to evaluate a variety of covariates that might affect waterthrush nest survival at multiple spatial scales (*i.e.* nest, territory, watershed).

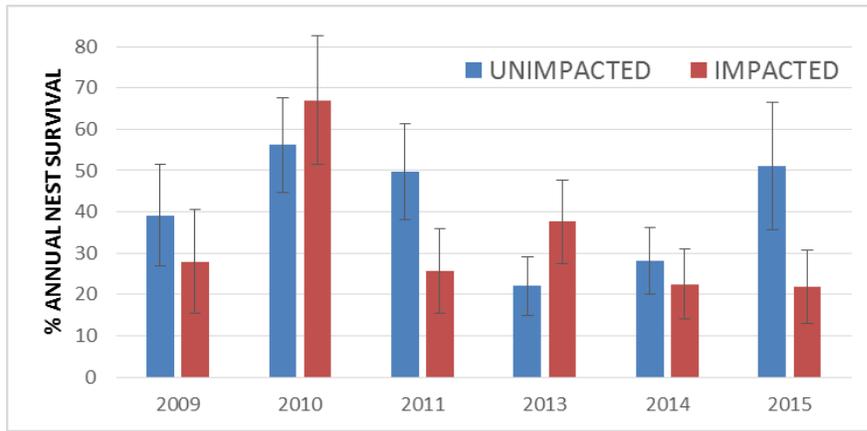


Figure 1. Annual survival of nests in impacted and unimpacted territories.

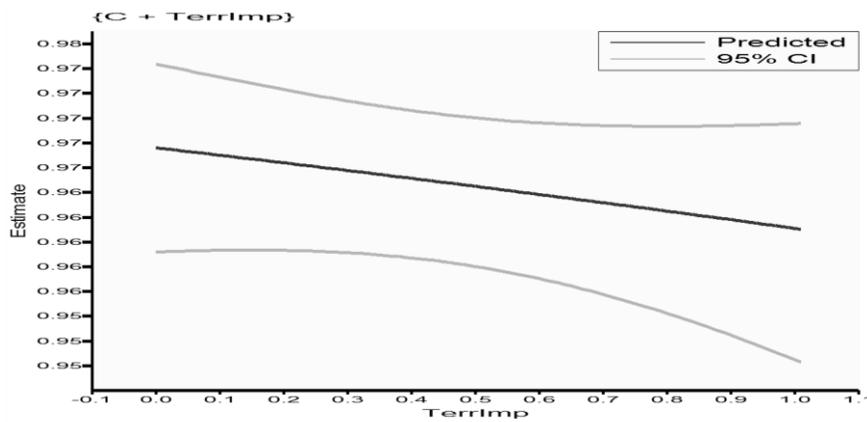


Figure 2. Preliminary analyses suggest that daily survival rate (y-axis) decreased as % of territory impacted by shale gas development increased (x-axis).

We banded 435 individual waterthrush and mapped 400 territories during 2009-2015. Annual return rates based on resightings of banded adults have declined during the study (Fig. 3 left). Territory density (#terr/km) per stream also has declined annually (Fig. 3 right). Analyses of these data will be completed this fall and winter.

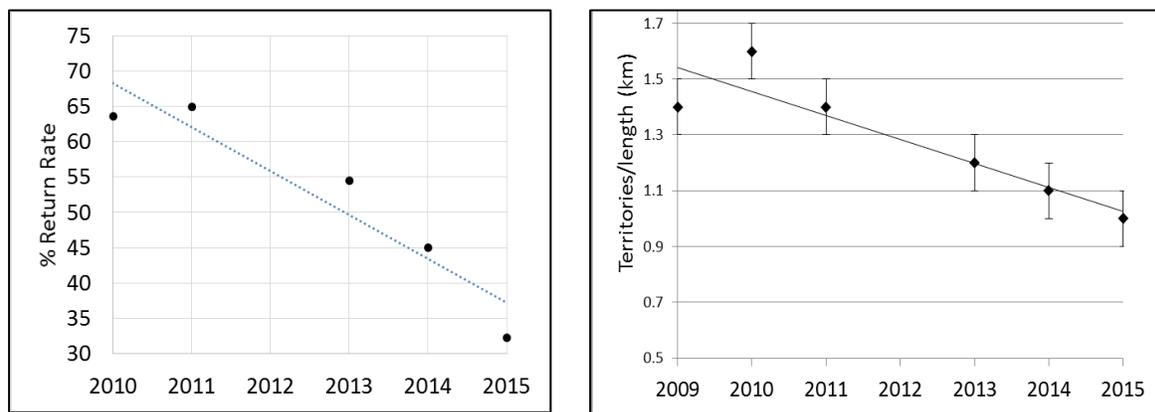


Figure 3. Louisiana Waterthrush return rates (left) and territory density (#terr/km, right).

