West Virginia Cooperative Fish and Wildlife Research Unit

FY 2013
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1 October 2012 - 30 September 2013

COOPERATING AGENCIES:

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

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Cover Graphics: Cedar Waxwing – Doug Becker
WVCFWRU logo by Marianne Dewey

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 on wildlife populations such as the effects of timber harvesting on birds, woodrats, and bats, and mountaintop mining on terrestrial 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, 2013, 104 students have completed their degree requirements: 84 Masters and 20 Ph. D. The Unit scientists are currently supervising 5 Master's students and 13 Ph.D. students.

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UNIT LEADER

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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 Associate Professor of Fisheries
Ph.D., West Virginia University, 1997. Fisheries ecology, zoogeography and systematics.

STAFF

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PROJECT COOPERATORS

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Zac Loughman, West Liberty University
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J.R. Shute, Conservation Fisheries, Inc.
Steve Summerfelt, Freshwater Institute
T. Bentley Wigley, NCASI

STUDENTS

STUDENT	DEGREE	GRADUATION DATE	ADVISOR
Kyle Aldinger	Ph.D.	Expected May 2014	Petra Wood
Melissa Braham	M.S.	Completed Dec 2012	Stuart Welsh
Ryan Braham	M.S.	Completed Dec 2012	Pat Mazik
Ryan Braham	Ph.D.	Expected Dec 2016	Pat Mazik
Ryan Davis	M.S.	Expected May 2015	Petra Wood
Sheila Eyler (NCTC)	Ph.D.	Expected May 2014	Stuart Welsh
Laura Farwell	Ph.D.	Expected Aug 2016	Petra Wood
Steve Foster (NCTC)	Ph.D.	Expected Dec 2015	Stuart Welsh
Mack Frantz	Ph. D.	Expected May 2016	Petra Wood
Cassidy Hahn	Ph.D.	Expected May 2015	Pat Mazik
Corbin Hilling	M.S.	Expected May 2015	Stuart Welsh
Lauren Kesslak	Ph.D.	Expected Dec 2016	Pat Mazik
Carlos Martinez (NCTC)	M.S.	Expected Dec 2014	Pat Mazik
Grtechen Nareff	Ph. D.	Expected May 2016	Petra Wood
Joni Riffle	M.S.	Expected May 2015	Stuart Welsh
Crystal Ruble	M.S.	Completed May 2013	Stuart Welsh
Jim Sheehan	Ph.D.	Expected May 2014	Petra Wood
Dustin Smith	Ph.D.	Expected Dec 2015	Stuart Welsh
Daniel Sparks (NCTC)	Ph.D.	Expected Dec 2014	Pat Mazik
Nate Taylor	M.S.	Completed May 2013	Stuart Welsh
Jeff Thomas (NCTC)	Ph.D.	Expected May 2016	Stuart Welsh
Thomas Waldrop (NCTC)	M.S.	Expected Dec 2014	Pat Mazik

UNIT STAFF COURSES TAUGHT

Patricia M. Mazik, Adjunct Associate Professor of Fisheries

Fish Physiology	Fall 2013	3 credits

Stuart A. Welsh, Adjunct Associate Professor of Fisheries

Advanced Ichthyology	Fall 2013	3 credits

Petra Bohall Wood, Adjunct Professor of Wildlife

Wildlife and Fisheries Graduate Seminar	Spring 2013	1 credit
Conservation Biology	Spring 2013	3 credits

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.

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

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

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

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

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

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

COMPLETED PROJECTS

AQUATIC

AN EVALUATION OF THE OCCURRENCE OF MICRONUCLEI AND OTHER NUCLEAR ABNORMALITIES IN FISHES FROM THE GREAT LAKES BASIN, UNITED STATES

Student Investigator: Ryan Braham, M.S.
Principal Investigators: Dr. Patricia Mazik
Collaborators: Dr. Vicki Blazer, USGS Leetown Science Center
Degree Program: MS
Years Ongoing: 2010 - 2012
Completed: December 2012
Funding Source: U. S. Geological Survey (RWO 55)

Objectives:

The primary objective of this study is to take an investigative approach to quantifying non-specific genetic damage to fish at various Areas of Concern located in the Great Lakes Basin, United States. Peripheral blood samples were collected from brown bullhead, white sucker and largemouth and smallmouth bass and examined for and nuclear abnormalities (specifically micronuclei) in the erythrocytes. The specific objectives of this study are to evaluate 1. Evaluate micronuclei and nuclear abnormality occurrence rates among 10 sites throughout the Great Lakes basin; 2. Evaluate micronuclei and nuclear abnormality occurrence rates among 4 possible species sampled within each site; 3. Evaluate micronuclei and nuclear abnormality occurrence rates among 2 sampling seasons within sites; 4. Examine the land use/land cover, point source discharge sites, as well as collaborate with the USGS Minnesota Water Science Center (Grand Rapids, Minnesota) to examine water and sediment chemistry data for any possible trends that may exist among micronuclei and/or nuclear abnormality occurrence rates; and 5. Statistically evaluate micronuclei and nuclear abnormality presence/absence among *a priori* biological explanatory variables with the objective of formulating a predictive model explaining any observed variation.

The specific land cover/land use metrics used will include but may not be limited to the percent of total land that is used for agriculture, industrial, forested, and wetland. The number and type of industrial facilities such as wastewater facilities, landfills and industrial plants were evaluated, as well as the number and location of confined animal feedlot operations (CAFO) and combined sewer overflow (CSO) points for any possible correlation to micronuclei abundance. Otoliths were used to evaluate any age-effect observed among sampling locations. The final result provided a biological endpoint of areas that are experiencing non-specific genetic disorders and possible causes for that endpoint.

Results:

Micronuclei and NA were expressed at differing rates within species among sites. At sites in which brown bullhead were collected during the spring, 35% of the individuals captured in the Genesee River expressed MN. The Ashtabula, Detroit and Niagara Rivers all expressed MN at a frequency of approximately 10%, while the Conneaut Creek and combined Duck/Otter Creek sites expressed MN at 0%. The highest frequency of occurrence of MN (presented as the mean number of micronuclei observed per 1000 erythrocytes, ‰) was observed in the Genesee River, followed by the Niagara River. The

Detroit and Ashtabula River sites expressed MN at a relatively lower rate. No site was statistically significantly different, however the Genesee River trended to have a higher MN occurrence rate as compared to the Ashtabula, Conneaut, Detroit, Duck/Otter, and Niagara sites. Micronuclei were not observed at the Ashtabula River or Conneaut Creek during the fall sampling.

During the spring sampling season, the highest frequency of occurrence of NA was observed at the Ashtabula and Detroit Rivers (75% and 50%, respectively). Conneaut Creek and the Duck/Otter Creek sites expressed NA at 21% and 28%, respectively. The Genesee River expressed NA at 13%, while no NA was observed at the Niagara River. The highest frequency of occurrence of NA (‰) was observed in the Detroit and Ashtabula River sites. Nuclear abnormalities were observed at relatively lower rates at the Genesee, Duck/Otter, and the Conneaut Creek sites. Nuclear abnormalities were not observed at the Niagara River site. Nuclear abnormalities were observed at significantly higher rate at the Ashtabula River as compared to the remaining sites. During the fall sampling season, NA were expressed in 47% of the brown bullheads collected at the Ashtabula River as compared to 17% of the bullheads collected at Conneaut Creek. There was a significantly higher occurrence rate observed at the Ashtabula River as compared to Conneaut Creek.

Among sites where largemouth bass were collected during the spring sampling season, all sites expressed MN and NA. The Ashtabula and Genesee Rivers expressed MN at approximately the same frequency (~58%), while the Detroit expressed MN at 45%. The Genesee, Detroit, and Ashtabula River all expressed MN at approximately the same occurrence rate (~0.9‰). There was no significant difference in the mean MN occurrence rates among any of the spring sites. During the fall sampling season, the Ashtabula River expressed MN at a higher frequency as compared to Conneaut Creek (58% and 11%, respectively). The Ashtabula River also expressed MN at a significantly higher occurrence rate than Conneaut Creek. The Genesee, Detroit, and Ashtabula River all expressed NA at 100%, as well as at a relatively high occurrence rate (>30.0‰). There was no significant difference in the mean NA occurrence rates among any of the spring sites. During the fall sampling season, the Ashtabula River and Conneaut Creek sites both expressed NA at a relatively high frequency (100% and 84%, respectively). The Ashtabula River expressed MN at a significantly higher occurrence rate as compared to Conneaut Creek.

Among sites where smallmouth bass were collected, all sites expressed MN and NA. The Saint Louis and Milwaukee River sites expressed MN at a higher frequency as compared to the Fox River and the Duck/Otter Creek sites. The Saint Louis and Milwaukee River sites also expressed MN at a significantly higher occurrence rate as compared to the Fox and Duck/Otter Creek sites. The Saint Louis, Milwaukee and Duck/Otter Creek sites ass expressed NA at 100% frequency of occurrence. Forty seven percent of the smallmouth bass collected at the Fox River expressed NA. The Saint Louis, Milwaukee and Duck/Otter Creek sites expressed NA at a relatively higher occurrence rate than the Fox River site. The Milwaukee and Duck/Otter sites expressed NA at a significantly higher rate than the Milwaukee and Saint Louis River sites.

Among sites where white sucker were collected, all sites expressed MN and NA. The Saint Louis River site had a higher frequency of individuals expressing MN as compared to the Swan and Milwaukee River sites. The Saint Louis River site also expressed MN at a relatively higher occurrence rate as compared to the Swan and Milwaukee River sites. There was no statistically significant difference for mean MN occurrence rates among any of the sites. The Swan Creek site had a higher frequency of individuals expressing NA as compared to the Saint Louis and Milwaukee River sites. The Swan Creek site also trended to express NA at a relatively higher rate than the Saint Louis and Milwaukee River sites; however this trend was not significant.

Micronuclei and NA were differentially expressed among species. For the 3 spring sites in which brown bullhead and largemouth bass were collected, MN frequency of occurrence rates were 0.189‰ for brown bullhead and 0.963‰ for largemouth bass. Nuclear abnormality occurrence rates were 1.594‰ for brown

bullhead and 32.724‰ for largemouth bass. For the 2 spring sites in which white sucker and smallmouth bass were collected, MN occurrence rates were 0.264‰ for white sucker and 3.430‰ for smallmouth bass. Occurrence rates for NA were 0.589‰ for white sucker and 23.923‰ for smallmouth bass. For the 1 spring site in which brown bullhead and smallmouth bass were collected, MN occurrence rates were 0.000‰ for brown bullhead and 0.598‰ for smallmouth bass. Nuclear abnormality occurrence rates were 0.532‰ for brown bullhead and 16.077‰ for smallmouth bass. For the 2 fall sites where brown bullhead and largemouth bass were collected, MN occurrence rates were 0.000‰ for brown bullhead and 0.576‰ for largemouth bass. Occurrence rates for NA were 0.807‰ for brown bullhead and 13.706‰ for largemouth bass.

A seasonal comparison was able to be examined for both brown bullheads and largemouth bass. Brown bullheads were collected at both the Ashtabula River and Conneaut Creek during the spring and fall of 2011. Largemouth bass only were collected at the Ashtabula River during both the spring and fall sampling seasons. There was no significant difference in the mean MN occurrence rate for the 2 sampling seasons. There was however, a significant decrease in the number of NA observed in the fall sampling as compared to the spring sampling season.

Age Analysis

Age analysis was conducted on a total of 549 individuals collected during the spring and fall of 2011. There was no trend observed among different sexes collected within species and sites. For sites where brown bullhead were collected, females' ages ranged from 5-7 years ($\bar{x}$ = 6.62; $SE_{\bar{x}}$ = 0.21) while males ages ranged from 6-8 ($\bar{x}$ = 6.68; $SE_{\bar{x}}$ = 0.20). The overall age of brown bullhead collected at the Genesee, Ashtabula, Conneaut, and Niagara River sites was >6 years old. Individuals collected at the Detroit River and Duck/Otter Creek sites were between 5 and 6 years old. There was no correlation between age and MN or NA frequency of occurrence.

Among sites where largemouth bass were collected, females ranged from 5-6 years ($\bar{x}$ = 6.10; $SE_{\bar{x}}$ = 0.20), while males ranged from 5-7 years ($\bar{x}$ = 5.96; $SE_{\bar{x}}$ = 0.20). There was no appreciable difference in age among sites. A significant positive correlation was observed for the frequency of occurrence of MN as a function of age. No trend was observed for NA frequency as a function of age.

Among sites where smallmouth bass were collected, females ranged from 3-6 years ($\bar{x}$ = 6.05; $SE_{\bar{x}}$ = 0.20), while males ranged from 3-9 years ($\bar{x}$ = 5.76; $SE_{\bar{x}}$ = 0.28). The age structure among smallmouth bass collected at the Milwaukee and Saint Louis River sites was similar. Individuals collected at the Duck/Otter Creek site were considerably younger than those collected at the other two sites. A positive trend was observed in the frequency of occurrence of MN as a function of age. This trend was not significant. No trend was observed for NA frequency as a function of age.

Among sites in which white sucker were collected, females ranged from 6-10 years ($\bar{x}$ = 8.08; $SE_{\bar{x}}$ = 0.21), while males ranged from 5-11 years ($\bar{x}$ = 8.70; $SE_{\bar{x}}$ = 0.26). Individuals collected at the Saint Louis and Swan Creek sites ranged from 6.5-9 years while fish collected at the Milwaukee River were approximately 11 years old. There was a significantly negative correlation in the frequency of occurrence of NA as a function of age. No trend was observed for MN frequency as a function of age.

Spatial Analysis

Due to the higher likelihood of genotoxic and/or mutagenic chemicals to be introduced from developed or agricultural land cover classifications, we focused our analysis of these two land cover types. The combined developed land use types account for approximately 90% of the total land cover in the combined Duck/Otter Creek site watersheds. It accounts for over 60% of the total land cover for the Detroit River site watershed. Approximately 25% of the Swan Creek and Milwaukee River site watersheds can be categorized as developed. The respective remaining sites have approximately 5-10% of

their site watersheds' land cover defined as developed. When we examine only the basin area within 1.5 km upstream of our respective sampling sites, the overall percent of land cover classified as developed generally increases. For example, greater than 70% of the basin area within 1.5 km of the Ashtabula, Duck/Otter, Milwaukee, Saint Louis and Swan Creek sites are classified as developed. Greater than 20% of the Conneaut, Fox, and Genesee sites are classified as developed within 1.5 km upstream of the sampling site. The combined agriculture use types account for approximately 40-60% of the Ashtabula, Fox, Genesee, Milwaukee, Niagara, and Swan Creek site watersheds. Approximately 36% of the Conneaut Creek site watershed is defined as agriculture. Approximately 10% and 5% of the Saint Louis River and pooled Duck and Otter Creek site watersheds, respectively, are defined as agriculture. When we constrain our analysis extent to only include the basin area within 1.5 km upstream of our sampling location, we generally observe a reduction in the percent of the basin area that can be classified as agricultural. The exceptions are the Duck/Otter and Fox River sites which are observed to have an increase. Greater than 65% of the Fox River within 1.5km of the sampling location can be classified as agricultural. Between 10% and 15% of the basin area within 1.5 km of the sampling sites for the Duck/Otter and Genesee River sites are classified as agricultural. Less than 5% of the remaining sites' respective basin areas within 1.5km of the sampling sites are classified as agricultural.

The majority of industrial sites occur within the Fox, Genesee, Milwaukee, and Saint Louis River site watersheds. The combined sewerage and land fill sites account for approximately 1-5% of the total industrial sites for nearly all of the respective site watersheds. Conneaut Creek is the exception with approximately 11% of the total industrial sites defined as sewerage facilities. Combined animal feedlot operations were only observed within the Genesee, Fox River, and Milwaukee River site watersheds (88, 39, and 7, respectively). No CAFO's were observed in the respective remaining site watersheds. The majority of CSO's were observed in the Milwaukee River and Swan Creek watersheds (70 and 21, respectively). Seven CSO's were observed in the Genesee River site watershed; while a relatively low number were observed in the pooled Duck and Otter Creek and Saint Louis River site watersheds (2 and 1, respectively). There were no CSO's observed in the Ashtabula, Conneaut Creek, or Fox River site watersheds. We observed a proportional reduction in the number of industrial discharge, CAFO, and CSO sites that were observed within 1.5km of the sampling locations based on the reduction in overall basin area.

<u>Chemical Analysis</u>

Lee et al. (In Prep.) conducted an intensive chemical analysis in both the water column and sediment at seven of the sites in which biological samples were collected. Six of the chemicals sampled have been shown in the literature to induce MN and/or NA expression. These chemicals are Atrazine, Chlorpyrifos, 4-Nonylphenol, Benzo[a]pyrene, Bisphenol A, and 17β-Estradiol. For these 6 chemicals, all were detected in either the water column and/or sediment at all sites.

Ryan Braham necropsying a large mouth bass

CAPTIVE PROPAGATION, REPRODUCTIVE BIOLOGY, AND EARLY LIFE HISTORY OF THE DIAMOND DARTER (*Crystallaria cincotta*)

Student Investigator: Crystal Ruble
Principal Investigators: Stuart A. Welsh
Collaborators: Pat Rakes, J.R. Shute
Years Ongoing: 2008-2013
Degree Program: MS
Completed: Spring 2013
Funding Sources: West Virginia Division of Natural Resources, U.S. Fish and Wildlife Service

Objectives:

Document parameters of reproduction and characteristics of early life history including water temperature range during spawning, egg size and development, egg viability, and the development, growth, and survival of larvae.

Results:

The Diamond Darter *Crystallaria cincotta* is a rare and recently-described fish of the Ohio River drainage. Currently, only a single population is known from the lower 37 km of the Elk River, Kanawha County, WV. Based on museum specimens, this species occurred previously over a wider range within the Ohio River drainage, but populations are considered extirpated from Kentucky, Ohio, and Tennessee. In Nov. 2009, the Diamond Darter was included as a candidate for listing as endangered or threatened. On 26 July 2012, the U.S. Fish and Wildlife Service (USFWS) published a proposed rule in the Federal Register to list the diamond darter and to designate critical habitat under the Endangered Species Act. On 26 July 2013, the USFWS published a final rule in the Federal Register to list the diamond darter as an endangered species.

Data on reproduction and early life history of the Diamond Darter are needed for conservation efforts, and can be obtained through propagation studies of captive breeding and rearing. During 2008–2012, captive propagation studies of the Diamond Darter were conducted at Conservation Fisheries, Inc., Knoxville, TN (CFI, Co-directors J.R. Shute and Pat Rakes; http://conservationfisheries.org/). For this study, a total of 17 Diamond Darters were collected from Elk River during 2008, 2009, and 2011. Aquarium design and recirculation systems used during this study are described in Crystal Ruble's MS thesis (available at http://wvuscholar.wvu.edu:8881/R?RN=508855129).

Water temperatures during spawning ranged from 11.1–23.3°C. Females and males spawned with quick vibrations, burying eggs in fine sand in relatively swift clean depositional areas (http://www.youtube.com/watch?v=tQSvqXX_az8). Egg size was 1.8–1.9 mm, and embryos developed within 7 to 11 days (Figure 1). Diamond Darters were 6.7–7.2 mm TL at hatch (Figure 1). Larvae ranged from 9.0–11.0 mm TL following a 5–10 day period of yolk sac absorption (Figure 1). Larvae were provided brine shrimp *Artemia sp.*, *Ceriodaphnia dubia* neonates, marine *Brachionus* rotifers, and powdered foods (50–400 µm), but did not feed in captivity, except for cannibalizing other larvae. Larvae survived for a maximum of 11 days. Larvae had relatively large mouth gapes and teeth, suggesting possible larval piscivory and a need for alternative food sources during captive propagation (Figure 2).