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 impacted and unimpacted areas. Individuals with high Barium and Strontium contaminant loads had a lower number of methylated DNA sites (n=47, Fig.

4). 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.

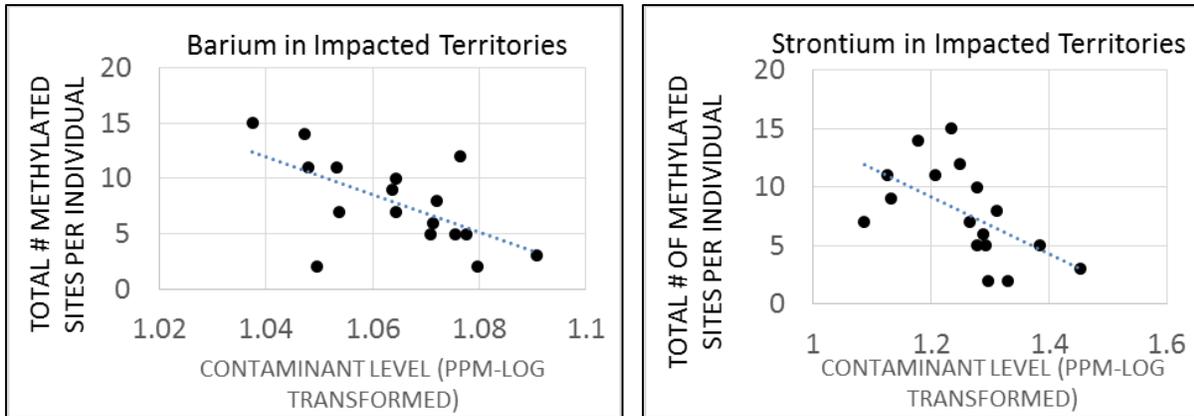


Figure 4. High levels of Barium and Strontium were correlated with a lower number of methylated DNA sites from individuals in impacted areas ($n=47$).

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. In general, preliminary analyses suggest lower stream and habitat quality scores at impacted nest sites. We also collected 168 and 146 macroinvertebrate samples (314 total) in 2013 and 2014 at foraging and nesting locations that will be compared between impacted and unimpacted areas. 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 GAS WELL DEVELOPMENT

Student Investigators: Laura Farwell, Jim Sheehan, Greg George

Principal Investigator: Petra B. Wood

Years Ongoing: 2008-2015

Expected Completion: May 2016

Funding Source: Department of Energy, US Fish and Wildlife Service

Objectives:

1. Determine how oil and gas activities influence long-term distribution and relative abundance of songbirds.
2. Determine how oil and gas activities influence habitat and landcover metrics.

Progress: The central Appalachian region is heavily forested and of high importance to forest songbirds but is also experiencing rapid growth in Marcellus shale gas development (Fig. 1). The Lewis Wetzel Wildlife Management Area in north-central WV had shale gas development beginning in 2008. This project is 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 these disturbances.

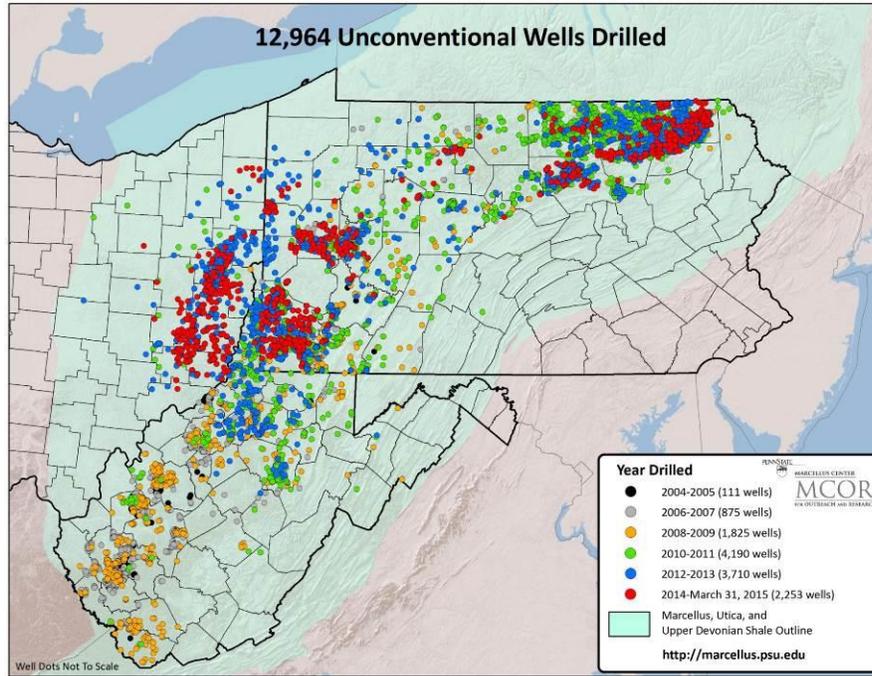


Figure 1. Marcellus gas wells as of March 2015.

We established 142 point count locations throughout the ~3,416 ha study area in northwestern West Virginia in both ridgetop 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 activity, and mature forest reference areas. Across the study area, forest loss and fragmentation have increased over the study period. The amount of mature forest cover has declined about 4% (Fig. 2). The increase in edge density (Fig. 2) is due primarily to the construction of new access roads and pipelines (Fig. 3).

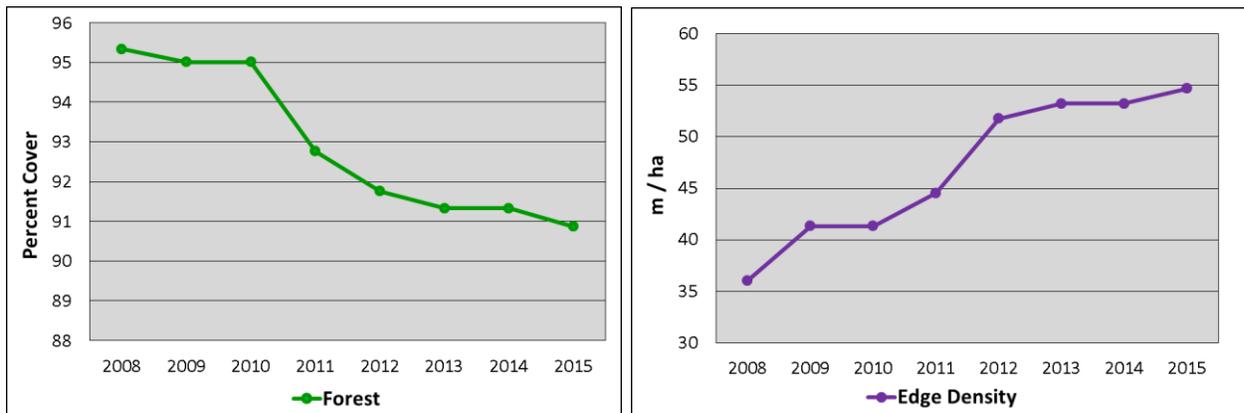


Figure 2. Mature forest cover and amount of forest edge on the management area during 2008-2015.



Figure 3. Ridge-top access road and gas well site (top) and pipeline corridor through mature forest (bottom).

Relative abundance of avian species dependent on mature forest habitats has generally declined across the study area (Fig. 4). Cerulean Warblers (CERW, 37% decrease) have had the largest declines followed by Louisiana Waterthrush (LOWA, 35% decrease), Worm-eating Warblers (WEWA, 30% decrease) and Black-and-White Warblers (BAWW, 29% decrease). During the 2015 breeding season, there was no active development at the WMA. Many mature forest species showed a slight rebound in relative abundance in 2015, possibly in response to the reduction in human disturbance. We plan to investigate this further.