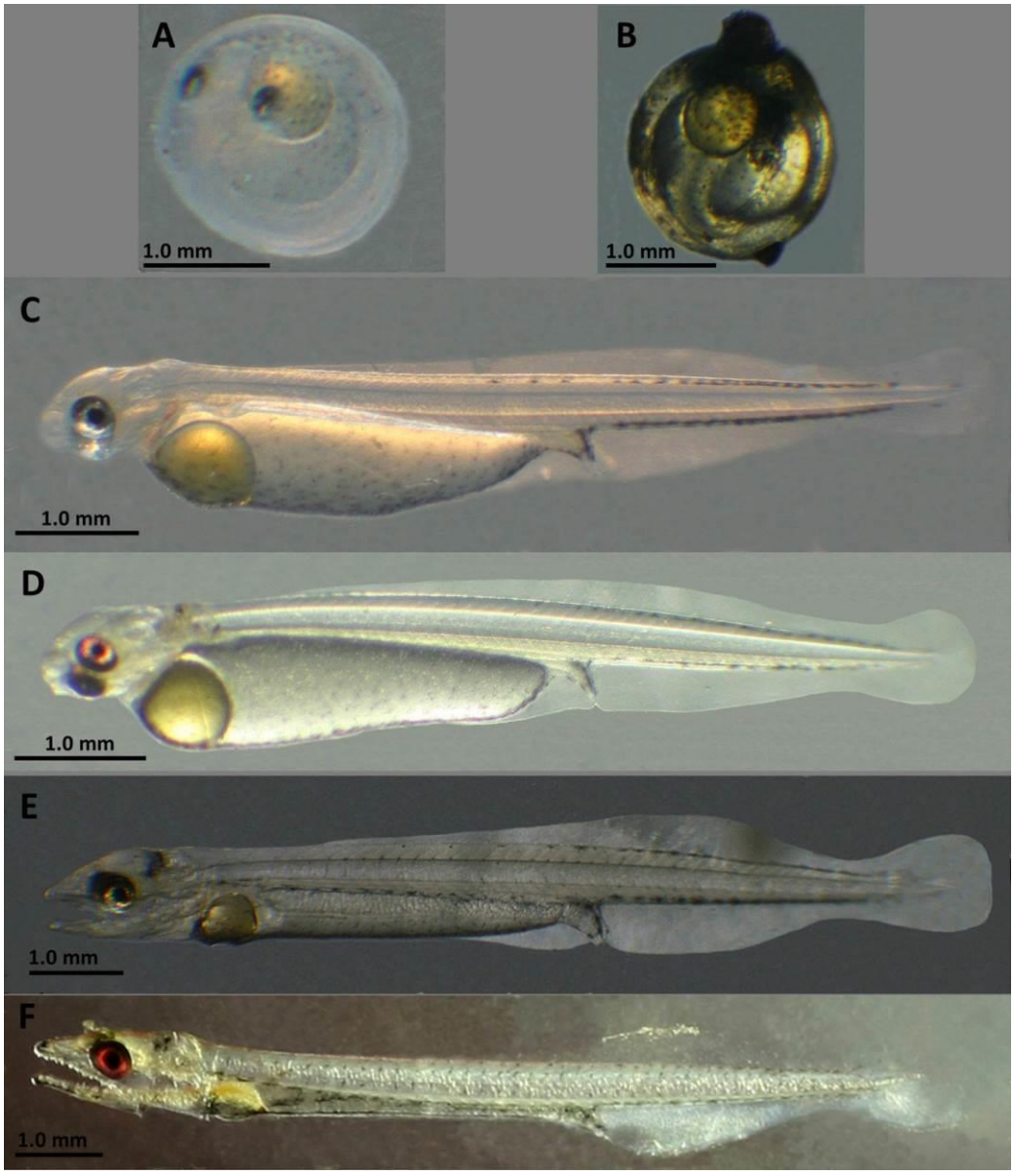


Figure 1. Photographs of eggs and larvae from a captive propagation study of the Diamond Darter: (A) eyed egg, (B) well-developed egg, (C) one-day old larva with heavy yolk sac, (D) two-day old larva, (E) five-day old larvae, (F) 10-day old larva with large gape size and large teeth.

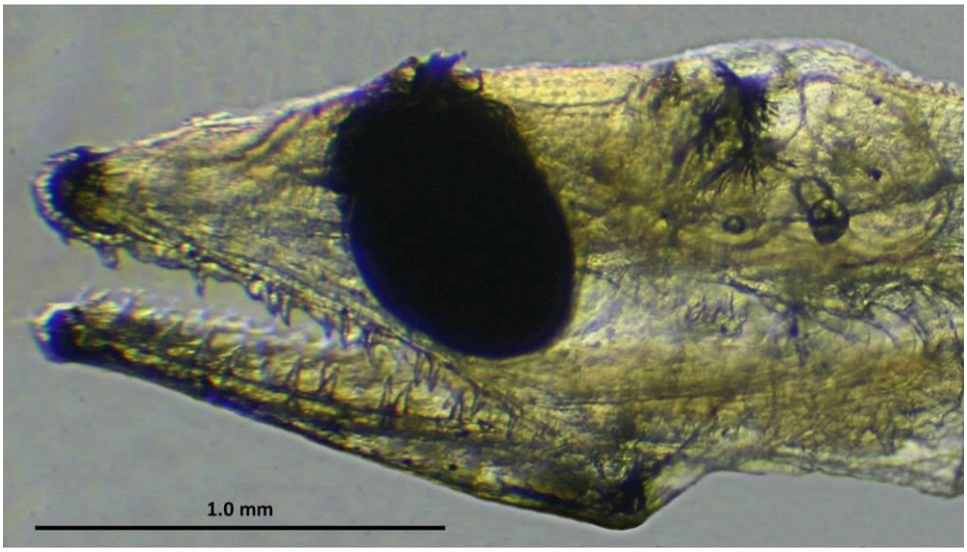


Figure 2. Large gape size and teeth of a 10-day old Diamond Darter larva photographed during a captive propagation study.

CAPTIVE PROPAGATION, REPRODUCTIVE BIOLOGY, AND EARLY LIFE HISTORY OF THE BOULDER DARTER, WOUNDED DARTER, AND SPOTTED DARTER

Student Investigator: Crystal Ruble
Principal Investigators: Stuart A. Welsh
Collaborators: Pat Rakes, J.R. Shute
Years Ongoing: 2008-2013
Degree Program: MS
Completed: Spring 2013
Funding Sources: West Virginia Division of Natural Resources, U.S. Fish and Wildlife Service

Objectives:

Document parameters of reproduction and characteristics of early life history including water temperature range during spawning, egg size and development, egg viability, and the development, growth, and survival of larvae.

Results:

Etheostoma wapiti (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter) are members of the *E. maculatum* species group of the darter subgenus *Nothonotus*. Species within this group are of conservation concern, in part, because of small or fragmented distribution ranges. The Boulder Darter occurs only within the Elk River drainage, TN. In comparison, the Wounded Darter is found within the upper Tennessee River drainage including NC, TN, and VA, and the Spotted Darter occurs in isolated populations within six states: IN, KY, OH, NY, PA, and WV. These darters typically occur within or near riffle habitat of medium to large rivers.

Data on reproduction and early life history of darter species are needed for conservation efforts, and can be obtained through propagation studies of captive breeding and rearing. In 2008, captive propagation

studies of the Boulder Darter, Wounded Darter, and Spotted Darter were conducted at Conservation Fisheries, Inc., Knoxville, TN (CFI, Co-directors J.R. Shute and Pat Rakes; http://conservationfisheries.org/). Boulder Darters were obtained from Elk River and Richland Creek, TN. Wounded Darters were collected from Little Tennessee River, NC. Spotted Darters were from Elk River, WV. Aquarium design and recirculation systems used during this study are described in Crystal Ruble's MS thesis (available at http://wvuscholar.wvu.edu:8881/R?RN=508855129).

The length of spawning period and associated range of water temperatures for the Wounded Darter (89 days, 16.0 – 24.0°C) exceeded that of the Spotted Darter (46 days, 17.0 – 22.5°C) and Boulder Darter (48 days, 17.0 – 22.5°C). Eggs produced per female were least in the Boulder Darter (163), intermediate in the Spotted Darter (191), and highest in the Wounded Darter (345). Egg diameters at deposition and prior to hatch were least for Spotted Darter (1.9–2.0 mm, and 1.9–2.1 mm), intermediate for Wounded Darter (2.1–2.2 mm, 2.4–2.5 mm), and largest for Boulder Darter (2.2–2.4 mm, 2.4–2.6 mm; Figure 1). Lengths of larvae at hatch and at the start of first fin development varied in a similar pattern: Boulder Darter (8.5–9.1, 14.0–15.0), Wounded Darter (7.7–8.0, 13.5–14.0), and Spotted Darter (6.5–7.0, 12.0–13.0 mm; Figure 1). Overall production per female was lowest in the Boulder Darter (61 juveniles/ female), intermediate in the Spotted Darter (86 juveniles/ female), and highest in the Wounded Darter (90 juveniles/ female).

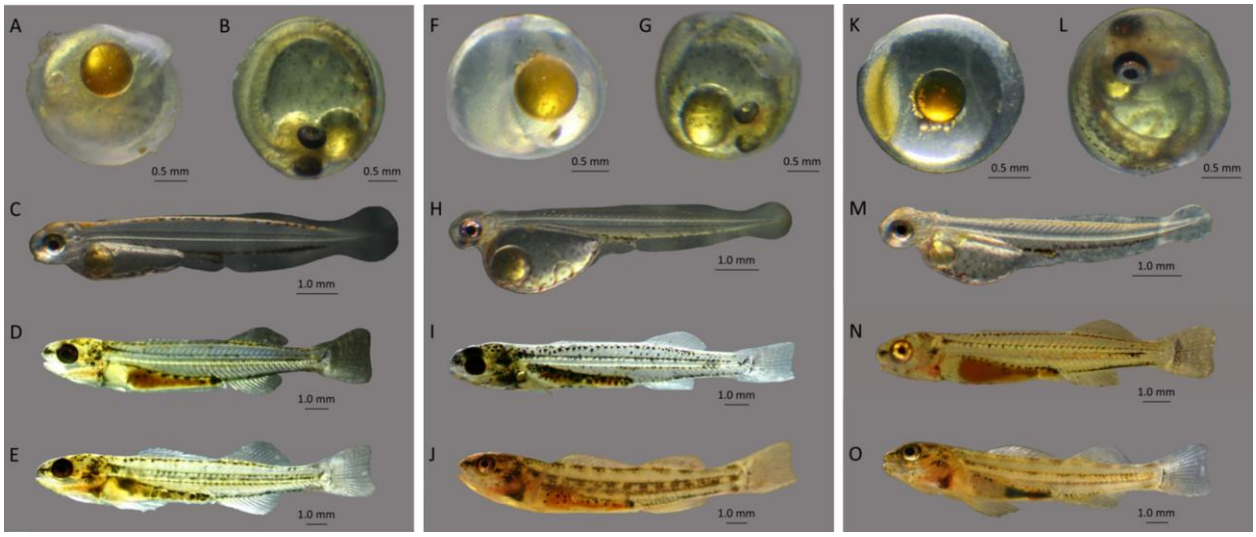


Figure 1. Photographs of eggs at deposition, eggs prior to hatch, yolk-sac larvae, larvae during fin development, and fully transformed juveniles for Etheostoma wapiti (A–E), E. vulneratum (F–J), and E. maculatum (K–O).

ASSESSMENT OF AGE, DIET, AND GROWTH OF YELLOW PERCH IN CHEAT LAKE, WEST VIRGINIA

Student Investigator: Nate Taylor
Principal Investigator: Stuart A. Welsh
Years Ongoing: 2012-2013
Degree Program: MS
Completed: May 2013
Funding Source: West Virginia Division of Natural Resources, FirstEnergy

Objectives:
The objectives of this research were to examine age and growth, and diet composition of Yellow Perch in Cheat Lake, WV.

Results:
Yellow Perch (*Perca flavescens*) provide economically-important sport fisheries throughout much of North America. Although an extensive literature base exists on Yellow Perch biology and ecology, geographic variability among populations requires a need for region-specific research. Information is limited on Yellow Perch populations in West Virginia. Although Yellow Perch are present in many rivers and reservoirs in West Virginia, population sizes are often limited or are comprised of small individuals. Cheat Lake, a 700 ha reservoir located in Monongalia County, supports one of the most productive Yellow Perch fisheries within West Virginia. However, limited information exists on this Yellow Perch population, and fishery-independent data are needed for species management. Particularly, information is needed on age and growth, as well as diet of Yellow Perch in Cheat Lake.

A total of 271 Yellow Perch was collected between 9 July and 4 September 2012. The sample included 39 young-of-year individuals ranging from 66–96 mm TL. The oldest individuals collected were Age-9 females (n=2, 316 mm and 277 mm TL). The most common age class was Age-2 (n=81), but lower sample sizes of Age-0 and Age-1 Yellow Perch likely resulted from gear bias associated with electrofishing. The sample size of females (n=175) exceeded that of males (n=57). Also, females were generally larger than males, with females ranging in size from 115–320 mm TL (mean = 211.50) and males ranging from 128–280 mm TL (mean = 198.89). Length-frequency histograms visually represented size structure (Figure 1).

Growth in fishes is commonly estimated by fitting growth models to age and length data. The von Bertalanffy growth model is commonly used for growth analysis, but other models include Gompertz, logistic, and power function. Four candidate models (von Bertalanffy, Gompertz, logistic, and power) were fitted to mean length-at-age data. Akaike's Information Criterion (AIC_c) was used for model selection. The von Bertalanffy growth model was supported by the data as the best approximating model, but the data also provided some evidence supporting the Gompertz model.

We used the standard length equation and relative growth index to compare growth rates observed in this study to other populations of Yellow Perch. The von Bertalanffy growth model was fitted to length-at-age data with parameter estimates constrained to match those of the standard length equation. Rapid growth was observed from Cheat Lake individuals, and asymptotic lengths were achieved at an earlier age than those reported from other populations (Figure 2). Based on the relative growth index, somatic growth in Cheat Lake individuals exceeded growth rates documented from more than 75% of other populations.

Yellow Perch are opportunistic foragers, consuming various prey items including zooplankton, benthic macroinvertebrates, and other fishes. Additionally, gape limitation plays a critical role in structuring the

diet of Yellow Perch, restricting diet of smaller individuals to smaller prey types. To examine diet of Yellow Perch in Cheat Lake, stomachs were dissected, and gut contents were identified from 246 individuals. Individuals with empty stomachs (n=54) and those containing unidentifiable prey items (n=2) were excluded from further analyses, reducing the total sample to 190 individuals. Individuals were grouped into three age categories: Age-0 (n=19), Age-1 (n=41), and Age-2^{+} (n=130). Percent frequency of occurrence (O_i), mean percent composition by number (MN_i), and prey-specific abundance (P_i) were calculated as quantitative measures describing diet composition among the three age groups (Table 1). Multivariate statistics were also used to detect similarities in diet and overlap of prey resources among the three age groups through permutational testing.

Zooplankton (mostly Copepods) was the primary prey type of Age-0 individuals. Trichoptera and Diptera (*Chaoborus spp*.) were also frequently consumed by Age-0 yellow perch, but numerically accounted for less than 10% of the diet. Trichoptera, Diptera, and Bivalvia comprised the majority of prey utilized by Age-1 individuals. The diet composition of Age-2^{+} individuals was more diverse than that of other age groups, possibly reflecting the effects of gape limitation on prey items consumed by Yellow Perch. Of Age-2^{+} individuals, 41% were piscivorous. Trichoptera, Diptera (Chironomidae), and Sialidae were also frequently identified from Age-2^{+} individuals.

Diet and mean annual water temperatures from Cheat Lake likely play a significant role in producing the exceptional growth rates observed from this Yellow Perch population. Further research is being conducted to better estimate growth parameters through model averaging procedures.

Nate Taylor holding a Cheat Lake Yellow Perch.

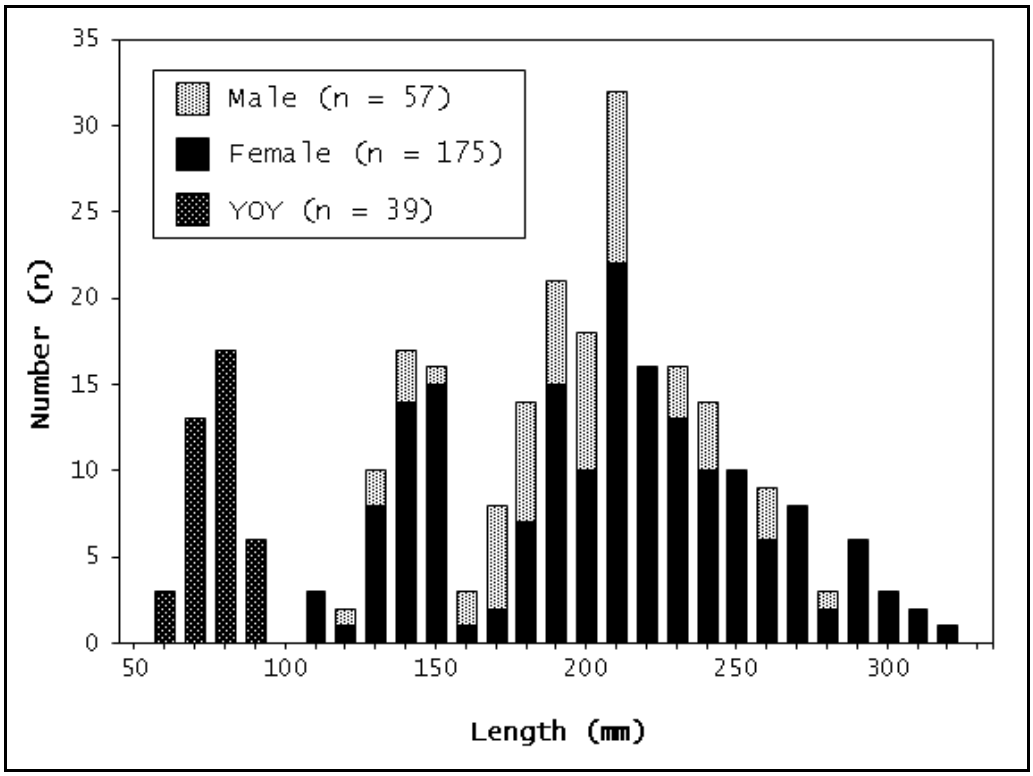


Figure 1. Length-frequency distributions for male, female, and young-of-year (YOY) Yellow Perch from Cheat Lake, West Virginia (n=271). Individuals grouped into 10–mm size categories.