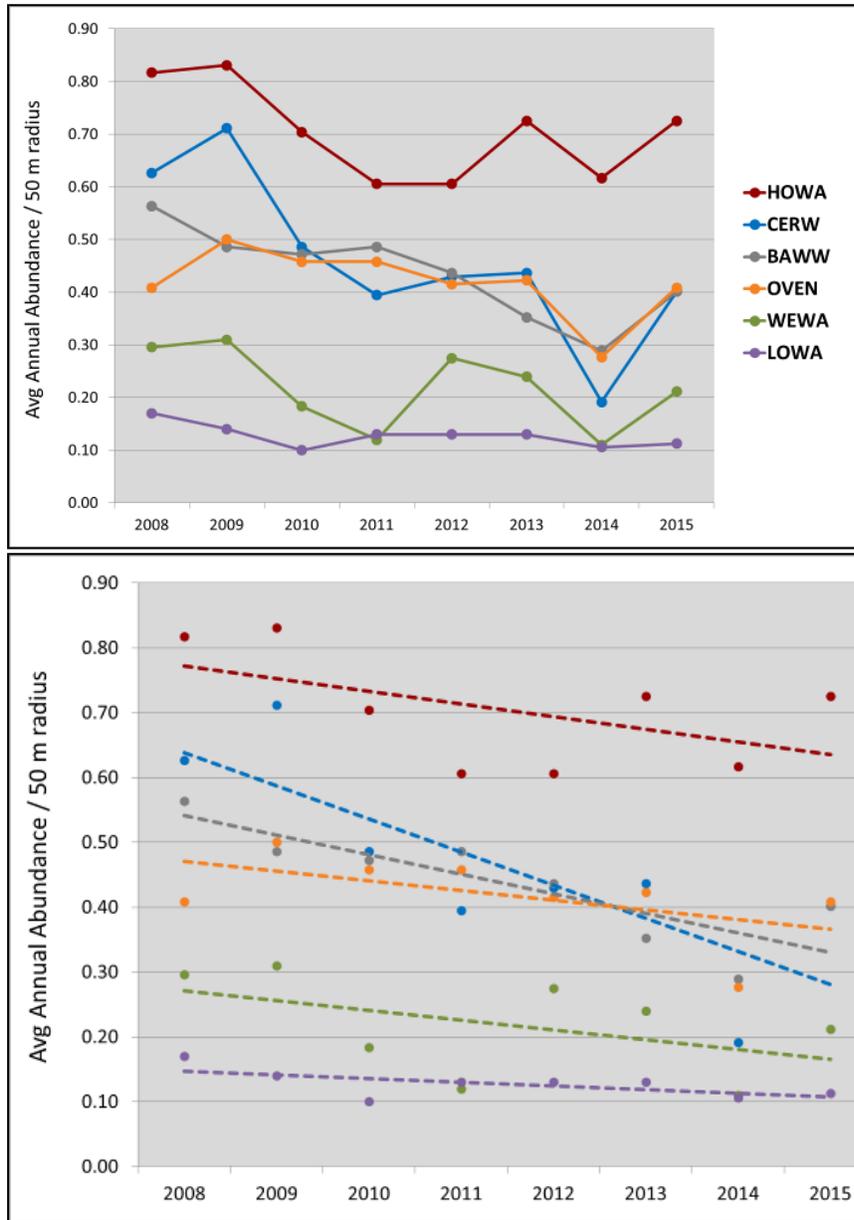


Figure 4. Annual relative abundance of mature forest dependent avian species (top), and trend lines showing general patterns of decline over the 8-year study period (bottom).

Conversely, early successional and edge-associated species have increased in abundance and distribution (Fig. 5), particularly in association with new impacts on the study area. Eastern Towhees (EATO, 22% increase) and Indigo Buntings (INBU, 40% increase) were the most abundant edge-associated species, while Chipping Sparrows (CHSP, 60% increase) and Common Yellowthroats (COYE, 50% increase) had very steep increases. Brown-headed Cowbirds (BHCO, 15-fold increase), a nest parasite that has been implicated in declines of other songbird species, increased sharply over the course of the study period.

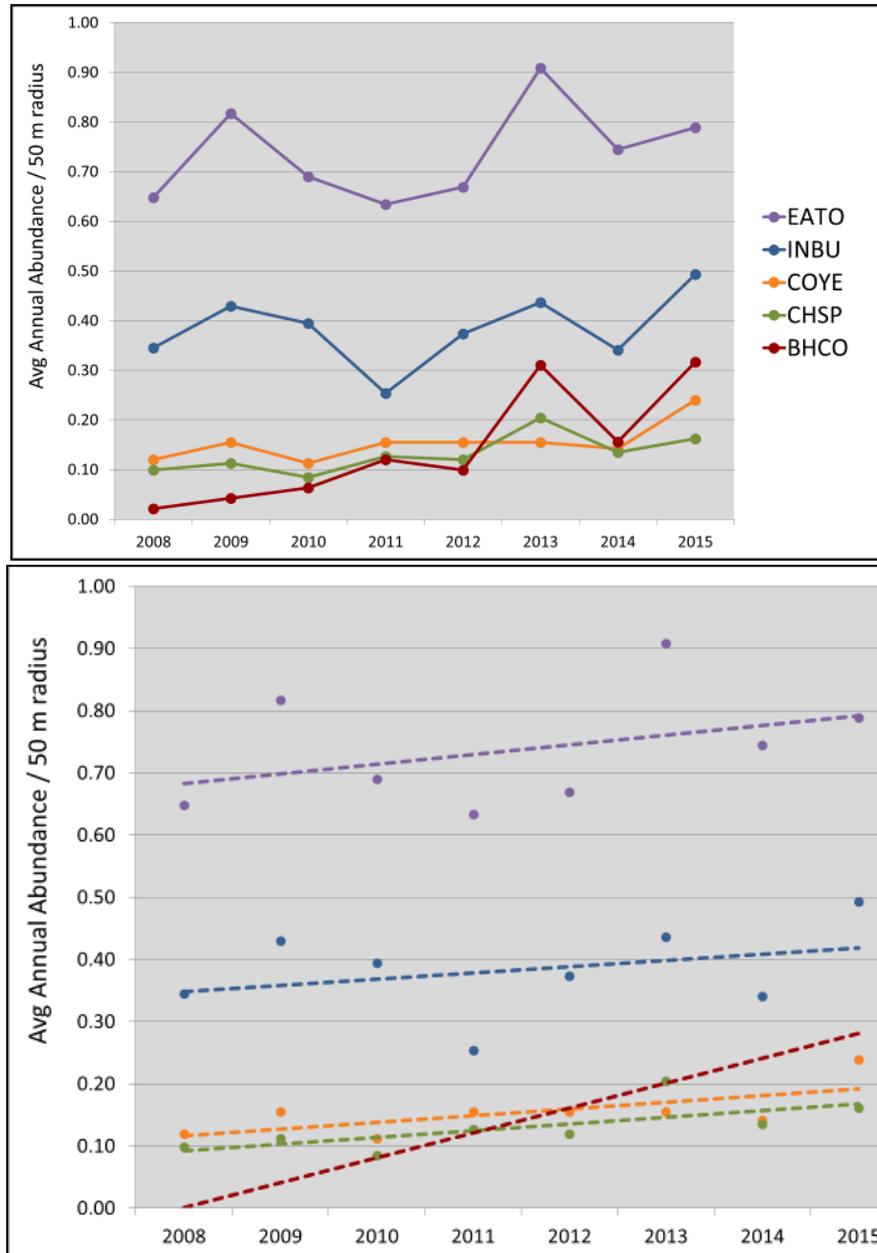


Figure 5. Annual relative abundance of edge-associated avian species (top), and trend lines showing general patterns of increase over the 8-year study period (bottom).

We are currently finalizing analyses of the 8-year dataset, using generalized linear mixed models (GLMM) to relate changes in land cover and forest edge density to relative abundances of focal bird species and habitat guilds. We have specified a Poisson distribution, since our count data are non-normally distributed, right-skewed, and bounded by zero. We are including year and survey point as random effects to account for variation associated with repeated measures over space and time. Lastly, we will use Akaike's information criterion with a second-order bias correction (AIC_c) to evaluate and rank GLMM outputs.

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

SCIENTIFIC PUBLICATIONS

Aldinger, K. R. and P. B. Wood. 2015. Variables associated with detection probability, detection latency, and behavioral responses of Golden-winged Warblers (*Vermivora chrysoptera*). *Condor* 117:364-375.

Aldinger, K. R., T. M. Terhune II, P. B. Wood, D. A. Buehler, M. H. Bakermans, J. L. Confer, D. J. Flaspohler, J. L. Larkin, J. P. Loegering, K. L. Percy, A. M. Roth, and C. G. Smalling. 2015. Variables associated with nest survival of Golden-winged Warblers (*Vermivora chrysoptera*) among vegetation communities commonly used for nesting. *Avian Conservation and Ecology* 10(1): 6. <http://dx.doi.org/10.5751/ACE-00748-100106>