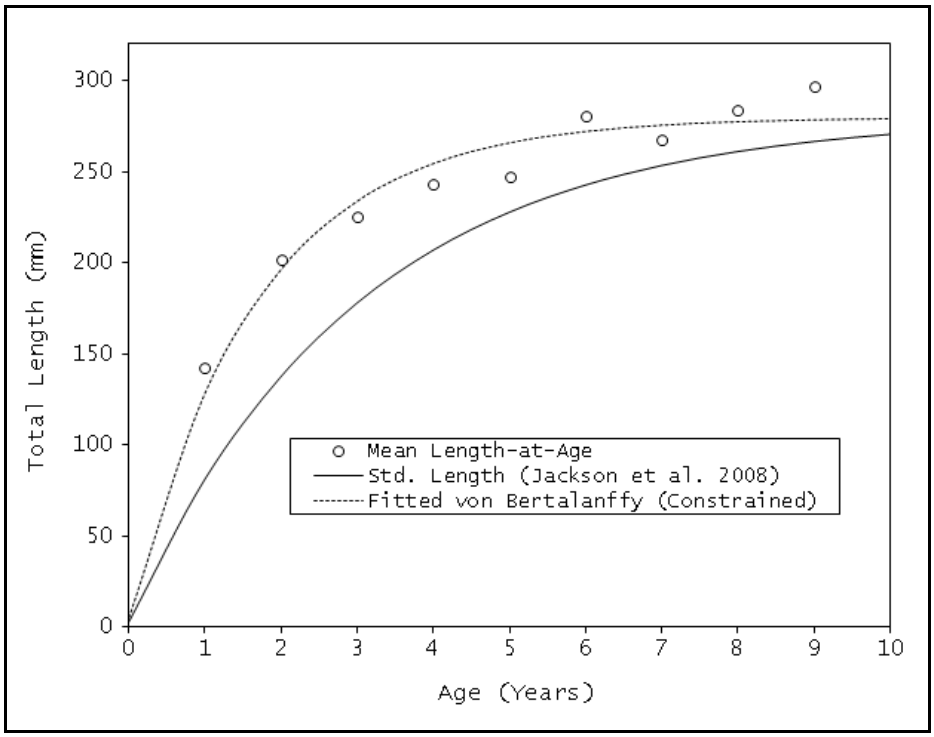


Figure 2. Comparison between growth rates of Cheat Lake individuals and the standard growth model. Standard growth model is representative of typical growth patterns documented from other North American populations.

Table 1. Summary of summer diet composition among age groups of yellow perch (n=190). Data are presented as frequency of occurrence (O_i), mean composition by number (MN_i), and prey-specific abundance (P_i). Yellow perch with empty stomachs and individuals containing unidentifiable prey items were excluded from diet analyses.

Prey Type	O_i			MN_i			P_i		
	Age-0	**Age-1**	**Age-2$^+$**	**Age-0**	**Age-1**	**Age-2$^+$**	**Age-0**	**Age-1**	**Age-2$^+$**
Zooplankton	**100**	**7.32**	**1.54**	**85.25**	**1.39**	**0.92**	**98.99**	**21.62**	**70.00**
Amphipoda	15.79	4.88	-	3.98	0.66	-	25.64	14.29	-
Cladocera	36.84	4.88	0.77	10.02	0.58	0.66	4.90	12.00	85.71
Copepoda	68.42	2.44	-	67.26	0.15	-	99.59	6.25	-
Ostracoda	10.53	-	0.77	3.99	-	0.26	29.41	-	33.33
Annelida	**-**	**-**	**1.54**	**-**	**-**	**0.21**	**-**	**-**	**4.26**
Hirudinea	-	-	0.77	-	-	0.02	-	-	2.33
Oligochaeta	-	-	0.77	-	-	0.19	-	-	25.00
Bivalvia	**-**	**17.07**	**15.38**	**-**	**3.78**	**5.40**	**-**	**10.81**	**24.27**
Corbiculidae	-	9.76	0.77	-	0.57	0.64	-	3.59	83.33
Sphaeriidae	-	12.20	14.62	-	3.21	4.76	-	16.06	23.56
Coleoptera	**-**	**-**	**3.08**	**-**	**-**	**0.92**	**-**	**-**	**25.00**
Crayfishes	**-**	**-**	**10.00**	**-**	**-**	**7.14**	**-**	**-**	**58.14**
Diptera	**10.53**	**60.98**	**36.15**	**5.09**	**20.78**	**12.11**	**45.45**	**20.63**	**22.04**
Chaoborus spp.	10.53	14.63	1.54	4.04	6.36	0.29	36.36	35.45	5.45
Chironomidae	5.26	56.10	34.62	1.05	13.35	11.69	20.00	13.72	21.94
Culicidae	-	4.88	0.77	-	0.67	0.02	-	7.27	2.70
Tipulidae	-	4.88	2.31	-	0.41	0.12	-	4.17	4.82
Ephemeroptera	**5.26**	**-**	**8.46**	**0.44**	**-**	**3.13**	**8.33**	**-**	**8.28**
Fishes	**-**	**19.51**	**41.54**	**-**	**9.05**	**30.65**	**-**	**5.37**	**22.04**
Gastropoda	**-**	**17.07**	**3.08**	**-**	**6.46**	**0.36**	**-**	**15.89**	**7.92**
Physidae	-	7.32	0.77	-	0.42	0.01	-	7.09	1.92
Planorbidae	-	14.63	2.31	-	6.04	0.34	-	9.27	9.39
Lepidoptera	**-**	**-**	**0.77**	**-**	**-**	**0.38**	**-**	**-**	**50.00**
Nematomorpha	**-**	**14.63**	**2.31**	**-**	**1.92**	**2.21**	**-**	**6.15**	**90.00**
Odonata	**5.26**	**14.63**	**9.23**	**0.38**	**12.54**	**3.68**	**7.14**	**72.73**	**21.68**
Anisoptera	-	14.63	7.69	-	12.54	3.43	-	72.73	23.93
Zygoptera	5.26	-	2.31	0.38	-	0.25	7.14	-	9.09
Sialidae	**-**	**12.20**	**24.62**	**-**	**0.68**	**12.57**	**-**	**4.59**	**52.99**
Trichoptera	**21.05**	**63.41**	**33.08**	**8.85**	**43.40**	**20.31**	**46.67**	**74.64**	**73.05**

Major taxonomic groupings of prey items and associated values are in bold font.

DISTRIBUTION AND HABITAT USE OF THE DIAMOND DARTER

Principal Investigator: Stuart Welsh,
Collaborators: Dustin Smith, Nate Taylor
Years Ongoing: 2012
Completed: August 2012
Funding Source: WVDNR

Objectives:

To document distribution and quantify habitat use of Diamond Darters within the Elk River, West Virginia

Results:

The Diamond Darter *Crystallaria cincotta*, a recently-described darter of the Ohio River drainage, is known from the lower 37 km of the Elk River, Kanawha County, WV (Figure 1). The species occurred previously over a wider range within the Ohio River drainage, but populations are thought to be extirpated from Kentucky, Ohio, and Tennessee. The Diamond Darter is a rare species, given that the only extant population is restricted to the Elk River drainage in West Virginia. Further, relatively few individuals have been collected from the Elk River, and observations of the species have been largely restricted to two sections of the river at Mink Shoals and Clendenin, WV. In Nov. 2009, the Diamond Darter was included as a candidate for listing as endangered or threatened. On 26 July 2012, the U.S. Fish and Wildlife Service (USFWS) published a proposed rule in the Federal Register to list the diamond darter and to designate critical habitat under the Endangered Species Act. On 26 July 2013, the USFWS published a final rule in the Federal Register to list the diamond darter as an endangered species.

A diamond darter photographed at night with underwater camera within Elk River, near Elk View, WV during July 2012.

Data are lacking on distribution and habitat use of the Diamond Darter, in part, because the species has been difficult to collect with conventional sampling gears, such as seines and electrofishers. Sampling efforts throughout the lower Elk River drainage have largely been unsuccessful at finding this species, but unsuccessful collections may result from either species absence or non-detection by sampling gear. During 13-14 September 2011, 25 Diamond Darters were observed using a new sampling method which used spotlights to locate individuals at nighttime. The 25 individuals observed during this two-night sampling period exceeded the previous total number of Diamond Darter observations. This spotlight sampling method, given its high success rate, provided a promising new approach to further document the distribution of this species within the lower Elk River. Furthermore, the ability to find Diamond Darters provided an opportunity to quantify habitat used by this species.

We quantified microhabitat use of Diamond Darters based on measurements of water depth, water velocity, and percent substrate composition. Using spotlights at nighttime, we sampled 16 sites within the lower 133 km of Elk River, and observed a total of 82 diamond darters at 10 of 11 sampling sites within the lower 37 km (Figure 2). Glides, located immediately upstream of riffles, were the primary habitats sampled for Diamond Darters, which included relatively shallow depths (< 1 m), moderate to low water velocities (often < 0.5 m•s-1), and a smooth water surface. Microhabitat use (mean ± SE) of Diamond Darters was estimated for depth (0.47 ± 0.02 m), average velocity (0.27 ± 0.01 m•s-1), and bottom velocity (0.15 ± 0.01 m•s-1). Substrate used (mean ± SE) by Diamond Darters was predominantly sand intermixed with lesser amounts of gravel and cobble; % sand (52.1 ± 1.6), % small gravel (12.2 ± 0.78), % large gravel (14.2 ± 0.83), % cobble (19.8 ± 0.96), and % boulder (1.6 ± 0.36). Based on our microhabitat use data, conservation and management efforts for this species should consider preserving glide habitats within Elk River. Spotlighting, a successful sampling method for Diamond Darters, should be considered for study designs of population estimation and long-term monitoring.

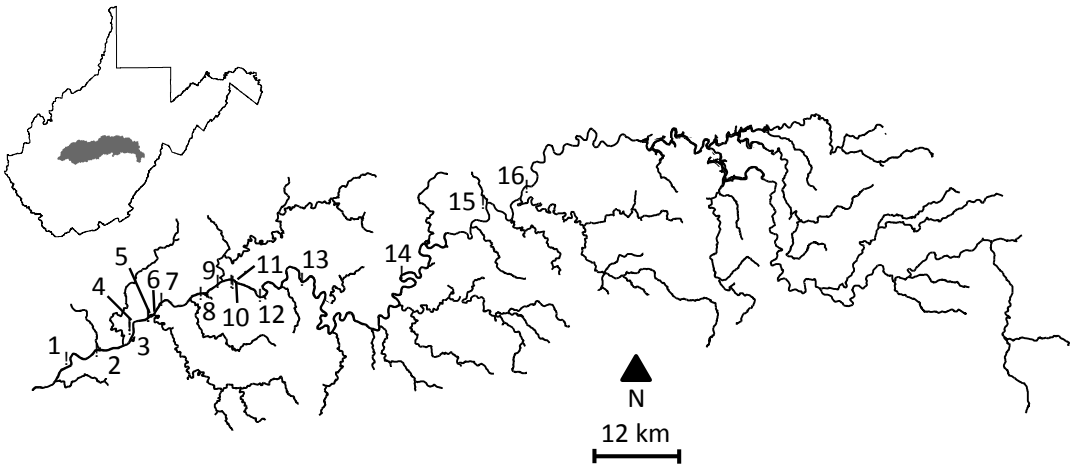


Figure 2. Locations of 16 sites sampled for diamond darters within Elk River.

WILDLIFE

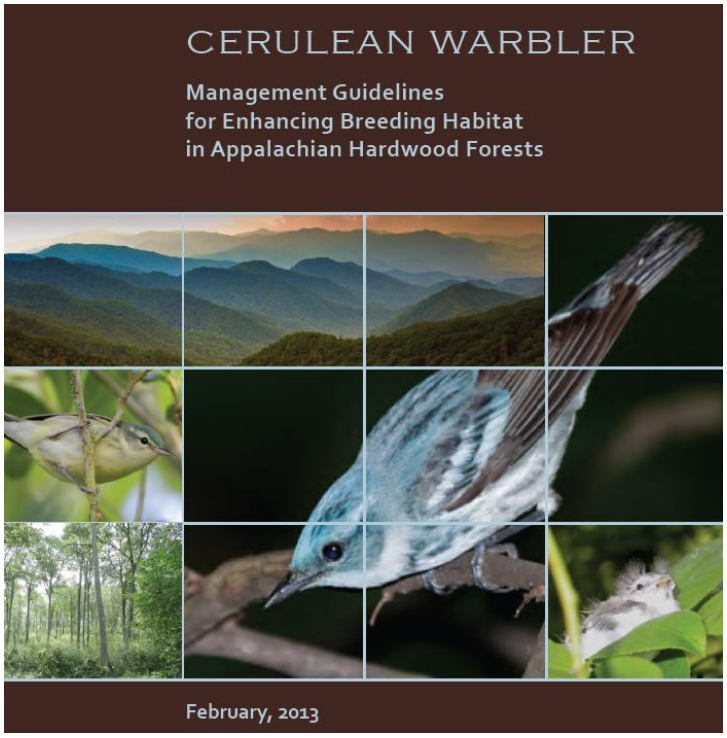
DEVELOPMENT OF TIMBER HARVESTING MANAGEMENT GUIDELINES FOR CERULEAN WARBLERS

Principal Investigator: Petra Bohall Wood
Cooperators: Randy Dettmers, Ben Wigley, David Buehler, Pat Keyser, Jeff Larkin, Amanda Rodewald
Years Ongoing: 2012-2013
Completed: Feb 2013
Funding Source: US Fish and Wildlife Service

Objective:
Develop timber harvesting management guidelines that will benefit Cerulean Warbler and associated species and that can be implemented throughout the Central Appalachian region.

Progress:

We developed timber harvesting management guidelines that benefit Cerulean Warblers and associated species and that can be implemented throughout the central Appalachian region. The guidelines are based primarily on a recently completed 6-year study in the central Appalachian Region evaluating response of Cerulean Warblers and associated avian species to a variety of timber harvesting practices common in the region. We also incorporated other relevant literature and input from cerulean experts and land managers.



The document includes sections addressing:
1. Conservation status of the Cerulean Warbler
2. Cerulean Warbler habitat associations with landscape-scale forest cover and configuration, topography, and stand-level vegetation structure and composition
3. Results from the forest management research project with respect to responses of Cerulean Warbler and associated forest birds to different forest harvest treatments.
4. Forest management recommendations including landscape-scale, stand-scale, and temporal considerations.

These guidelines have been distributed to forest and wildlife managers throughout the Appalachian Region. They are being implemented by state agencies and has led to funding for a follow-up study to evaluate management success. The management guidelines recently were highlighted in the AMJV spring newsletter. The conservation importance of the work summarized in the guidelines was recognized through a Partners in Flight Research Award presented to the research team at the 2013 PIF Conference.

CONTINUING PROJECTS

AQUATIC

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

Student Investigator: Ryan Braham, M. S.
Principal Investigators: Dr. Patricia Mazik
Collaborators: Dr. Vicki Blazer - USGS Leetown Science Center, Jim Hedrick - WVDNR
Years Ongoing: 2013 – 2016
Degree Program: Ph. D.
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. Investigate potential correlations between the cyanotoxins present in the water column with the potential toxin available in both the water column and the periphyton community at selected sites in West Virginia, Maryland, and Virginia; 2. Investigate potential correlations between the phytoestrogens present in the water column with the potential toxin available in both the water column and the periphyton community at selected sites in West Virginia, Maryland, and Virginia; 3. Examine the effects of MC-LR exposure to hepatocyte cultures of smallmouth bass (*Micropterus dolomieu*) and golden redhorse sucker (*Moxostoma erythrurum*) using differential gene expression as an endpoint for disruption; 4. Examine gene expression markers for exposure to MC-LR in wild fish at selected sites in West Virginia, Maryland, and Virginia; 5. Investigate possible accumulation of cyanotoxins in the liver and skin of fishes as a function of the amount of cyanotoxins present in the aquatic environment at selected sites in West Virginia and Maryland; and 6. Investigate the presence of histological endpoints associated with exposure to MC (such as necrosis) in fish at selected sites West Virginia and Maryland.

Progress:

Fifteen sites were identified in West Virginia, Maryland, and Virginia for analysis. Beginning in July, 2013, water and periphyton was collected and will continue to be collected monthly for 24 months. Preliminary samples have been shipped to an independent contractor for preliminary analysis for the presence of cyanotoxins in the water column and periphyton. Subsequent analysis will be performed at the United States Geological Survey, Leetown Science Center (Kearneysville, WV). Water is also being collected for estrogenicity. Fish were collected at selected sites in West Virginia and Maryland at 2 times during 2013. Hepatic tissue was retained for cyanotoxin, gene expression, and histological analysis. All analysis is ongoing.

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
Collaborator: David R. Smith
Years Ongoing: 2007 – 2013
Degree Program: PhD
Expected Completion: December 2013
Funding Source: WVDNR, FirstEnergy

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.

Progress:

The American eel (*Anguilla rostrata*) is a catadromous fish found in rivers and streams along the Atlantic slope from Canada to northern South America. Eels spawn in the Sargasso Sea and young eels migrate up Atlantic Coast rivers and stay in upstream areas for up to 20 years. Dams along coastal rivers can impact eel migrations, both upstream and downstream. When hydroelectric facilities are associated with a dam, eels can suffer high mortality when attempting to pass downstream as adults. Because eels have been found to travel in the deeper sections of the river, they may be more likely to enter the headrace of a hydroelectric facility rather than surface and pass via the spillway. Once in the headrace, eels can continue downstream toward hydroelectric turbines which can result in mortality.

There are five hydroelectric facilities on the Shenandoah River. All facilities are run-of-the-river and are operated by FirstEnergy. Eels are present in the watershed above all five dams and the power company conducts nightly turbine shutdowns through the fall in an attempt to reduce turbine-related mortality on adult eels migrating downstream. Our study determined when and how adult eels pass through the hydroelectric stations.

During the falls of 2007 through 2010 we collected large eels in various locations of the South Fork of the Shenandoah River, all above the Luray Dam. Most eels were collected during boat electrofishing in impounded sections of the river, but several fish were caught using backpack electrofishing in the smaller tributaries and one fish was caught in a hoop-net. We implanted internal radio tags in 151 eels and released them at their capture location within two hours of capture. Ninety-six of the eels tagged were silver phase (mature), 30 were an intermediate phase between immature and mature, and the remaining 25 were large yellow (immature) eels (Photo 1). We monitored eel locations using stationary arrays and manual tracking. The stationary arrays consisted of between fourteen and eighteen radio receiver/antenna combinations that passively listened for radio tags in the vicinity of the Shenandoah, Newport, Luray, Warren, Millville Dams on the Shenandoah River, and at the Little Falls Dam and Dalecarlia Reservoir on the Potomac River.

As of June 2011, 102 eels had made some downstream migration, although not all migrated out of the Shenandoah River. At the time of tagging, most of migrants were silver phase, except for ten that were intermediate phase and seven that were yellow phase. Fifty-nine eels had successfully migrated past Millville dam (lowermost dam on the Shenandoah), and 25 of those had also passed the Little Falls

Pumping Station on the Potomac River. Three eels have been recorded at the Dalecarlia Reservoir of the Washington Aqueduct System.

Eels migrated past dams from early November 2007 through mid June 2008, from August 2008 through June 2009, from September 2009 through January 2010, and September through May 2011. Peak movements occurred from late fall through winter and spring. These peaks appeared to be correlated with spikes in discharge following periods of low flows based on data from the USGS gauging station in Luray, Virginia. All downstream passage occurred when water temperatures were between 3^{o}C and 23^{o}C with most passage (79%) occurring between 4^{o}C and 13^{o}C. Most (85%) eels passed the dams between 5:00 pm and 5:00 am. Most (70%) eels passed over the dam spillway versus passing through the hydro plant during their downstream migration. Radio-tagged eels passed dams during all lunar phases.