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- Smith, B.W., A.N. Tri, C.A. Dobony, J.W. Edwards, and P.B. Wood. Behavior and nesting ecology of Appalachian ruffed grouse. *Canadian Field Naturalist (In Press)*
- Smith, G.D., V.S. Blazer, H.L. Walsh, L.R. Iwanowicz, C. Starliper, and A.J. Sperry. (2015) The effects of disease-related mortality of young-of-year Smallmouth Bass *Micropterus dolomieu* on population characteristics in the Susquehanna River Basin, Pennsylvania and potential implications to conservation of black bass *Micropterus* spp. diversity. Pages 319-332 in Tringali, M. D., M. S. Allen, T. Birdsong, and J. M. Long. 2014. Black Bass Diversity: Multidisciplinary Science for Conservation. Proceedings of the Symposium Black Bass Diversity: Multidisciplinary Science for Conservation Held in Nashville, Tennessee, February 8-10, 2013. American Fisheries Society, Bethesda, Maryland.
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- Welsh, S.A. and Z.J. Loughman. In press. Upstream dispersal of an invasive crayfish aided by a fish passage facility. *Management of Biological Invasions*.

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- Eyler, Sheila M. 2014. Timing and survival of American Eels migrating past hydroelectric dams on the Shenandoah River. PhD Dissertation, West Virginia University, Morgantown, WV.
- Hilling, Corbin D. 2015. Evaluation of age, growth, and diet of Channel Catfish (*Ictalurus punctatus*) in Cheat Lake, West Virginia. M.S. thesis, West Virginia University, Morgantown, WV.

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- Aldinger, K. R., M. H. Bakermans, D. A. Buehler, J. L. Larkin, C. G. Smalling, P. B. Wood, J. A. Jones, J. A. Lehman, D.J. McNeil, and A. Tisdale. Nesting ecology of Golden-winged Warblers in vegetation communities resulting from different NRCS conservation practices. The Wildlife Society Annual Conference. Pittsburgh, PA. 27 October 2014.
- Aldinger, K. R. and P. B. Wood. Golden-winged Warbler density and priority species richness in the West Virginia highlands. Association of Field Ornithologists, Society of Canadian Ornithologists, and Wilson Ornithological Society join meeting. Wolfville, NS. 17 July 2015. (poster)
- Braham, M., R. P. Braham, J. Brandt, L. Mendenhall, J. Hall, T. Miller, M. Lanzone, A. Duerr, A. McGann, and T. E. Katzner. 2015. Wing loading and correlates of flight behavior of California condors (*Gymnogyps californianus*). Wilson Ornithological Society – Association of Field Ornithologists – Canadian Ornithological Society Annual Meeting, Wolfville, NS. July 16-19, 2015. (poster)
- Braham, R., P. Mazik, J. Hedrick, L. Iwanowicz, and V. Blazer. 2015. Spatial and temporal variation among microcystin toxins and environmental estrogens within the Potomac River Drainage. USGS Priority Ecosystem Chesapeake Bay Annual Meeting. Shepherdstown, West Virginia. May 27-28, 2015. (poster)
- Braham, R. P., V. S. Blazer, J. Hedrick, and P. M. Mazik. 2015. Spatial and temporal variations in microcystin toxins and environmental estrogens within the Potomac River Drainage. American Fisheries Society Fish Health Section Annual Meeting. Ithaca, New York. July 13-15, 2015.
- Becker, D.A., P.B. Wood, M.P. Strager, and C. Mazzarella. Identifying landscape thresholds for avian species in the mountaintop mining region. The Wildlife Society Annual Conference, Pittsburgh, PA.
- Eyler, S., S. Welsh, D. Smith, and M. Rockey. 2015. Passage method, turbine mortality, and migratory delay of silver American Eels (*Anguilla rostrata*) at five hydroelectric dams on the Shenandoah River. Presented at the Connecticut River Atlantic Salmon Commission (CRASC) Research Forum, MADFW Westborough Field Office, 3 March 2015, Westborough, MA.
- Eyler, S., S. Welsh, D. Smith, and M. Rockey. 2015. Environmental variables associated with American Eel (*Anguilla rostrata*) downstream migrations in the Shenandoah River. Presented at the 145th Annual Meeting of the American Fisheries Society, 17 August 2015, Portland, OR.
- Farwell, L.S., Wood, P.B., Sheehan, J., George, G. 2015. Trends in avian response to shale gas development: a long-term case study from West Virginia. 100th Annual Meeting of the Ecological Society of America. Baltimore, MD. August 9-14, 2015.