Timing and duration of silver eel migration out of the Shenandoah River was highly variable. For eels that moved past more than one dam, the length of time to complete downstream migration was highly variable. Migrating eels moved in the Shenandoah River between 50 to 123 miles after they passed their first dam. Movement between two dams occurred over a range less than one day to 208 days. Sixty-eight percent of the eels completed their downstream migrations in less than 30 days. The fastest movement between dams was an average rate of 74 miles per day (3 mph) for a fish that moved between the Shenandoah and Millville dams in 43 hours. The slowest movement was less than 0.1 miles per day for an eel that took 7 months to move between the Shenandoah and Newport Dams.

We suspect turbine related mortality had occurred at all five dams on the Shenandoah River. At least 28 of 102 migrating eels were suspected of being killed while attempting to pass through hydro stations on the Shenandoah River. Outside the shutdown period, when plants were able to operate turbines 24-hours per day, between 41% and 89% of passage events occurred through turbines. The proportion of eels passing through hydro plants was reduced to between 3% and 17% when plants turned off the turbines at night during the period of September 15 to December 15. Despite the efforts of the shut-down period, suspected turbine mortalities occurred throughout the study period, including during the night-time shutdown periods. Based on the overall mortality rates for each dam, currently there is a cumulative 65% survivorship for large eels that migrate out of the Shenandoah River from above Shenandoah Dam.

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.

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

Student Investigator: Cassidy Hahn, M.S.
Principal Investigators: Dr. Patricia Mazik
Collaborators: Dr. Vicki Blazer, Dr. Luke Iwanowicz - USGS Leetown Science Center
Years Ongoing: 2011 – 2015
Degree Program: Ph D.
Expected Completion: December 2015
Funding Source: U. S. Fish and Wildlife Service (RWO 55, 61)

Objectives:

The primary objective of this study is to evaluate molecular techniques to assist in the assessment of the health of fish populations exposed to emerging environmental contaminants in Great Lakes Areas of Concern. Liver samples will be collected from either brown bullhead or white sucker, and either largemouth bass or smallmouth bass at each sampling site. These samples will then be used to evaluate gene expression changes through the use of Nanostring technology. Peripheral blood samples will also be collected from these individuals for use in population assessment via microsatellite analysis of genomic DNA.

Progress:

Samples have been collected in the fall of 2010, spring of 2011, and spring of 2012 from a total of 8 AOCs throughout the Great Lakes region. Using pooled tissue from collection sites, whole transcriptome databases have been created for each species of interest at the Leetown Science Center (Kearneysville, WV). Initial databases for smallmouth bass and brown bullhead were created by CoFactor Genomics (St. Louis, MO) on an Illumnia. Assembly and annotation of these databases was also provided. An additional smallmouth bass run was then performed at Duke Institute for Genome Science and Policy (Durham, NC) using 454 sequencing on a GS Junior. A hybrid assembly was then completed at the Leetown Science Center to integrate data from both runs into a single database for the smallmouth bass. White sucker transcriptome sequencing was performed at the Leetown Science Center. Genes from this run were identified through BLASTn and BLASTx searches on a local database and assembled using CLC workbench. These databases have been used for gene discovery and the creation of probes for gene expression analysis.

Liver samples from smallmouth bass for use with gene expression analysis have been processed by NanoString Technologies. This method of evaluating gene expression is similar in sensitivity and specificity to real time PCR but allows for the examination of multiple genes and therefore a reduction in cost per gene. Currently, quantitative data from each gene of interest for smallmouth bass liver samples is undergoing statistical analysis. Analysis will include comparison of gene expression levels across all sampling sites, seasonal variation, and variation between sexes. Gene expression data will also be compared with data from other areas of the study including histological and water quality results. Liver samples from brown bullhead, largemouth bass, and white sucker will also be sent to Nanostring Technologies in large batches pending the completion of gene discovery in order to be as cost effective as possible.

Genomic DNA extraction from blood samples is completed for both smallmouth bass and white suckers. The formerly mentioned transcriptome databases were used to create microsatellite markers for smallmouth bass and will be used to create additional markers for white sucker. Testing of microsatellite

markers for use with smallmouth bass is complete and analysis of all smallmouth bass samples should be completed by the end of the year. Testing of microsatellite markers for white sucker is underway and has been successful thus far. Additional markers will need to be created in the coming year as few published markers are available for the species.

INVESTIGATION OF DIET, AGE AND GROWTH OF CHANNEL CATFISH IN CHEAT LAKE, WV

Student Investigator: Corbin Hilling
Principal Investigator: Stuart A. Welsh
Years Ongoing: 2013
Degree Program: MS
Expected completion: May 2015
Funding Source: West Virginia Division of Natural Resources, FirstEnergy

Objectives:

To examine the age/size structure, growth rates and food habits of channel catfish *Ictalurus punctatus* in Cheat Lake, WV

Progress:

Cheat Lake was created as a hydropower reservoir in 1926. Over time, mining operations throughout the Cheat River watershed caused high levels of acid mine drainage to accumulate in Cheat Lake. After decades of poor water quality, the reservoir as a fishery has drastically improved as a result of mitigation efforts and regulations controlling operation of the Lake Lynn dam. Currently, 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 managing this fishery.

We plan to capture Channel Catfish for this study using low-frequency boat electrofishing and baited hoop-nets. Channel Catfish will be measured (mm), weighed (g), and sexed. Pectoral spines will be removed for age analysis and a subsample of fish will be sacrificed for age analysis validation using otoliths. Stomach contents will also be removed using gastric lavage and will be taken to laboratory for identification of diet items. Data on sex, age, and size structure will be used to estimate growth curves and examine growth rates.

Corbin Hilling holding a channel catfish

EXAMINING THE EFFECTS OF LAND USE AND REPRODUCTIVE ENDOCRINE DISRUPTING CHEMICALS ON PHYSIOLOGICAL AND REPRODUCTIVE CHANGES IN SMALLMOUTH BASS AND GOLDEN REDHORSE IN THE CHESAPEAKE BAY DRAINAGE

Student Investigator: Lauren Kesslak, M.S.
Principal Investigator: Dr. Patricia Mazik
Collaborators: Dr. Vicki Blazer, Dr. Luke Iwanowicz – USGS Leetown Science Center
Years Ongoing: 2012-2015
Degree Program: Ph. D
Expected Completion: December 2015
Funding Source: U.S. Geological Survey (RWO 60)

Objectives:

The main objective of this research is to examine the effects of land use and reproductive endocrine disrupting chemicals on physiological and reproductive changes in smallmouth bass and golden redhorse. This will be assessed by examining the effects of reproductive endocrine disruption between the two species by comparing intersex occurrence and severity using histopathology, hepatocyte vitellogenin assays for vitellogenin expression, and a gonad explant assay. Samples from the hepatocyte vitellogenin assay will be analyzed using real time PCR methods for detection of vitellogenin and estrogen receptor alpha, as well as an ELISA for detection of vitellogenin proteins in supernatant.

Progress:

Tissue and blood samples were taken from both smallmouth bass and redhorse sucker from the Chesapeake drainage system for a vitellogenin plasma ELISA and intersex analysis in the spring of 2013. Smallmouth bass were brought in from the South Branch of the Potomac River and acclimated in ponds at the Leetown Science Center in Kearneysville, WV for validation of a hepatocyte vitellogenin assay. Optimal culture conditions, viability, and cell density were determined through a kinetics experiment featuring controls for culture media, vehicle, and high, medium, and low concentrations of estradiol. Exposures occurred over a 96 hour period and collected every 24 hours. Samples were analyzed using real time PCR for vitellogenin, estrogen receptor alpha, and housekeeping gene elongation factor alpha, as well as an ELISA for vitellogenin protein. Methods for using the plasma vitellogenin ELISA on supernatant from the assay were developed to compare results and assay sensitivity to PCR methods.

A second kinetics experiment was conducted over a 48 hour incubation period with cells exposed to an estradiol standard as well as high and low concentrations of glyphosate, genistein, and metolachlor as well as vehicle and culture medium controls. This experiment will allow me to test the use of the assay to detect estrogenicity of regionally relevant chemicals and eventually extractions from water sources as well as examine species differences in vitellogenin and estrogen receptor expression within the hepatocyte assays. Estrogen receptor antagonists ICI 182 780 and Tamoxifen were used to explore estrogen receptor activation and deactivation in the presence of an estradiol standard. Analysis of initial assay samples and assay validations will be complete by the end of summer 2013.

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

Student Investigator: Dustin M. Smith, M.S.
Principal Investigator: Stuart A. Welsh
Years Ongoing: 2011 – 2013
Degree Program: PhD
Expected completion: Dec 2015
Funding Source: WVDNR, FirstEnergy

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 will focus on Cheat Lake (main lake and embayments).

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 Cheat 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: the Cheat 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).

Currently, objectives 1-8 are ongoing, and objective 9 is complete. Quarterly progress reports are provided to the funding agencies. In summary, water samples have been collected bi-monthly from Cheat Lake tailwater, Grassy Run, and Cheat River downstream of Grassy Run. Water quality characteristics have been measured at 1-meter profiles at three water quality stations within Cheat Lake monthly. Electrofishing surveys and gill net surveys have been conducted at all study stations within both the lake and downstream of the dam. Benthic macroinvertebrates have also been collected downstream of Cheat Lake in the tailwater section. Also, a bathymetric map of Cheat Lake has been created using sonar, GPS technology, and GIS-based interpolation techniques. Currently, we are working on creating an aquatic vegetation map of Cheat Lake to document the distribution and abundance of aquatic plant species within the lake.

The water quality of the Cheat Lake tailwaters and Cheat River have been monitored bi-monthly 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 6.7, average dissolved oxygen of 8.3 mg/l, and average specific conductivity of 101 µs/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 2.9 and conductivity of 1128 µs/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 5.4 and an average conductivity of 253 µs/cm.

Physical and chemical water quality profiles have been conducted monthly (except during periods of ice cover) from 2011-2013. The primary focus of these limnological profiles is 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. Additionally, limnological profiles will be used to help determine summer depth usage by tagged walleyes (which have temperature coded tags) and to look for movements related to water quality changes. 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 been ongoing since the initiation of lake profiles by WVDNR in 2005. However, in 2012 and 2013, 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 has been satisfactory, remaining above 6.0 the majority of the time. Stratification of water temperature and dissolved oxygen does occur in lower Cheat Lake from approximately June-September. In general, the upper 6-10 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 thus far in 2013, Cheat Lake has not experienced the severity of stratification that normally occurs during summer months.

Night boat electrofishing and gill netting have been conducted during May 2011, October 2011, May 2012, October 2012, and May 2013 in Cheat Lake. The primary focus of these surveys is to monitor the health of the fish communities of Cheat Lake. We are also planning to analyze trends in fish species abundances over time to determine if significant changes have occurred to the fish community and if so, what environmental factors appear to have effected these changes. In total, 522 fishes have been captured with gill nets, while 3,547 have been collected using electrofishing. The upper lake, which retains many riverine characteristics, has consistently produced a greater abundance of fish compared to both the lower lake and embayment areas. The embayment areas have produced the lowest fish abundances. Largemouth bass and spotted bass have been most abundant in embayment areas, while smallmouth bass are more abundant in the upper lake. Green sunfish, bluegill, and pumpkinseed are most abundant in the lower lake. Walleye, yellow perch, white bass, and channel catfish are typically most abundant in the upper lake. Smaller forage species also differ dependent on lake area. Mimic and emerald shiners are very abundant in the upper lake and fairly abundant in the lower lake, but are uncommon in embayments. Conversely, logperch and brook silversides are most abundant in the embayments and lower lake. Gizzard shad are typically most abundant in the lower and upper lake, while golden redhorse are most abundant in the upper lake.

Night boat electrofishing, tow barge (pram) day electrofishing, and gill netting have been conducted during July, September (pram only), and October 2011 in the tailwaters and river downstream of Cheat Lake. In total, 967 fishes have been captured with boat electrofishing, 113 with gill nets, and 745 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. There were also several species of game fishes collected in both the tailwaters and river section including smallmouth bass, spotted bass, largemouth bass, channel catfish, walleye, sauger, yellow perch, and white

bass. 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 2011. 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 are 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-spinnning caddisflies) account for most of the macroinvertebrates at these downstream stations.

Research on adult walleye movement was started in early December 2011. We have successfully implanted 45 adult walleyes (33 males; 12 females) with acoustic transmitters. Each transmitter also relays the water temperature an implanted fish is occupying at each location. Currently, 38 tagged walleye are active, with 7 of the fish currently missing. Fish locations have been determined using both submerged, stationary receivers and active tracking. Walleyes were captured primarily with boat electrofishing. While the entire lake area was sampled in attempts to capture adult fish large enough to tag (> 16 inches), the majority of our tagged fish (77 %) were captured from the middle lake area. Twenty-eight of the 45 walleye were implanted at the Cheat Lake Park and released in the main lake adjacent to Morgans Run Embayment. Tagged fish have displayed strong site fidelity, with all active fish released at Cheat Lake Park returning to near their capture location upstream (5-7 miles) within a few weeks. During winter months, tagged fish have normally remained near their original capture locations until late February (2012)/mid-March (2013) when fish start to make upstream movements, likely in order to reach spawning habitat. By early March (2012) to early April (2013), most tagged fish have moved to the head of Cheat Lake to spawn. Preliminary analysis of the data suggests that upstream movements correspond to a combination of increased water temperature, an increase in incoming river flow, and increases in lake elevation. A minimum of 13 tagged fish have proceeded to move upstream of the first riffle and into the Cheat River, possibly to spawn in the next riffle/run area upstream of the lake approximately 1 mile. Several tagged fish continue to use this area upstream of the first riffle during the spring and summer months. Most male fish remain near the spawning location until June when downstream movement typically increases. Most females move back towards the main lake shortly after spawning has likely finished. Also, during non-spawning periods, increases in incoming river flow and water temperature appear to trigger upstream movements of many tagged fish. There is currently some evidence to suggest that 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. Currently (Summer 2013), 14 of the 38 active fish have commonly used the upper lake area, while the remaining 24 active fish have usually stayed in the lower/middle lake area.

Walleye population monitoring and stocking assessment surveys have been conducted in Cheat Lake during March/April 2012, November 2012, and March/April 2013. Gill nets are 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 and Spring 2013. However, it is most likely that the walleye population was greatly underrepresented during our surveys. Most adult walleye were likely upstream of our netting areas near the head of the lake. Information from our acoustic tagged walleyes indicated that most fish were occupying this area in preparation for spawning during both 2012 and 2013. Supporting this assumption, our CPUE during Fall 2012 was much higher with 1.5 fish per net night, which equals the highest CPUE has been since the initiation of walleye stocking assessment surveys in 2005.

Additional walleye studies that will be ongoing in Fall 2013 are a laboratory study examining competitive interactions between juvenile walleye, yellow perch, and largemouth bass and an age/growth study of walleye in Cheat Lake. The laboratory study will aim to determine if juvenile walleyes are at a competitive disadvantage to yellow perch and largemouth bass. Additionally, we will seek to determine if the presence of these species results in prey switching behavior by juvenile walleye. The age and growth study will examine the growth of walleye in Cheat Lake and if certain environmental correlates (lake area, yearly water temperatures, lake hydrology, etc.) have an impact on yearly growth.

Currently, an aquatic vegetation map of Cheat Lake is being created. Areas of Cheat Lake that harbor aquatic vegetation are being visually assessed to determine species composition and relative abundance. This information will be incorporated into a lakewide map using GIS, and will depict species presence/abundance information. 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 is being used to help determine habitat preferences of walleye and also areas that are vulnerable to water level fluctuations.

Dustin Smith holding a 28-inch Cheat Lake walleye.

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

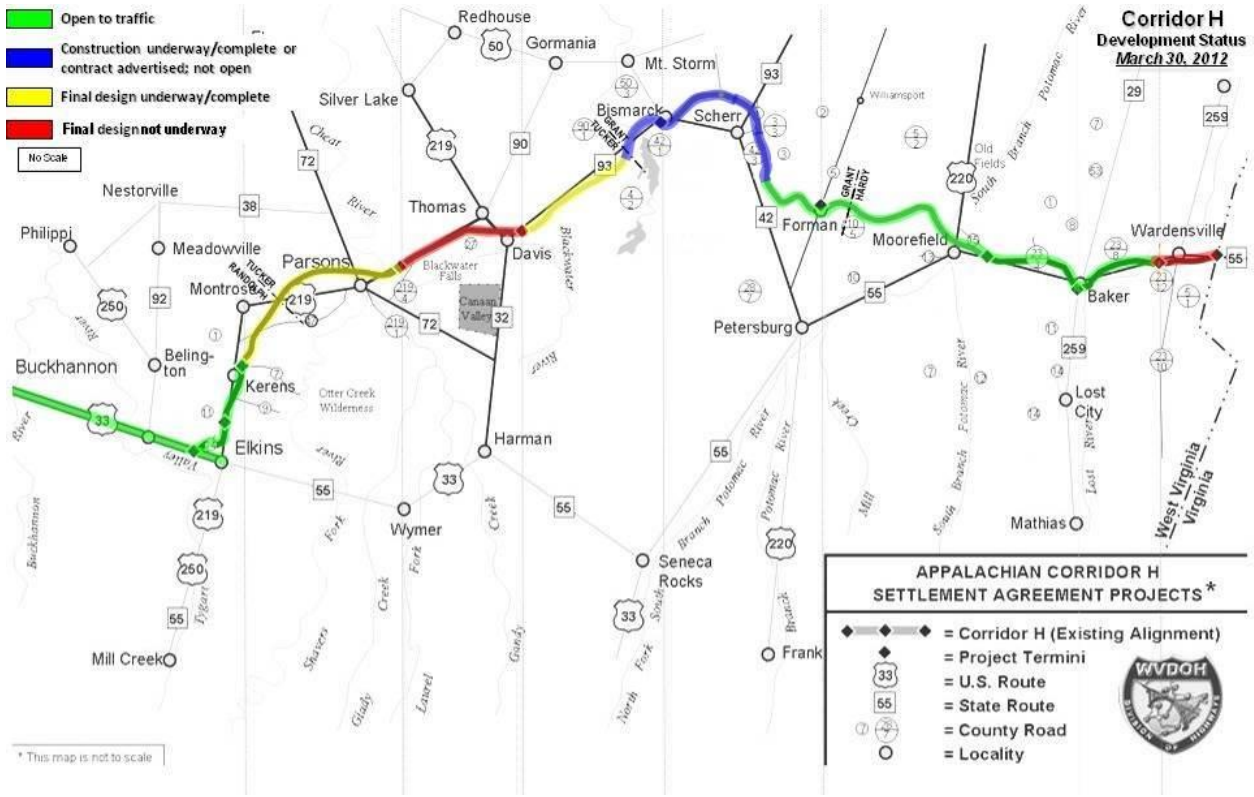
Principal Investigators: Stuart Welsh
Collabortaors: Jim Anderson, and Lara Hedrick
Years Ongoing: 2002-2013
Expected Completion: May 2014
Funding Sources: West Virginia Division of Highways

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: Baker Run/Long Lick and tributaries, Beaver Creek, Big Run, Lost River, Pheasants Run, Patterson Creek, tributaries of Elk Lick, Middle Fork of Patterson, Walnut Bottom Run, and Waites Run. In 2005 two new watersheds were added to the study as part of the Parsons Avoidance Study - Mill Run and Big Run, located east of Parsons, WV.



Corridor H alignment: http://www.wvcorridorh.com/engineer/construct_map0312.html

In fall 2012 and spring 2013, benthic macroinvertebrate samples were collected in three areas, Patterson Creek, Walnut Bottom, and Beaver Creek. Sample collection in the Big Run and Mill Run watersheds have been suspended at this time. Samples in the three areas were collected on the main stem of Patterson Creek, Middle Fork of Patterson Creek, Elklick Run, tributaries of Elklick Run, the main stem and tributaries of Walnut Bottom, the main stem and tributaries of Beaver Creek, and the main stem and tributaries of Mill Run and Big Run. Benthic macroinvertebrate samples from 2012-2013 are currently being picked and identified in the laboratory.

Work to be completed in 2013-2014 includes identification of benthic macroinvertebrate samples collected during 2011-2012. Selected metrics associated with the West Virginia Stream Condition Index (WV-SCI) will be calculated for these benthic macroinvertebrate samples. Benthic macroinvertebrate collections will be made in the fall of 2013 and the spring of 2014 at all sites. An annual report will be submitted to the WVDOH.

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

Principal Investigator: Stuart A. Welsh
Years Ongoing: 2003 – 2013
Expected Completion: 2014
Funding Source: WVDNR, FirstEnergy

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 FirstEnergy. An eel ladder was installed in 2003 on Millville Dam within the lower Shenandoah River (Figure 1). This ladder has passed over 20,000 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.

Based on data collected at the Millville ladder, upstream migrant eels average 30 cm in length (primarily ranging from 19 cm to 50 cm), and range from 3 to 10 years in age. Eels often use the ladder during time periods near the new moon or periods of increasing river discharges (Figure 2). Eels are nocturnal and primarily use the ladder at night. The largest numbers of eels have passed the Millville ladder during high river discharges of spring and fall, and relatively few eels move upstream during low flow periods of summer.

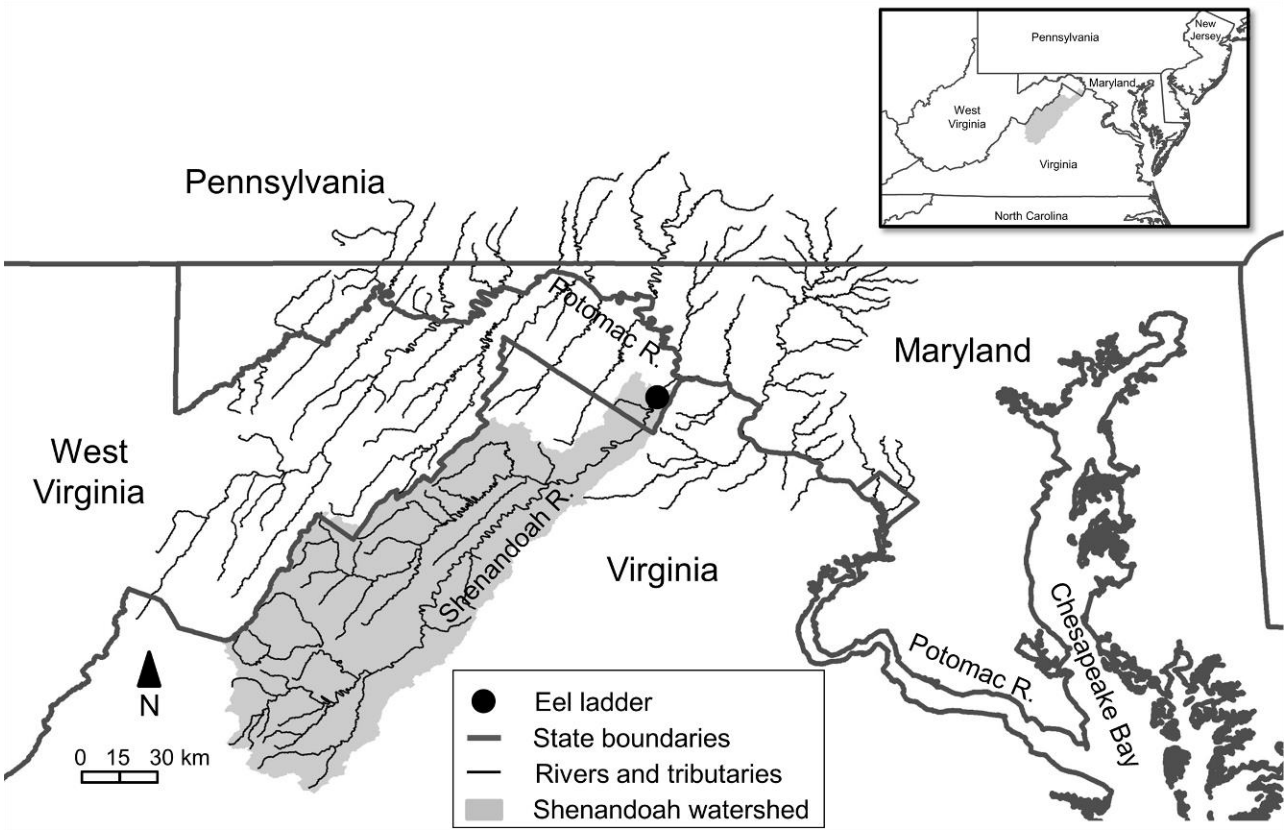


Figure 1. Map of the Potomac River drainage and the location of the Millville Dam eel ladder on the Shenandoah River.

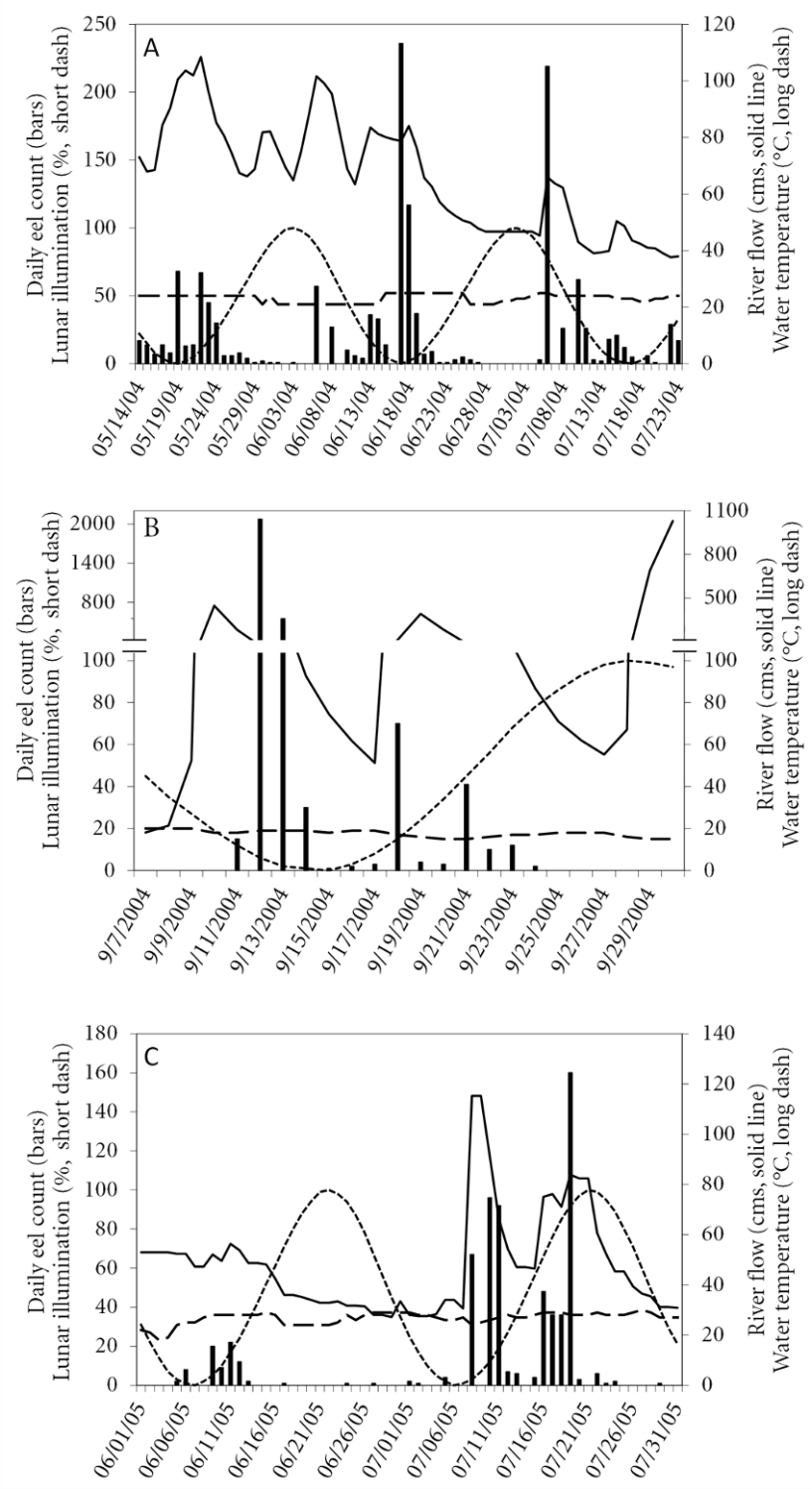


Figure 2. Daily counts of eels at the Millville Dam eel ladder and associated environmental variables (lunar illumination, river discharge, and water temperature) for three sampling periods: (A) 14 May – 23 July 2004, (B) 7 Sept – 30 Sept 2004, and (C) 1 June – 31 July 2005.

A FACTORIAL STUDY TO INVESTIGATE THE EFFECTS OF SWIMMING SPEED AND DISSOLVED OXYGEN CONCENTRATION ON RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND ATLANTIC SALMON (*SALMO SALAR)* PERFORMANCE, PRODUCT QUALITY, AND WELFARE

Student Investigator: Tom Waldrop
Principal Investigators: Dr. Patricia Mazik
Collaborators: Dr. Chris Good, Conservation Fund Freshwater Institute
Years Ongoing: 2009-2014
Degree Program: MS
Expected Completion: December 2014
Funding Source: Self Funded (National Conservation Training Center student)

Objectives:

The objectives of this study are to determine the impacts, both alone and in combination, of swimming speed and dissolved oxygen concentration on the performance (growth, survival, and feed conversion), product quality (proximate analysis, fillet and cook yield, and texture) and welfare (fin condition and survival) of Rainbow trout, *Oncorhynchus mykiss*, and Atlantic salmon, *Salmo salar*.

Hypothesis:

Reduced dissolved oxygen and low swimming speeds will be associated with reduced performance, product quality, and welfare of these two salmonid species.

Progress:

The Rainbow trout portion of the experiment concluded in January 2010. The study was carried out over 225 days, and by end-of-study rainbow trout from all treatments weighed approximately 838 to 1049 grams. Analysis of variance was used to assess the effects of swimming speed and dissolved oxygen on fish performance outcomes. No significant ($p<0.05$) interactions were detected between the two treatments. However, Dissolved oxygen had a significant influence on final weight. In addition, swimming speed had a significant influence on cardiosomatic index, which increased with exercise. There were no significant differences between mortalities, length, feed conversion rate, condition factor, or fillet yields among treatment groups.

The Atlantic salmon portion of the experiment concluded in April 2011. The study lasted approximately 253 days and by the end of study fish Atlantic salmon from all treatments weighed approximately 292 to 343 grams. Analysis of variance was used to assess the effects of swimming speed and dissolved oxygen on fish performance outcomes. No significant ($p<0.05$) interactions were detected between the two treatments, however, dissolved oxygen as well as swimming speed both had a significant influence on final fish weight. Data is still be statistically analyzed on the effects of swimming speed and dissolved oxygen on product quality, fish welfare, and performance parameters such as fin condition and survival.

WILDLIFE

ECOLOGY AND MANAGEMENT OF GOLDEN-WINGED WARBLERS ON HIGH-ELEVATION PASTURELANDS ON THE MONONGAHELA NATIONAL FOREST, WEST VIRGINIA

Student Investigator: Kyle Aldinger
Principal Investigator: Petra Bohall Wood
Cooperators: Rich Bailey, Cathy Johnson, Rob Tallman
Years Ongoing: 2008-Present
Degree Program: PhD
Expected Completion: May 2014
Funding Sources: US Natural Resources Conservation Service, US Geological Survey, US Fish and Wildlife Service, US Forest Service, WV Division of Natural Resources, National Fish and Wildlife Foundation,

Objectives:

1. Evaluate the response of Golden-winged Warblers to habitat management.
2. Quantify site-fidelity, survival, and movements of banded males, females, and nestlings.
3. Monitor broad-scale Golden-winged Warbler and associated bird species populations on pastures with regards to vegetative characteristics and grazing intensity.

Progress:

Golden-winged Warblers (*Vermivora chrysoptera*, GWWA) are one of the most rapidly declining songbird species in North America, with especially dramatic declines in West Virginia (1966-2011 Breeding Bird Survey annual average decline -9.2% year^{-1}, n = 44 routes). Key limiting factors for this species may include habitat availability on breeding and wintering grounds, migratory obstacles, Brown-headed Cowbird (*Molothrus ater*) brood parasitism, climate change, and hybridization, genetic introgression, and competition with the closely-related Blue-winged Warbler (*V. cyanoptera*, BWWA). Complicating conservation efforts, GWWA and BWWA produce fertile hybrids, the Brewster's Warbler (BRWA) and the rarer Lawrence's Warbler (LAWA). Our research primarily has focused on active and abandoned pasturelands on and around the Monongahela National Forest in Pocahontas and Randolph counties, West Virginia. The West Virginia study is part of two larger range-wide investigations of GWWA breeding ecology. Range-wide, GWWA breeding territories consistently contain a mixture of grasses, herbaceous vegetation, raspberry/blackberry (*Rubus spp*.), shrubs, saplings, and a few trees in a forested landscape.

We located and monitored 102 GWWA nests during 2008-2013 that reached at least egg-laying. Average annual Mayfield nest survival was 30.5 – 61.2% (Fig. 1). Complete clutch size (4.6 ± 0.1 SE) and young fledged per nest attempt (2.0 ± 0.2) were similar to other studies. We found 18 nests of mixed species parentage with GWWA x BRWA hybrid pairings most common (Table 1). BWWA began to colonize the pastures near Elkins, Randolph County in 2010. The first two ♂ BWWA x ♀ BWWA nests on our sites were discovered in 2012 and 2013.

Table 1. Parental phenotypes of 142 Vermivora nests discovered during 2008-2013.

	♀ GWWA	♀ BWWA	♀ BRWA	♀ LAWA
♂ GWWA	122	2	5	0
♂ BWWA	1	2	0	0
♂ BRWA	8	1	0	1

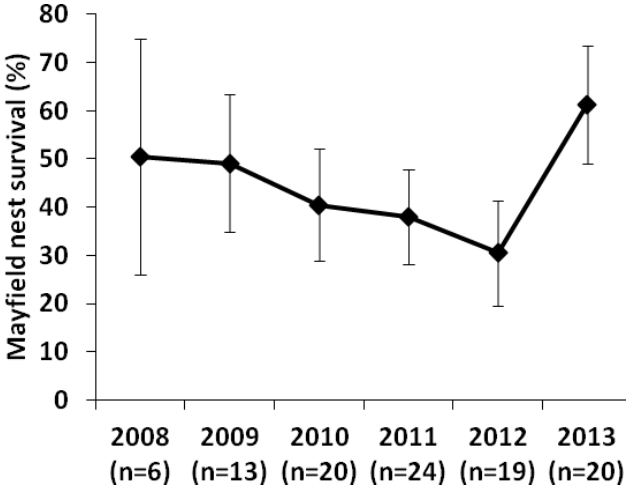


Figure 1. Annual average Golden-winged Warbler nest survival.

Brewster's Warbler hybrid male (Kyle Aldinger)

The Elkins GWWA population experienced precipitous declines during 2008-2013, while the number of BWWA and hybrid individuals increased, although they occur at relatively low densities (Fig. 2a). The population of GWWA, BWWA, and hybrids showed less variation during 2009-2013 at the Marlinton study area (Fig. 2b).

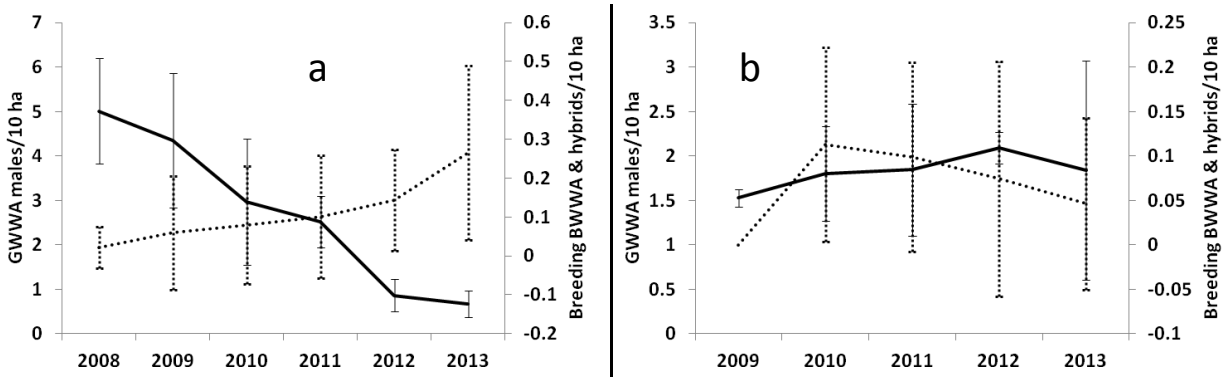


Figure 2. Population trends of territorial GWWA males (solid lines) and breeding BWWA and hybrid individuals (dotted lines) at the Elkins (a) and Marlinton (b) study areas.

We mapped 219 territories of GWWA (89%), BWWA (6%), and BRWA (5%) males during 2008-2013. Average territory sizes for GWWA, BWWA, and BRWA were 1.5 ± 0.1 ha, 3.3 ± 1.2 ha, and 3.0 ± 1.1 ha, respectively. Compared to their spot-mapped territories, telemetry-delineated territories for 8 male GWWA were significantly larger, overlapped more surrounding territories, had greater canopy tree abundance, and contained more mature forest. Current conservation plans for Golden-winged Warblers that are based on habitat characteristics measured within spot-mapped territories and at the landscape-scale using GIS are missing recommendations for an intermediate spatial scale.

The probability of a GWWA returning to the study area decreased by about 50% each year after initial capture and banding (Fig. 3). Attaching transmitters did not appear to negatively affect return rates; 5 of 8 males equipped with transmitters in 2012 returned in 2013.