- Farwell, L.S., Wood, P.B., Sheehan, J., George, G. 2015. Avian community response to shale gas development at Lewis Wetzel Wildlife Management Area. Joint WV Department of Natural Resources / U.S. Forest Service Meeting. Canaan Valley State Park, WV. 1 April 2015.
- Farwell, L.S., Wood, P.B., Dettmers, R., Fearer, T., Brittingham, M. 2014. Songbird response to forest disturbance due to Marcellus shale gas development. Joint Conference of Northeast and Southeast Partners in Flight. Virginia Beach, VA. Oct 6-9, 2014.
- Farwell, L.S., Wood, P.B., Sheehan, J., George, G. 2014. Early trends in avian response to Marcellus gas development in a Central Appalachian forest. 21st Annual Conference of the Wildlife Society. Pittsburgh, PA. October 25-30, 2014.
- Frantz, M., A. Welsh, and P. B. Wood. Epigenetic (DNA methylation) variation in the Louisiana Waterthrush due to shale gas development in West Virginia. The Wildlife Society, 21st Annual Conference. Pittsburgh, PA, 25-30 Oct. 2014 (poster)
- Frantz, M. W., P. B. Wood, J. Sheehan, G. George, A. Welsh, and S. Latta. Response of Louisiana Waterthrush to shale gas development. Ecological Society of America (ESA) Annual Meeting. Baltimore, MD, 10-13 Aug. 2015.
- Hahn, C.M., Iwanowicz, L.R., Blazer, V.S., Cornman, R.S. 2014. The First Report of a Hepadnavirus Isolated from Fishes: Evidence of Hepatitis B Virus infection in White Sucker (*Catostomus commersoni*) from the Great Lakes Region. International Association of Great Lakes Research. May 2015. Burlington, VT. (poster)
- Hahn, C.M., Iwanowicz, L.R., Blazer, V.S., Walsh, H.L., Braham, R.P., Mazik, P.M. 2015. Effects of Contaminants on Gene Expression Endpoints in Micropterus from Great Lakes Areas of Concern. International Association of Great Lakes Research. May 2015. Burlington, VT.
- Hilling, C, S. Welsh, and D. Smith. 2015. Evaluation of age, growth and condition of Channel Catfish in Cheat Lake, West Virginia. Presented at the Joint Meeting of VA and WV AFS Chapters, 18 February 2015, Blacksburg, VA.
- 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. 2015. Migration and movement ecology of Golden-winged Warblers derived from light-level geolocators. Joint Meeting of The American Ornithologists' Union & The Cooper Ornithological Society, Norman, Oklahoma. 30 July 2015.
- Leuenberger, W., Aldinger, K.R., M.H. Bakermans, D.A. Buehler, J.A. Jones, J.L. Larkin, J.A. Lehman, D.J., McNeil, C.G. Smalling, A.C. Tisdale, P.B. Wood. Avian species associated with habitat created for the Golden-winged Warbler through the NRCS Working Lands for Wildlife program. The Wildlife Society Annual Conference, Pittsburgh, PA.
- Loughman, Z, N. Sadecky, and S. Welsh. 2015. Determination of crayfish occupancy rates across mined watersheds in eastern Kentucky. Presented at the Southern Division American Fisheries Society meeting, 30 January 2015, Savannah, GA.
- McNeil, D.J., Aldinger, K.R., M.H. Bakermans, D.A. Buehler, J.A. Jones, J.L. Larkin, J.A. Lehman, C.G. Smalling, A.C. Tisdale, P.B. Wood, J.L. Larkin. Nesting, territoriality, and morphometrics of Golden-winged Warblers in vegetation communities resulting from different NRCS conservation practices. NE-SE Partners In Flight. Virginia Beach, VA. Oct 2014