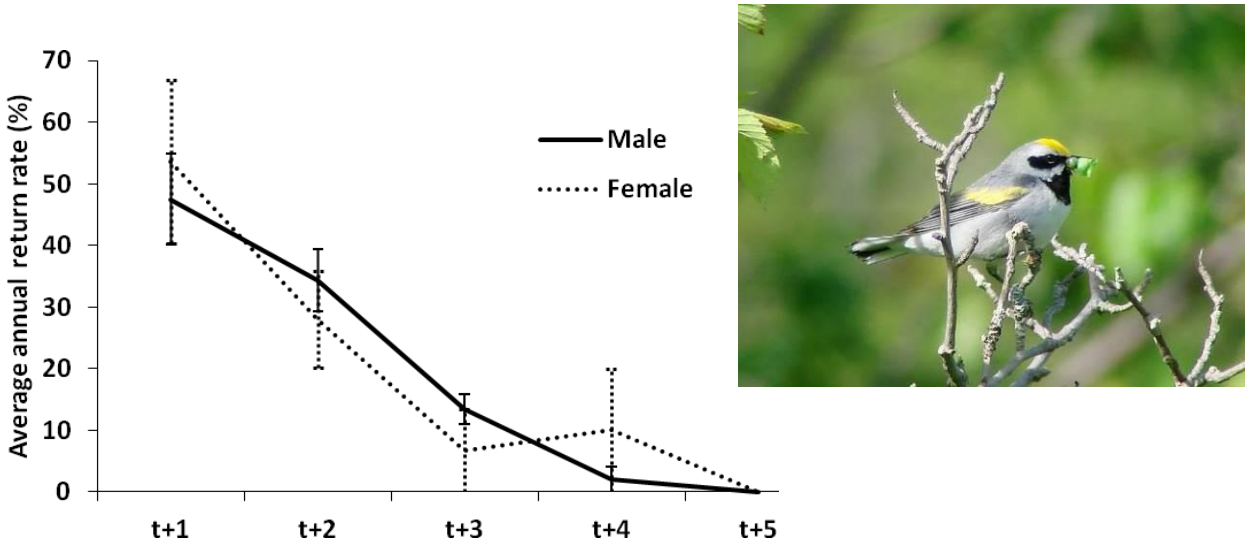


Figure 3. Average annual return rates during 2008-2013 for male and female Golden-winged Warblers following the initial banding year (t).

We conducted point counts at 157 unique locations during 2008-2013 and recorded a total of 19,715 detections and 122 different species. GWWA, BWWA, BRWA, and LAWA occurred at 40.8%, 8.9%, 7.0%, and 0.6% of locations, respectively. Chestnut-sided Warbler (*Setophaga pensylvanica*), Eastern Towhee (*Pipilo erythrophthalmus*), Indigo Bunting (*Passerina cyanea*) and Red-eyed Vireo (*Vireo olivaceus*) were the only species to co-occur at all of the locations where GWWA were present.

Our results and those from the range-wide study were used to develop the GWWA Conservation Plan and regional habitat management plans. Our current work is evaluating the effectiveness of the NRCS Working Lands for Wildlife program in providing quality habitat for GWWA.

IMPACTS OF MOUNTAINTOP MINING ON TERRESTRIAL ECOSYSTEM INTEGRITY: IDENTIFYING LANDSCAPE THRESHOLDS FOR AVIAN SPECIES

Postdoctoral Research Associate: Doug Becker
Principal Investigators: Petra Bohall Wood and Michael Strager
Cooperator: Christine Mazzarella
Years Ongoing: 2012-2013
Expected Completion: January 2014
Funding Source: Environmental Protection Agency

Objective:

Identify avian community abundance landscape thresholds in response to changing landcover and landscape metrics from mountaintop mining.

Progress:

Largely missing from the environmental assessments of mountaintop mining are its effects on terrestrial resources. Environmental assessments discuss the numerous forest types found in the region, but do not articulate likely effects of mountaintop mining on the forest types themselves and do not rigorously quantify the effects of mountaintop mining on the numerous terrestrial species. The specific goal of our research is to identify how changes in landcover affect avian species abundance in the mountaintop removal/valley fill region (MTRVF) in southwestern West Virginia and northeastern Kentucky.

Using existing datasets, we conducted a preliminary *Threshold Indicator Taxa ANalysis* (*TITAN*). TITAN is a relatively novel approach to threshold analysis which has been used effectively in aquatic ecosystems. However, due to limitations of the available data the thresholds generated were not precise. More broadly sampled metrics representative of the complete natural range of available habitats on the landscape were needed. Therefore, we initiated a study to identify precise thresholds in the amount of mature forest and mining habitat that could be used to inform assessments for mountaintop mining.

We conducted point count surveys in May-July of 2012 and 2013 (353 in West Virginia and 354 in Kentucky) using standard avian sampling protocols as well as acquired data for 142 points completed by the WVDNR in 2012. We are working on acquiring data for ~300 additional points in Kentucky from 2013. Specific mines sampled were chosen to maximize the geographic distribution within this region given limitations with access to some locations and to maximize sampling of internal forest patches (Fig. 1) within the greater mineland matrix allowing us to sample the full range of percent mature forest cover available in the landscape.

Figure 1. Forest patch on a mountaintop mine in Kentucky.

The avian count data collected in 2012 and 2013 were combined with the data used in the preliminary analysis after performing removal models to diagnose any differences in detectability among different data sets. This more comprehensive dataset (excluding the yet to be received Kentucky points) was analyzed with *TITAN* threshold analysis to evaluate avian species response to landscape habitat metrics derived using feature extraction in ArcMap. The landcover around each point was classified into five groups (mature forest, mining barren, mining shrub/grass, other barren, other grass/shrub). We calculated 6 metrics (forest edge density, 100 - % core mature forest, 100 - % mature forest, % total mineland, % total barren, % total grassland and shrubland) within a 1-km radius of each count location and determined avian thresholds for each metric separately. We used a random forest modeling technique to test each metric's relative importance for each significant species, ranking the metrics from 1 to 6, and then calculating the average importance rank across all species.

Community (total) thresholds for Z- species (those with a negative response) tended to be near the lower end of each metric's distribution except for core mature forest loss, while, Z+ species (those with a positive response) were located within the middle and upper ends of the distributions except for mature forest loss (Table 1). A preliminary analysis of mature forest loss thresholds based on habitat groupings reveals increasing thresholds for Z- species as taxa transition from forest interior to early successional species (Table 1). In contrast, interior edge Z+ species had lower thresholds than the other two groups, responding more immediately, most likely to the opening of the forest and the creation of edges.

Table 1. Community relative abundance thresholds for Z- and Z+ species for all species (Total), forest interior species (FI), interior edge species (IE), and early successional species (ES). A dash (-) indicates analyses that have not yet been completed.

	Z- Species				Z+ Species			
Metric	Total	FI	IE	ES	Total	FI	IE	ES
100 - % Mature Forest	4.7	3.9	10.4	18.1	0.0	48.7	17.4	52.0
100 - % Core Mature Forest	97.9	-	-	-	88.1	-	-	-
Forest Edge Density (m/ha)	25.0	-	-	-	278.8	-	-	-
% Mineland	5.3	-	-	-	77.0	-	-	-
% Total Barren	0.3	-	-	-	20.7	-	-	-
% Total Grassland/Shrubland	0.2	-	-	-	47.9	-	-	-

These community threshold patterns are supported by a review of individual thresholds for significant species (Fig. 2). Z- species exhibited an almost immediate negative response, while Z+ species were variable, likely due to the differing responses of interior edge and early successional species to different degrees of habitat change. The average random forest MSE importance ranks emphasized mature forest loss and total shrubland across all, Z-, and Z+ species with less emphasis on total mineland and total barren (Fig. 3) except for an increased emphasis for total mineland for Z+ species.

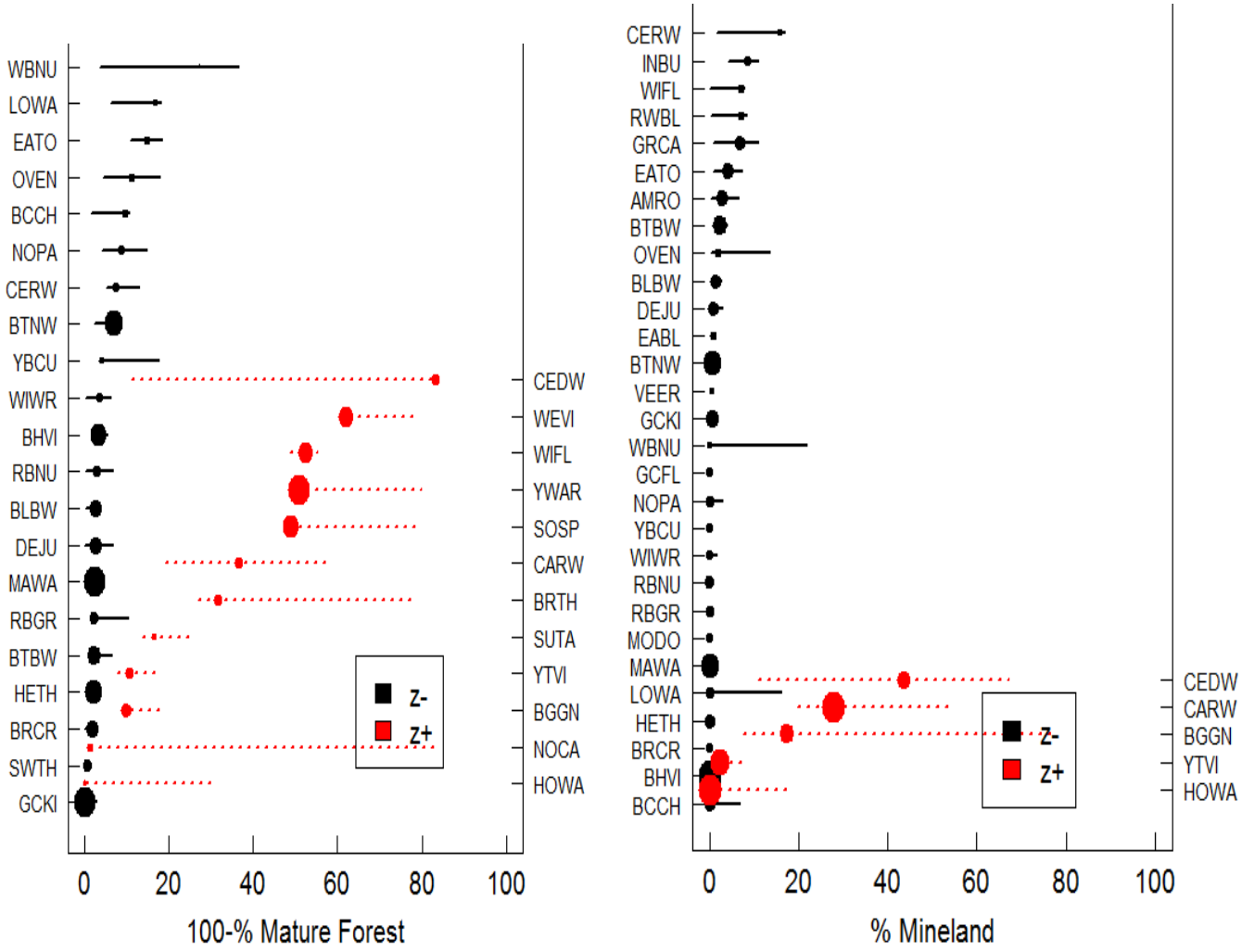


Figure 2. Preliminary TITAN output indicating relative abundance thresholds and 95% confidence intervals for significant avian species. Z+ species had a positive response to exceeding their threshold, while Z- species had a negative response. Species with larger circles are more significant.

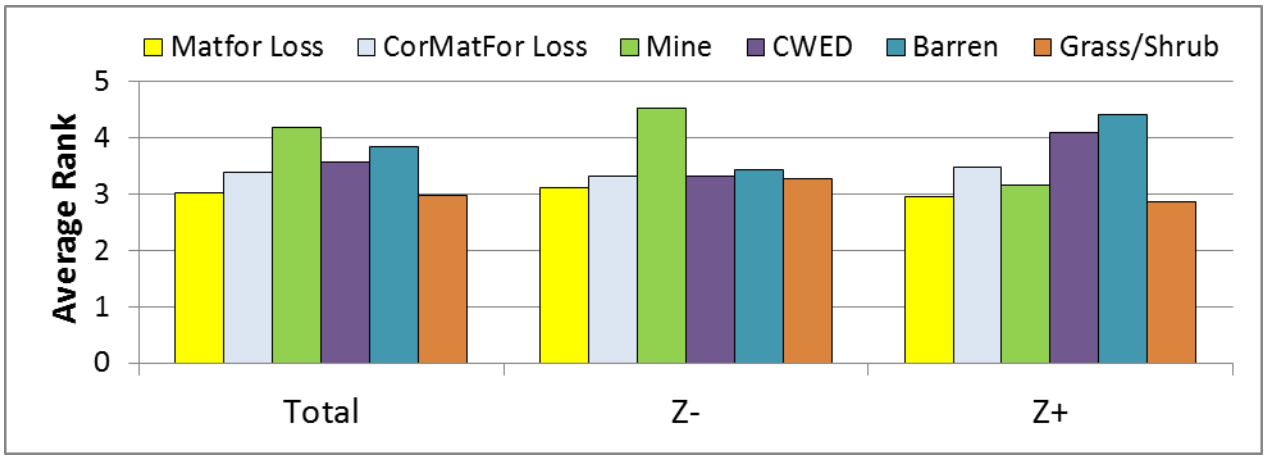


Figure 3. Average random forest importance ranks based on 5,000 random regression trees for each significant TITAN species for all species (Total), negatively (Z-), and positively (Z+) responding species.

IMPACT OF MARCELLUS SHALE DEVELOPMENT ON SHRUBLAND SONGBIRD NEST SUCCESS AND ABUNDANCE

Student Investigator: Ryan Davis
Principal Investigator: Petra Bohall Wood
Cooperators: Rick Hammack, Harry Edenborn
Years Ongoing: 2012-Present
Degree Program: M.S.
Expected Completion: May 2014
Funding Sources: U.S. Department of Energy

Objectives:

1) Quantify avian abundance, community composition, and nest success rates on shrubland sites before and after development of a Marcellus shale gas well.
2) Gather data on luminance and ambient noise levels before, during, and after development.
3) Determine if changes in avian abundance, community composition, and nest success occurred after development of the Marcellus Shale well.
4) Examine the relationship of these changes to the accompanying changes in luminance, ambient noise levels, and vegetative composition.

Progress:

Extraction of gas from the Marcellus shale formation has increased dramatically in recent years due to technological advancements, namely hydraulic fracturing ("fracking"), that enable companies to reach these previously untapped deposits. These new techniques leave a much larger footprint than conventional oil and gas infrastructure does, and there is growing concern over the potential environmental impacts of hydraulic fracturing methods. Avian concerns mostly relate to habitat fragmentation and loss due to the large area of land that is converted from forest to well pads, gas lines,

and roads. There has been an upswing in research being conducted on the various impacts of Marcellus shale gas extraction and development commensurate with growing implementation of the practice, but most examines the changes in avian communities on forested landscapes. We will be assessing the local-scale impacts in an already highly-fragmented landscape context to fill the gap in research.

Shrubland songbirds are a highly imperiled guild across much of North America due to reductions in forest disturbance and thus rapidly-diminishing habitat. We will accordingly focus on this suite of species, quantifying abundance, community composition, and nest success because they are important indices to avian community health. Changes in avian metrics on the study area in years following well development will suggest that Marcellus development in early-successional habitats may threaten shrubland songbird communities in already-fragmented landscapes. If impacts are not detected, it may be the case that development should occur on these highly-impacted areas rather than on forested landscapes where drilling has been suggested to be more detrimental.

Data on avian abundance, community composition, nest success, vegetative characteristics, and luminance and noise levels were gathered in the 2012 and 2013 breeding seasons. Our study areas are on shrublands located in southwestern Pennsylvania and the northern panhandle of West Virginia. In 2012 data were gathered on two non-developed sites and in 2013 on two non-developed sites and one site which was actively developing infrastructure and extracting natural gas.

We used 10-minute unfixed-radius point counts to measure avian abundance and composition. In subsequent analyses, we will account for detection probability using a time-of-detection removal method. Each point was sampled 3 times during the peak of the season. For initial data summaries, we excluded flyovers and detections greater than 70 meters from the observer. In 2012, this resulted in 1042 total detections of 55 unique species, 792 detections at one site (of 49 unique species) and 250 at the other (48 unique species). The 2013 results were 1087 detections of 55 unique species total, 323 detections of 40 unique species at the site being developed for natural gas, 397 detections of 39 unique species at one non-impacted site, and 367 detections of 34 unique species at the other.

We transformed the 2013 count data into a Bray-Curtis dissimilarity matrix and assessed avian community composition with Analysis of Similarity (ANOSIM) tests. Community structure differed significantly ($P=0.001$, $R=0.36$) by site, and all pair-wise tests between sites indicated that each site had different community structure than the others. At the site which is currently being developed for natural gas, we found that avian community composition was different at all points ($P=0.001$, $R=0.39$) which were arranged in increasing distances from the gaswell. Future analyses will examine if these differences are related to presence of the well or to other factors such as vegetation.

Figure 1. Focal species from left: Field Sparrow, Eastern Towhee (male), Blue-winged Warbler

Nests were found and monitored for 3 focal species: Field Sparrow (*Spizella pusilla)*, Eastern Towhee (*Pipilo erythrophthalmus*), and Blue-winged Warbler (*Vermivora cyanoptera*) (Figure 1). Nest success rates were calculated initially with the Mayfield method and will be analyzed further using logistic-exposure models. In 2012, 118 nests were found, 67 were at one site and 51 at the other. In 2013, 96 nests were found; 31 at the site being developed (CCWMA), 42 at one non-developed site, and 23 at the other. Our nest success rates (Table 1) are similar to those reported in the literature.

Table 1. Nest success results for each site and species. Success rate is the unadjusted ratio of nests that fledged. Probability of Survival is calculated with the Mayfield method which accounts for observation days. BWWA = Blue-winged Warbler, EATO = Eastern Towhee, FISP = Field Sparrow.

Site	# Nests		# Successful		Success Rate		Probability of Survival	
	2012	2013	2012	2013	2012	2013	2012	2013
BWWA								
All Sites	8	6	4	2	0.5	0.33	43%	21%
MTS	5	-	2	-	0.4	-	34%	-
HSP	3	3	2	1	0.67	0.33	21%	4%
CCWMA	-	3	-	1	-	0.33	-	35%
HWMA	-	-	-	-	-	-	-	-
EATO								
All Sites	19	12	3	3	0.15	0.25	7%	3%
MTS	14	-	1	-	0.07	-	6%	-
HSP	5	10	2	2	0.4	0.20	11%	2%
CCWMA	-	1	-	0	-	0.00	-	-
HWMA	-	1	-	1	-	1.00	-	-
FISP								
All Sites	91	78	22	24	0.24	0.31	15%	23%
MTS	48	-	6	-	0.13	-	10%	-
HSP	43	29	16	5	0.37	0.17	22%	15%
CCWMA	-	27	-	11	-	0.41	-	30%
HWMA	-	22	-	8	-	0.36	-	30%

Luminance levels were not associated with gas development activity, meaning that light pollution was not an issue at this particular site. In 2013, 10,743 hours of sound were recorded and we are currently analyzing these data to determine if there was a relationship between gas development activity and ambient noise levels. If we do find a relationship, we will incorporate noise levels into models to test for explaining variation in avian abundance, community structure, and nest success.

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

Student Investigator: Gretchen E. Nareff
Principal Investigator: Petra Bohall Wood
Cooperators: Mark Ford, Todd Fearer, Scott Stoleson, Jeff Larkin
Years Ongoing: 2013-Present
Degree Program: Ph D
Expected Completion: May 2016
Funding Sources: U.S. Geological Survey (WV RWO 62), WV Department of Natural Resources

Objectives:

1. Quantify and compare Cerulean Warbler abundance and territory density at four harvested sites in West Virginia, pre- and post-harvest.
2. Quantify and compare avian abundance and community composition pre- and post-harvest across five cooperating states.
3. Examine how Cerulean Warblers and associated species place territories in relation to silvicultural harvests with regards to slope, aspect, and vegetative structure in West Virginia and Pennsylvania, pre- and post-harvest.

Progress:

The Cerulean Warbler (*Setophaga cerulea*; hereafter, CERW) is a severely declining, late successional songbird species whose core breeding range is in the hardwood forests of the Appalachian Mountains. CERW use heavily forested landscapes with heterogeneous vegetation structure. Although not federally listed by the Endangered Species Act, it was petitioned for listing in 2000. The CERW 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 3.2% per year on the breeding grounds between 1966 and 2003 and a more severe decline of 4.6% per year between 2003 and 2008. 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 CERW preferences is critical in reversing this trend and conserving the species. This study is a region-wide, cooperative project with study sites in Kentucky, Ohio, Pennsylvania, Virginia, and West Virginia.

This project uses information from the recently published Cerulean Warbler Management Guidelines for Enhancing Breeding Habitat in Appalachian Hardwood Forests and applies it on a broader landscape scale. The overarching goal is to use adaptive management strategies to provide enhanced and revised management guidelines that can be implemented on public and private lands across the core breeding range of the CERW. Ideally, we will recommend harvest regimes that benefit CERW and associated Species of Greatest Conservation Need (SGCN) that can be integrated into existing harvest regimes. Ultimately we will look at the birds' selection of residual basal area and vegetation structure within harvest matrices within mature deciduous forests across five states in their core breeding range in order to provide adequate breeding habitat. We will look at structural selection within territories and compare pre- and post-harvest densities to detect differences in abundance. Fieldwork will be conducted within the host states by local teams and data management and analysis will be a cooperative effort. Global analyses will be completed by the West Virginia team.

The four West Virginia study sites are located within CERW Focal Areas designated by the Appalachian Mountains Joint Venture. Study sites are at Coopers Rock State Forest (CRSF; 180.6 ac) in Monongalia

and Preston counties, Stonewall Jackson Wildlife Management Area (WMA; 59.5 ac) in Lewis County, and two sites (Dynamite, 98.4 ac and Wolf Creek, 65.8 ac) at the Elk River WMA in Braxton County. Stonewall Jackson WMA and Wolf Creek will be harvested later in 2013 or early 2014; the remaining two sites, CRSF and Dynamite, were harvested in 2012. Harvests occur as a matrix at each site, including shelterwood harvests, clearcuts, singletree selection, and group selection, with unharvested areas throughout (Figure 1).

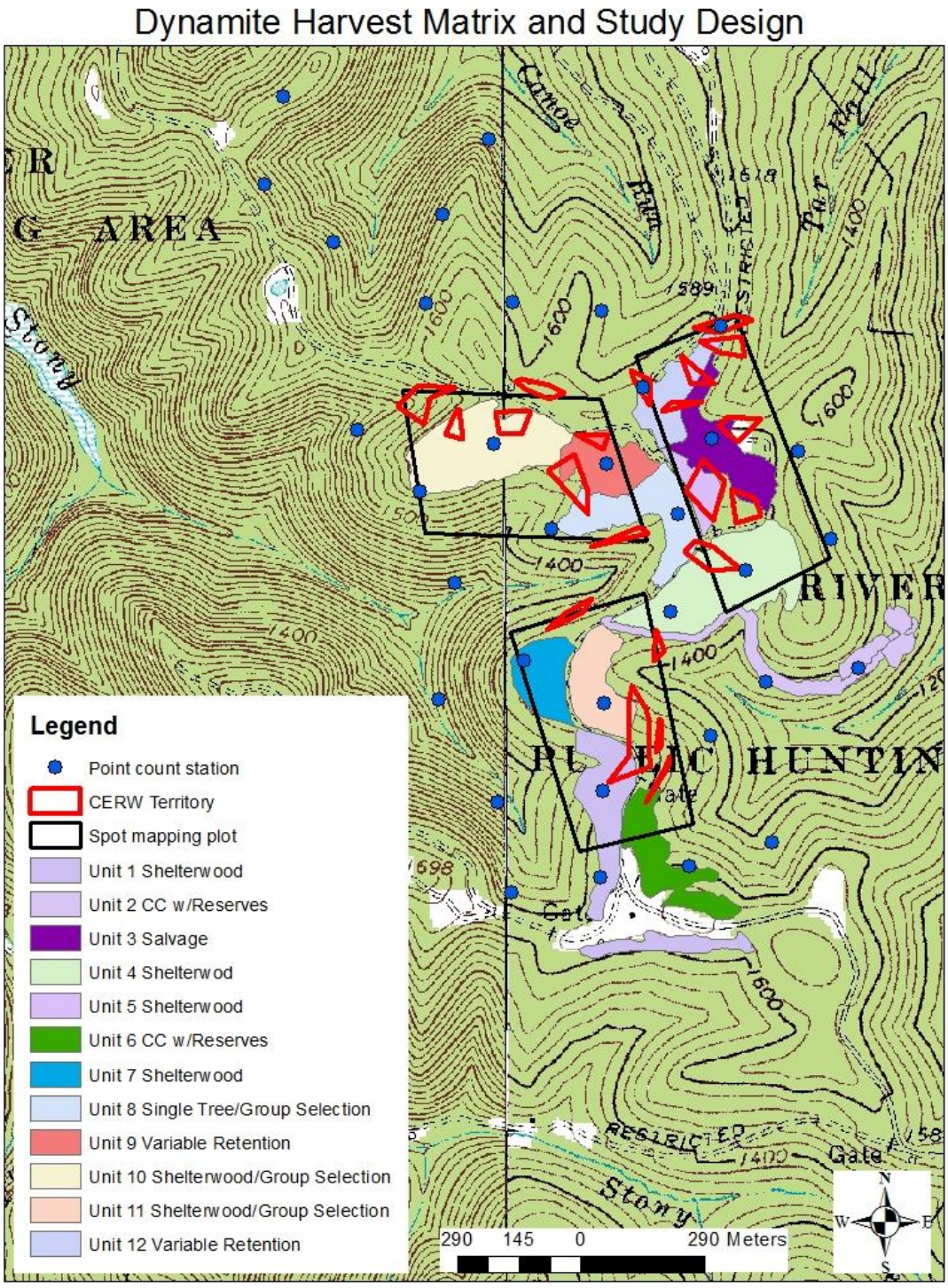


Figure 1. Dynamite Harvest within the Elk River Wildlife Management Area in Braxton County, WV.

Data collection during the first season included point counts for the entire avian community and territory mapping on CERW and a set of focal SGCN associated with CERW. Point count stations were placed within harvests at previously harvested sites and within proposed harvest boundaries at the pre-harvest sites. Additional point count stations were placed on the edges of harvests and in reference stands (Figure 1). Number of point count stations per study site ranged from 22 to 40. We used standardized 10-minute point counts conducted three times at each point to determine avian abundance and community composition. Spot mapping plots 16-18 ha in size were placed over the harvest matrices so they would include all intensities of harvest and no more than 20% unharvested stands. Study areas had either 2 or 3 spot mapping plots depending on the size of the harvest matrix (Figure 1). Prism plots were completed at all point count stations, 1 plot per ha within spot mapping plots, and 2-3 plots within each CERW territory, depending on size of territories and coverage needed for the harvest matrix. On each prism plot, we recorded tree species, diameter at breast height (dbh), canopy position, and canopy condition. Slope and aspect within each prism plot will be calculated with ArcMap.

For initial analyses, we excluded flyovers and birds detected greater than 75 m from the observer. Subsequent analyses will account for detection probability and time removal methods. We made 10,132 detections of 3,401 individuals of 68 species including an unidentified woodpecker group (Table 1). Red-eyed Vireos (*Vireo olivaceus*; REVI; 15.9%) and Ovenbirds (*Seiurus aurocapilla*; OVEN; 10.3%) accounted for the most detections. CERW were detected a total of 247 times at all four study sites with the most detections at Wolf Creek (4.2% of all detections), a pre-harvest site. CERW accounted for 2.3% of all detections at all sites. CERW were detected at point counts with total basal areas (BA) ranging from 1 to 50 m^2 /ha with the maximum number of CERW (4) detected at BAs of 27 and 33 m^2 /ha (Figure 2). The mean number of CERWs at all harvested point count stations was 0.38, while the mean number of CERWs at unharvested point count stations was 0.75. Table 1 shows the mean number of CERWs at harvested and unharvested point count stations per site.

Table 1. Summaries of first-season data at all sites in West Virginia: Coopers Rock State Forest, Dynamite within Elk Creek Wildlife Management Area (WMA), Stonewall Jackson WMA, and Wolf Creek within Elk River WMA.

	CRSF	**DYNO**	**SJ**	**WC**
# species	51	55	41	44
All species detections	2,982	2,706	1,839	2,605
CERW detections	19	55	63	110
% CERW detections	0.50	1.80	3.24	4.15
# CERW territories	1	20	7	12
Territory density (#/ha)	0.02	0.33	0.21	0.34
Mean # CERW/pt (harvested pts)	0.24	0.56	NA	NA
Mean # CERW/pt (unharvested pts)	0.05	0.44	0.91	1.35

Forty CERW territories were mapped within spot mapping boundaries and varied by study area (Table 1). One CERW nest was located incidentally at Dynamite and appeared to fledge, and CERW fledglings were observed at Wolf Creek. Territories appeared to align primarily along harvest boundaries (Figure 1). Detailed analyses will be completed this fall and winter of the WV data and the overall project data.

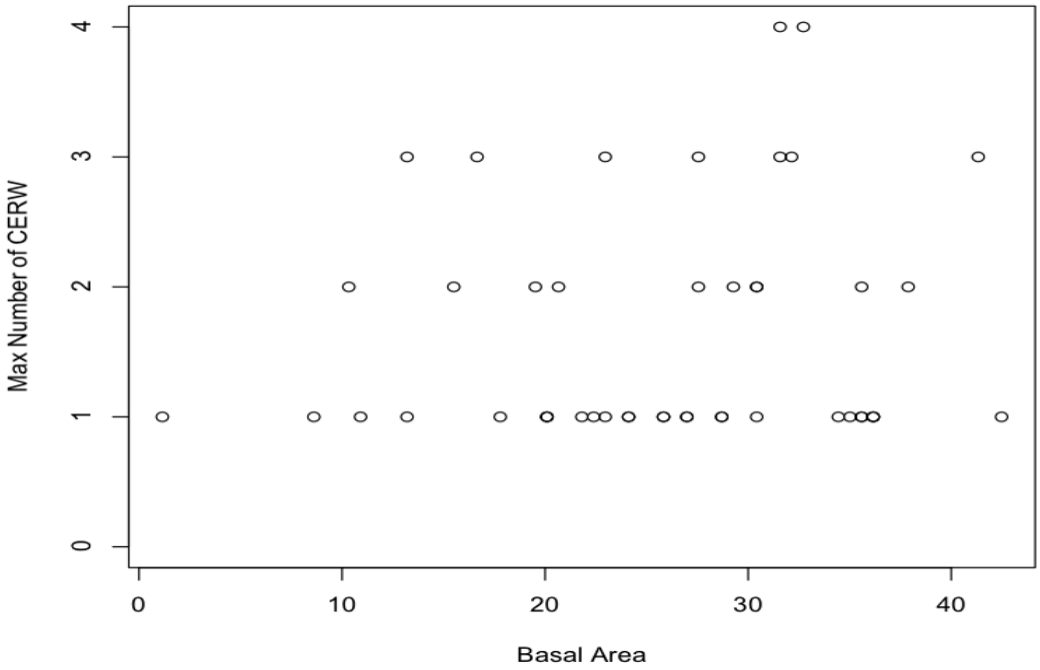


Figure 2. Number of Cerulean Warblers detected at point counts vs. basal area in m^2 /ha.

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 Bohall Wood
Cooperators: Harry Edenborn, Randy Dettmers, T. Bentley Wigley
Years Ongoing: 2010-Present
Degree Program: Ph.D.
Expected Completion: May 2014
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 successfully predict the avian community found on mature hardwood-forested ridges.
2. Use this remote sensing information along with field 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 ridgetops of the heavily forested region of northwestern West Virginia harbor a rich avian community important to the native biodiversity of the Central Appalachians. These ridges also are the focus of much energy industry activity, particularly development of Marcellus Shale natural gas. The Marcellus well pads and associated infrastructure (access roads and pipelines), and other anthropogenic impacts such as development of the region's abundant conventional gas and oil resources, have the potential to affect a significant portion of the region's ridgetops and associated breeding bird species, including large populations of the sharply declining Cerulean Warbler.

Use of remote sensing information has proven useful for studying avian habitat relationships. One objective of this project is to examine predictive relationships between two high-resolution datasets, a leaf-on 2009 multispectral Quickbird satellite image and a 3-meter digital elevation model (DEM), and a 2010-11 ridgetop avian point count survey of the Lewis Wetzel Wildlife Management Area in Wetzel Co. WV. Continuous forest in the image (Figure 1) appears to reflect a structural gradient from smooth, unbroken canopy with an open understory to rough, broken canopy with abundant gaps and high structural diversity found in the field. These two forest structural types support different avian communities, and textural and spectral information obtained from the image will be used in conjunction with fine-scale topographic variables calculated from the DEM for the point count locations to analyze the avian data. If predictive relationships are found, this remote sensing information could be an important tool applied at broader spatial scales and in lieu of intensive field surveys for examining implications of energy industry activities on the ridge forest bird community.

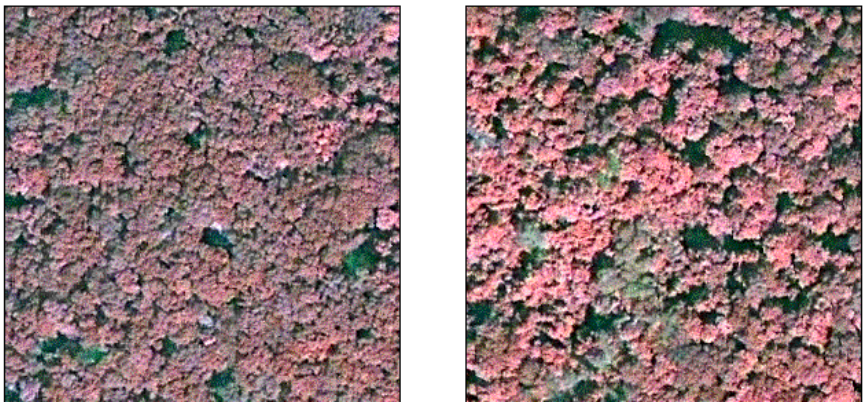


Figure 1. Two ridgetop forest canopy types: "smooth" unbroken canopy (left) and "rough" broken canopy (right).

The other two objectives of this project use the distribution patterns of three territorial songbirds, obtained from focal ridgetop transect surveys in 2010 and 2011. Ovenbirds, Hooded Warblers, and Cerulean Warblers are widespread and abundant at the site, and differ in the forest habitat components they require for breeding (Figure 2). Comparing and contrasting their distribution patterns will be used to examine potential underlying factors responsible for their occurrence. This analysis will use remote sensing information obtained for the ridge transects from the satellite image and the DEM, along with territory habitat information collected in the field. For the Cerulean Warbler, the final objective is to infer if habitat, sociality, or both, may be driving factors underlying where they occur, and in particular where they strongly cluster at the site. Understanding factors responsible for their observed distribution patterns could be particularly valuable in the management of this species of high conservation priority in the Central Appalachians.

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

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

Student Investigator: Laura Farwell
Principal Investigator: Petra Bohall Wood
Cooperators: Randy Dettmers, Todd Fearer, Margaret Brittingham
Years Ongoing: 2013-present
Degree Program: PhD
Expected Completion: Sept 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 adjacent mature forest.
   b. Quantify effects of well pad site size and age 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 to those using the coarser scale PBBA data to validate the TNC/PBBA models.
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. We will 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. Our analyses will potentially 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 will randomly select a sample of well sites from landscapes with high and low density gas well development and also will sample mature forest reference areas not impacted by Marcellus development. We will use standard protocols to quantify avian, vegetation, and landscape metrics and will develop statistical models to relate bird densities and abundance to the habitat and landscape metrics. These results can 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.

We currently are in the process of identifying potential study sites in WV, OH, and PA. Field sampling will occur in the 2014 and 2015 breeding seasons.

A new Marcellus well pad where there was previously mature forest. Photo by Jim Sheehan.

A Marcellus well pad, wastewater pond, and access road along a ridgeline.

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

Student Investigator: Mack Frantz
Principal Investigator: Petra Bohall Wood
Cooperator: Steve Latta
Years Ongoing: 2013-present
Degree Program: PhD
Expected Completion: December 2015
Funding Sources: USGS, WVDNR, Pittsburgh National Aviary

Objectives:

1. Determine how gas well development activities spatially influence Louisiana Waterthrush territory density, foraging locations or behavior, nesting location and success, and site fidelity.

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

3. Develop spatially explicit habitat models from Louisiana Waterthrush data to aid land managers in mitigating habitat disturbance from oil and gas activities.

Progress:

An emerging threat for wildlife is shale gasdevelopment in the Appalachian region. My research will determine how gas development activity and possible environmental stressors (such as stream contamination via heavy metals) influence Louisiana Waterthrush (*Parkesia motacilla*) DNA methylation between individuals nesting at impacted and unimpacted streams on the Lewis Wetzel Wildlife Management Area (LWWMA). In addition, stream and nesting features will be spatially assessed to determine if there is a spatial component to gas development activity that influences waterthrush demographics (eg. nesting success).

This study follows up on a study during 2008-2011 of Louisiana Waterthrush on LWWMA. In the original work, we observed no demographic response of waterthrush to the low amount of habitat disturbance from shale gas development although habitat quality decreased at impacted sites. The amount of shale gas development has continued to increase leading to this follow-up study which is using methods similar to the original study.

Figure 1. Louisiana Waterthrush fledgling.

We color-banded adult Louisiana Waterthrush to allow for recognition of individuals by sight. We banded nestlings (Figure 1) to ID individuals and to monitor dispersal. Adults were captured using active mist-netting techniques. We collected three flight feathers from each adult and 3-5 breast feathers from each nestling for analysis of contaminants. Blood (5-10 microliters) was sampled by venipuncture from the ulnar vein of the wing for analysis of epigenetic (DNA methylation) differences among individuals. Efforts will be made each year to resight color-banded individuals to allow for calculation of survival rates across and within years. We will assess stream and habitat quality by analyzing vegetation immediately surrounding the nest, use the EPA Rapid Bioassessment protocol for high gradient streams, and a Louisiana Waterthrush Habitat Suitability Index (HSI). Macroinvertebrates occurring in riffle habitat of each individual's territory and at each nesting location will be sampled with a Surber sampler to see if they vary among stream site (Figure 2).

Louisiana Waterthrush monitoring took place during March 28 – 31 July 2013 by searching 50 km of headwater streams on LWWMA. Ninety-four nests were found through our nest searching efforts with only two known fledged nests that were not found. We had our first recorded observations of Brown-headed Cowbird nest parasitism (n=4) at LWWMA. Two of the parasitized nests were successful, yielding one and three fledglings in each nest. We banded 112 nestlings and 72 adults over the breeding season (184 total). Ten of these banded individuals were recaptures from previous years with one being previously banded in 2008. From mist-netting efforts, we collected 183 feather and 184 blood samples that will be used for analysis of stream contaminants and epigenetic variation. We collected 633 territorial observations in order to determine if territorial size differs by the amount of gas development impact on habitat. Finally, we collected over 170 macroinvertebrate samples at foraging and nesting locations that will be compared between impacted and unimpacted areas. Data summaries and analyses will be completed this fall and winter.