- 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. 2nd Joint Northeastern and Southeastern Partners In Flight Meeting. Virginia Beach, VA, 6-9 October 2014. (poster)
- 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. The Wildlife Society 21st Annual Meeting. Pittsburgh, PA, 25-30 October 2014. (poster)
- Nareff, G.E., P.B. Wood, T. Fearer, W.M. Ford, J. Larkin, and S. Stoleson. Forest management for the cerulean warbler and response of associated songbird species. Joint WV Department of Natural Resources / U.S. Forest Service Meeting. Canaan Valley State Park, WV. 1 April 2015.
- Nareff, G.E., P.B. Wood, T. Fearer, W.M. Ford, J. Larkin, and S. Stoleson. Forest management for the cerulean warbler and response of associated songbird species. WVU Davis College Interdisciplinary Graduate & Professional Student Symposium. Morgantown, WV, 2-3 April 2015.
- Nareff, G.E., P.B. Wood, T. Fearer, W.M. Ford, J. Larkin, and S. Stoleson. Forest management for the cerulean warbler and response of associated songbird species. Brooks Bird Club Annual Foray. Terra Alta, WV, 8 June 2015.
- Nareff, G.E., P.B. Wood, and T. Fearer. Cerulean warbler territory habitat selection related to operational silviculture prescriptions in West Virginia. Ecological Society of America 100th Annual Meeting, Baltimore, PA, 9-14 August 2015
- Nicholson, M.C., J. Wickham, P.B. Wood, M. Strager, C. Mazzarella, W. Jenkins. July 2015. Landscape impacts of mountaintop mining in the Appalachian Mountains. International Association of Landscape Ecology World Congress
- Rolek, B.W., C. Loftin, D. Harrison, and P. Wood. Effects of Forest Management on Avian Abundance in Spruce-Fir Forests of New England. Joint meeting between Association of Field Ornithologists, Society of Canadian Ornithologists / Société des ornithologistes du Canada, and Wilson Ornithological Society. 15-19 July 2015.
- Sheehan, J. and P.B. Wood. Use of high spatial resolution remote sensing data to model avian and habitat field data from an Appalachian mature deciduous forest, with implications for remote sensing-based biodiversity assessment at larger spatial scales. Northeast and Southeast Partners in Flight, Wyndham Virginia Beach, Virginia, October 6-9, 2014. (poster)
- Smith, D.M., S.A. Welsh, N.D. Taylor, and C.D. Hilling. 2015. Population Dynamics and Seasonal Movement Patterns of Walleye in a Hydropower Reservoir. Presented at the Southern Division American Fisheries Society meeting, 30 January 2015, Savannah, GA.
- Smith, D.M., S.A. Welsh, N.D. Taylor, and C.D. Hilling. 2015. Population dynamics and seasonal movement patterns of Walleye in a hydropower reservoir. Presented at the Joint meeting of VA and WV AFS Chapters, 18 February 2015, Blacksburg, VA.
- Walsh, H.L., M. Keplar, G. Smith, and V.S. Blazer. 2015. The Use of Molecular Pathology Techniques for Understanding Mechanisms of Disease in Smallmouth Bass from the Chesapeake Bay Watershed. USGS Priority Ecosystem Chesapeake Bay Annual Meeting 2015, Shepherdstown, WV, May 27-28. (Poster)

Welsh, S.A. and J.L. Aldinger. 2014. Eel ladders and dam passage of upstream migrant American Eels in the Shenandoah River drainage. Presented at the annual meeting of the Southeastern Fishes Council, 14 November 2014, Asheville, NC.

Welsh, S.A. and Z.J. Loughman. 2015. Upstream dispersal of an invasive crayfish aided by a fish passage facility. Presented at the Southern Division American Fisheries Society meeting, Savannah, GA, 30 January 2015.

Welsh, S. and J. Aldinger. 2015. A 12-year time series of American eel passage at an eel ladder, lower Shenandoah River. Presented at the joint meeting of VA and WV Chapters of AFS, 18 February 2015, Blacksburg, VA.

Welsh, S.A. and J.L. Aldinger. 2015. Periodicity and timing of upstream migration of yellow-phase American Eels, Potomac River drainage, USA. The 95th Annual Meeting of the American Society of Ichthyologists and Herpetologists. University of Nevada, Reno, Nevada, 19 July 2015.

Welsh, S.A. and J.L. Aldinger. 2015. Upstream migrant American Eels: A 12-year time series from an eel pass on the lower Shenandoah River. Presented at the 145th Annual Meeting of the American Fisheries Society, 19 August 2015, Portland, OR.

AWARDS

Kyle Aldinger (PhD student) was selected as the Hoyt Outstanding PhD student in the Division of Forestry and Natural Resources at WVU, March 2015

Kyle Aldinger, Laura Farwell, Mack Frantz, 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 Wildlife Society Conference in Pittsburgh, PA in October 2015. Laura Farwell was awarded an additional travel grant from The Wildlife Society for this conference.

Laura Farwell (PhD student) received the AVMA Roger Latham Memorial Award from the Appalachian Vegetation Management Association, March 2015. Laura also participated in the 2015 Student Research & Creative Scholarship Conference for the Davis College of Agriculture, Natural Resources & Design and received 3rd place in the PhD Oral/Paper Presentations category, April 7, 2015.

Pat Mazik was named an American Fisheries Society Fellow at the AFS National Meeting in Portland, Oregon, August 17, 2015