Figure 2. Macroinvertebrates were collected at foraging and nest site locations to determine if habitat quality differs between impacted and unimpacted areas.

LONG-TERM SONGBIRD POPULATION RESPONSE TO GAS WELL DEVELOPMENT

Student Investigators: Jim Sheehan, Greg George
Principal Investigator: Petra Bohall Wood
Cooperator: Harry Edenborn
Years Ongoing: 2008-2013
Expected Completion: November 2014
Funding Source: Department of Energy

Objectives:

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

Progress:

West Virginia is known for its extensive tracts of mature hardwood forest and high diversity of flora and fauna. Oil and gas (O&G) exploration and development activities in West Virginia are increasing, particularly for Marcellus shale gas (Fig. 1). O&G activities can impact wildlife populations by changing habitat structure and through effects of noise and human presence. 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 habitat models that will aid land managers in mitigating these disturbances.

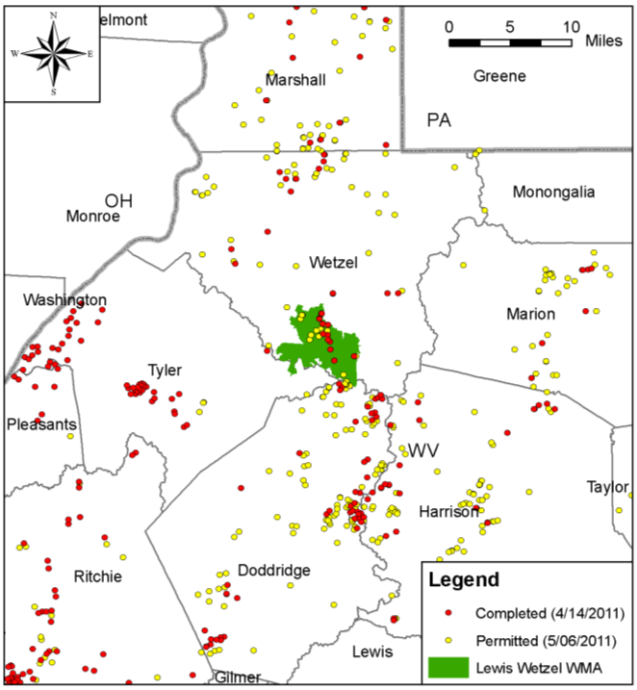


Figure 1. Permitted and completed Marcellus gas wells (as of April-May 2011) in the study area and surrounding region (obtained from the West Virginia Geological and Economic Survey http://www.wvgs.wvnet.edu/).

We established 144 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 2008-2013 breeding seasons to determine abundance, diversity, and distribution of songbirds. Point counts sampled areas with current Marcellus activity, areas with past O&G activity, and mature forest reference areas. Across the study area, habitat fragmentation and change (Fig. 2) has increased over the study period.

Figure 2. A ridgetop road/pipeline in previously unfragmented mature forest.

Relative abundance and distribution of Cerulean Warblers (CERW) has declined over time (Figs 3 and 4). The mature forest-associated Worm-eating Warbler (WEWA) also declined initially but increased in recent years. Brown-headed cowbirds (BHCO), a nest parasite, increased in abundance and distribution particularly in association with new O&G impacts. As O&G activities continue on the study area, including new Marcellus well pad construction and associated infrastructure (access roads and pipelines), we expect the avian community to continue to change.

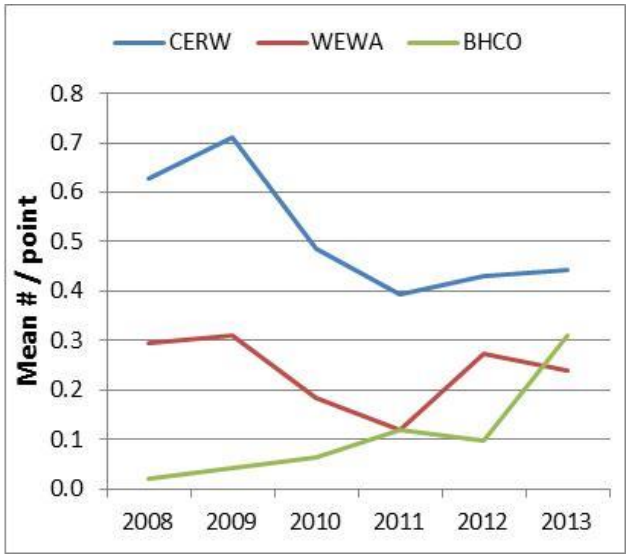


Figure 3. Avian species relative abundance.

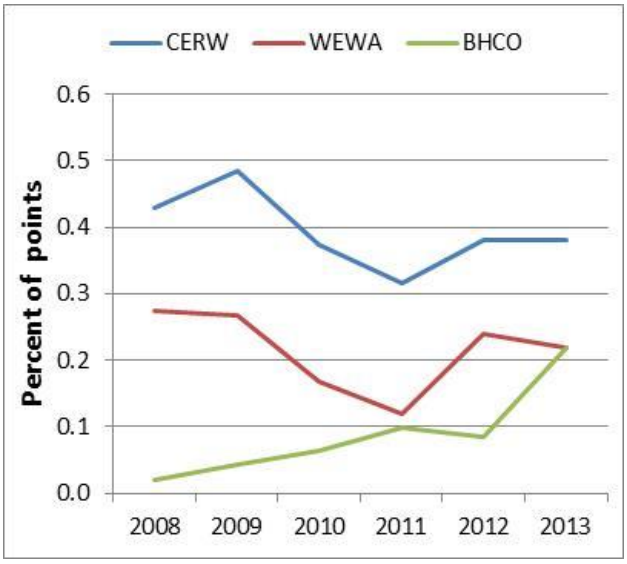


Figure 4. Avian species distribution.

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

SCIENTIFIC PUBLICATIONS

Anderson, J. T., A. K. Zadnik, P. B. Wood, and K. Bledsoe. 2013. Evaluation of habitat quality for selected wildlife species associated with island back channels. Open Journal of Ecology 3:301-310. doi:10.4236/oje.2013.34035 7/29/2013

Boves T.J., D.A. Buehler, J. Sheehan, P.B. Wood, A.D. Rodewald, J.L. Larkin, P.D. Keyser, F.L. Newell, G.A. George, M.H. Bakermans, A. Evans, T.A. Beachy, M.E. McDermott, K.A. Perkins, M. White, and T.B. Wigley. 2013. Emulating natural disturbances for declining late-successional species: a case study of the consequences for Cerulean Warblers (*Setophaga cerulea*). PLoS ONE e52107. 1/20/2013

Boves T.J., D.A. Buehler, J. Sheehan, P.B. Wood, A.D. Rodewald, J.L. Larkin, P.D. Keyser, F. L. Newell, A. Evans, G.A. George and T. B. Wigley. 2013. Spatial variation in breeding habitat selection by Cerulean Warblers (*Setophaga cerulea*) throughout the Appalachian Mountains. Auk 130(1): 46-59. 1/28/2013

Ekman, D.R., G.T. Ankley, V.S. Blazer, T.W. Collette, N. Garcia-Reyero, L.R. Iwanowicz, Z.G. Jorgenson, K.E. Lee, P.M. Mazik, D.H. Miller, E.J. Perkins, E.T. Smith, J.E. Tietge, and D.L. Villeneuve. 2013. Biological Effects-based Tools for Monitoring Impacted Surface Waters in the Great Lakes: A Multi-Agency Program in Support of the GLRI. Environmental Practice. Accepted for Publication.

Hedrick, L., J. Anderson, S. Welsh, and L-S. Lin. 2013. Sedimentation in mountain streams: a review of methods of measurement. Natural Resources 4: 92-104.

Loughman, Z., D. Foltz, N. Garrison, and S. Welsh. 2013. *Cambarus theepiensis* sp. n., a new species of crayfish (Crustacea, Decapoda, Cambaridae) from the coalfields region of Eastern Kentucky and Southwestern West Virginia, USA. Zootaxa 3641: 63-73.

Loughman, Z.J., D. Foltz, and S.A. Welsh. In press. Baited lines, an active nondestructive collection method for burrowing crayfish. Northeastern Naturalist.

Newell, F., J. Sheehan, P. Wood, et al. In Press. Do Different Methods Detect Treatment Effects? Comparison of Point Counts and Territory Mapping for Forest Songbirds. Journal of Field Ornithology

Sheehan, J., P.B. Wood, Buehler, D.A., Keyser, P.D., Larkin, J.L., Rodewald, A.D., Wigley, T.B., Boves, T.J., George, G.A., Bakermans, M.H., Beachy, T.A., Evans, A., McDermott, M.E., Newell, F.L., Perkins, K.A., White, M. 2013. Avian response to timber harvesting applied experimentally to manage Cerulean Warbler breeding populations. Forest Ecology and Management. online 8/19/2013

Sheehan, K., M. Strager, and S. Welsh. 2013. Advantages of geographically weighted regression for modeling benthic substrate in two Greater Yellowstone Ecosystem Streams. Environmental Modeling and Assessment 18:209-219.

Smith, B.W., J. Botero, J.L. Larkin, A.D. Rodewald, P.B. Wood, P.A. Angel, and S.E. Eggerud. 2012. Integrating conservation management, species protection, and economic viability into sustainable land use practives for the cerulean warbler in the Appalachian and northern Andes Mountains. Ornitologia Neotropical 23:329-334. 7/29/2013

Welsh, S.A., D.M. Smith, and N.D. Taylor. 2013. Microhabitat use of the diamond darter. Ecology of Freshwater Fish. doi: 10.1111/eff.12062

Welsh, S.A. and H. Liller. 2013. Environmental correlates of upstream migration of yellow-phase American eels in the Potomac River drainage. Transactions of the American Fisheries Society 142:483-491.

Welsh, S.A., D. A. Cincotta, and W.C. Starnes. In press. First records of *Nocomis biguttatus* (Hornyhead Chub) from West Virginia discovered in museum voucher specimens. Northeastern Naturalist.

Wickham, J., P.B. Wood, M.C. Nicholson, W. Jenkins, D. Druckenbrod, G.W. Suter, M.P. Strager, C. Mazzarella, W. Galloway, and J. Amos. 2013. The overlooked terrestrial impacts of mountaintop mining. BioScience 63:335-349. 7/29/2013

Wood, P.B. and K.A. Perkins. 2012. Behavioral activities of male cerulean warblers in relation to habitat characteristics. Wilson Journal of Ornithology 124(3):497-505. 10/1/2012

Wood, P.B., J. Sheehan, P. Keyser, D. Buehler, J. Larkin, A. Rodewald, S. Stoleson, T.B., Wigley, J. Mizel, T. Boves, G. George, M. Bakermans, T. Beachy, A. Evans, M. McDermott, F. Newell, K. Perkins, and M. White. 2013. Management guidelines for enhancing Cerulean Warbler breeding habitat in Appalachian hardwood forests. American Bird Conservancy. The Plains, Virginia. 28 pp. 2/28/2013

Wood, P.B. and J.M. Williams. 2013. Impact of Valley Fills on Streamside Salamanders in Southern West Virginia. Journal of Herpetology 47:119-125. 4/15/2013

Wood, P.B. and J.M. Williams. 2013. Terrestrial salamander abundance on reclaimed mountaintop removal mines. Wildlife Society Bulletin. online 7/29/2013

Zimmerman, J.L., and S.A. Welsh. 2012. Prevalence of *Anguillicoloides crassus* and growth variation in migrant yellow-phase American eels of the upper Potomac River drainage. Diseases of Aquatic Organisms 101:131–137.

THESES AND DISSERTATIONS

Braham, M. 2012. Selection of benthic habitat by yellow-phase American eels (*Anguilla rostrata*). MS Thesis, West Virginia University, Morgantown, WV.

Braham, R. 2012. An evaluation of the occurrence of micronuclei and other nuclear abnormalities in fishes from the Great Lakes Basin, United States. MS Thesis, West Virginia University, Morgantown, WV.

Ruble, C. 2013. Captive propagation, reproductive biology, and early life history of *Crystallaria cincotta* (Diamond Darter), *Etheostoma wapiti* (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter). MS Thesis, West Virginia University, Morgantown, WV.

Taylor, N.D. 2013. Assessment of Age, Diet, and Growth of Yellow Perch (*Perca flavescens*) in Cheat Lake, West Virginia. MS Thesis, West Virginia University, Morgantown, WV.

PRESENTATIONS

Aldinger, K. 11 October 2012. Golden-winged Warbler Breeding Ecology on Actively Managed Shrublands on the Monongahela National Forest. West Virginia Cooperative Stamp Meeting. Canaan Valley Resort, WV.

Aldinger, K., Terhune, Buehler, Wood, Bakermans, Confer, Flaspohler, Larkin, Loegering, Percy, Roth, and Smalling. 15 Aug 2013. Golden-winged Warbler nesting ecology and productivity: a range-wide assessment. Joint Annual Meeting American Ornithologists Union and Cooper Ornithological Society, Chicago, IL.

Anderson, J.T., J.L. Pitchford, S.M. Selego, K.R.P. McCoard, L. Lin, G.T. Merovich, and S.A. Welsh. 2013. Biotic and abiotic response to stream restoration on the Cacapon River. West Virginia Academy of Science, 6 April 2013, Canaan Valley Institute, Davis, West Virginia.

Becker, D., P.B. Wood, and M.P. Strager. 15 Aug 2013. Impacts of mountaintop mining on terrestrial ecosystem Integrity: identifying landscape thresholds for avian species. Joint Annual Meeting American Ornithologists Union and Cooper Ornithological Society, Chicago, IL.

Braham, R., V. Blazer, P. Mazik. 2013. An Evaluation of Genotoxic and Mutagenic Contamination in the Great Lakes Basin, a Case Study. AFS Southern Division Meeting, Nashville, Tennessee.

Frantz, M., K. Aldinger, J. Duchamp, T. Simmons, T. Nuttle, J. Larkin, A. Vitz, and P. Wood. 23 March 2013. Space and habitat use by breeding Golden-winged Warblers in the central Appalachians. PA Chapter of The Wildlife Society & PA Biological Survey 2013 Joint Annual Conference & Workshop. State College, PA.

Frantz, M.W., K. Aldinger, Duchamp, Simmons, Nuttle, Larkin, Vitz, and P.B. Wood. 15 Aug 2013. Space and habitat use by breeding Golden-winged Warblers in the central Appalachian Mountains. Joint Annual Meeting American Ornithologists Union and Cooper Ornithological Society, Chicago, IL.

Hahn, C.M., L.R. Iwanowicz, V.S. Blazer, P.M. Mazik. 2013. Biological Effects of Environmental Contaminants on Gene Expression Endpoints in Fishes of the Great Lakes. 56th Annual Conference on Great Lakes Research, West Lafayette, Indiana.

Hahn, C.M., L.R. Iwanowicz, V.S. Blazer, P.M. Mazik. 2013. Effects of Environmental Contaminants on Gene Expression Endpoints in Fishes of the Great Lakes. AFS Southern Division Meeting, Nashville, Tennessee.

Hahn, C.M., V.S. Blazer, P.M. Mazik. 2012. Genetic Divergence and Longitudinal Variation of Smallmouth Bass (*Micropterus dolomieu*) Populations in Lake Erie and its Tributaries. 2012 Student Colloquium at the Northeast Association of Fish and Wildlife Agencies. Charleston, West Virginia.

Larkin, J.L., P.B. Wood, T.J. Boves, J. Sheehan, D.A. Buehler, A.D. Rodewald, P.D. Keyser, T.A. Beachy, M.H. Bakermans, A. Evans, G.A. George, M.E. McDermott, F.L. Newell, K.A. Perkins, and M. White. 2013. Cerulean Warbler Response to Forest Management: Can forest management produce more breeding birds? Pennsylvania Chapter of The Wildlife Society Annual Conference. State College, PA. March 22-23.

Lucero, M., R. Fagundo, S. Welsh, and Z. Loughman. 2013. Distribution and conservation status of four *Orconectes* crayfishes occurring in Virginia's upper James and Maury River systems. Seventy-fourth Annual Meeting of the Association of Southeastern Biologists, 12 April 2013, Charleston, West Virginia.

Smith, D.M., S.A. Welsh, and N.D. Taylor. 2013. Investigating Seasonal Movements and Spawning Locations of Walleye in Cheat Lake, WV using Acoustic Telemetry. Annual meeting of the Southern Division American Fisheries Society, 10 February 2013, Nashville, Tennessee.

Smith, D.M., S.A. Welsh, and N.D. Taylor. 2013. Determining Seasonal Movements and Spawning Habitat Use of Walleye in Cheat Lake, WV using Acoustic Telemetry. Joint meeting of the Ohio and WV Chapters of the American Fisheries Society, 20 February 2013, Huntington, West Virginia.

Taylor, N.D., S.A. Welsh, and D.M. Smith. 2013. Assessment of age, diet, and growth of yellow perch In Cheat Lake, West Virginia. Annual meeting of the Southern Division American Fisheries Society, 10 February 2013, Nashville, Tennessee.

Taylor, N.D., S.A. Welsh, and D.M. Smith. 2013. Age structure, growth, and summer diet of yellow perch (Perca flavescens) in a West Virginia Reservoir. Joint meeting of the Ohio and WV Chapters of the American Fisheries Society, 20 February 2013, Huntington, West Virginia.

Terhune, Buehler, Aldinger, Bakermans, Confer, Larkin, Loegering, Percy, Roth, Smalling, Wood. 15 Aug 2013. Effects of Golden-winged Warbler habitat management on other avian species. Joint Annual Meeting American Ornithologists Union and Cooper Ornithological Society, Chicago, IL.

Terhune, Buehler, Aldinger, Bakermans, Confer, Larkin, Loegering, Percy, Roth, Smalling, Wood. 15 Aug 2013. Golden-winged Warbler breeding habitat selection at nest-site and territory scales. Joint Annual Meeting American Ornithologists Union and Cooper Ornithological Society, Chicago, IL.

Welsh, S.A., D.M. Smith, and N.D. Taylor. 2013. Nocturnal microhabitat use of the diamond darter. Annual meeting of the Southern Division American Fisheries Society, 9 February 2013, Nashville, Tennessee.

Welsh, S.A., and D. Wichterman. 2013. Angler and creel surveys of the walleye fishery at Summersville Reservoir, West Virginia. Joint meeting of the Ohio and WV Chapters of the American Fisheries Society, 20 February 2013, Huntington, West Virginia.

Welsh, S.A., H. Liller, and D.M. Smith*. 2013. Environmental Correlates of Upstream Migration of Yellow-phase American Eels in the Potomac River Drainage. Sixty-ninth Annual Northeast Fish

and Wildlife Conference, 8 April 2013, Saratoga Springs, New York [*Presented by D.M. Smith].

Williams, J. and P.B. Wood. 13 July 2013. Impact of mountaintop removal mines on terrestrial and aquatic salamanders in southern West Virginia. Energy Symposium, Annual Conference Society of Ichthyologists and Herpetologists, Albuquerque, NM. **[Invited]**

AWARDS

Cassidy Hahn received the student travel award from the WV Chapter of AFS to attend the annual AFS meeting in Little Rock, AR.

Secretary of Interior 2012 Partners in Conservation Award presented to OSM for the Appalachian Regional Reforestation Initiative and accepted by members of the Science Team of which Dr. Petra Wood is a member. 10/18/12

Partners in Flight Research Award presented to the cerulean warbler research team of which Dr. Petra Wood is a member and a lead PI. Aug 2013