INSTITUTE FOR THE ENVIRONMENT
HIGHLANDS FIELD SITE
2016 INTERNSHIP RESEARCH REPORTS

HIGHLANDS BIOLOGICAL STATION

HIGHLANDS, NORTH CAROLINA

Introduction

""Do you see it yet?"

...With these encouraging words, he added, "Well, what is it like?"

Chapter 2. The Nature of Learning: Recognition of Different Perspectives from *"In the Laboratory with Agassiz,"* by Samuel H. Scudder

The 2016 fall semester on the Highlands Plateau took us across the mountain landscape from classroom and lab to river and forest. The IE-Highlands Field Site students embraced the experience and came through with a set of research papers, presented in this volume, that demonstrates the breadth of their endeavors and the skills they developed. The experience and learning in a field school setting are habitually more intense, more in-depth, and more personal than campus classroom experiences. This year was no exception for the Highlands IE Program group in that they had the opportunity to live and work together along with benefit from working independently in shaping their research projects with their mentors.

We would like to thank those who have worked closely with the students and us this semester, including

Karen Kandl – former Associate Director
Steve Foster – capstone project
Gary Wein – Landscape Analysis course.

Also a hearty thanks is due the mentors who guided the students through their research projects, and gave them an appreciation for the systems that they worked with, including Rich Baird, Chelcy Miniat, Sandra Hawthorne, Jack Johnston, Gail Lemiec with Aimee Tomcho, Kyle Pursel and Gary Wein.

From dynamics of old-growth hemlock forests (and rhododendron thickets), radial growth analysis, chestnuts and green salamanders to sediment and OHVs, mountain camellias and golden-winged warblers, this group of students will also be able to reflect upon

- when they see a stream, swollen with a week's worth of rain, to remember the power of streams, the importance of streams, and how what we humans do in the watershed effects the streams,
- remember how to find salamanders and that this will remind them of the wilderness what was and still is a part of much of the southern Appalachians,
- remember the hard work they have put into writing these papers, and that will give them a sense that they can succeed with patience and dedication, and
- also, never lose the desire to learn and build upon the ideas that they have absorbed here in Highlands.

It is our hope that the IE students from the Highlands Field Site will use the experience and knowledge they gained from their semester in the mountains to help protect the diversity of life they have come to appreciate and to protect the richness of mountain highlands wherever they are.

~ Jim Costa and Sarah Workman
Highlands Field Site Directors

ACKNOWLEDGEMENTS

On behalf of the IE-HFS class of 2016, we would like to thank the many individuals who took time from their busy lives to help teach the IE courses, and to support and mentor the student research. We thank the internship mentors Rich Baird (Mississippi State University), Sandra Hawthorne (Coweeta Hydrologic Laboratory), Jack Johnston (Clayton, GA), Gail Lemiec (Highlands Nature Center), Chelcy Ford Miniat (Coweeta Hydrologic Laboratory), Kyle Pursel (Highlands-Cahiers Land Trust), Aimee Tomcho (North Carolina Audubon Society), and Gary Wein (Highlands-Cashiers Land Trust), whose knowledge and guidance made these research experiences possible.

We are grateful to Steve Foster (Watershed Science, Inc.) who taught us about streams and all things within and around streams as he helped us with our Capstone Project. His knowledge, expertise, and deep love for the rivers of western North Carolina guided us in the field and in the writing of the Capstone Project. Alyssa Fuller was instrumental in the capstone success and we thank her for contributing her expertise of macroinvertebrates to our Capstone Project.

We thank Gary Wein of the Highlands-Cashiers Land Trust for once again teaching the Landscape Analysis course and helping with the GIS components of this year's internships. We appreciate, too, the many guest instructors that contributed to this year's program, including JJ Apodaca (Warren Wilson College), Rich Baird (Mississippi State University), Patrick Brannon (Highlands Biological Station), Beverly Collins (Western Carolina University), Anya Hinkle (Asheville Botanist), Jeremy Hyman (Western Carolina University), Brent Martin (The Wilderness Society), Tom Martin (Western Carolina University), and James Reynolds (Brevard College).

Special thanks are merited for the benefactor who set up the Chestnut Trust Fund at UNC, which supported the chestnut blight work by Alex Kellogg and Alexandra Tribo. We also thank Mark Hopey at the Southern Appalachian Raptor Research for hosting two of our student researchers and mentors for a field day.

The HBS staff, Alyssa Fuller, Mike McMahan, Patrick Brannon, Katie Lapish, Sonya Carpenter and Russell Funderburk, helped make the living and learning experience at HBS enriching and comforting. We thank Amelia Kiechle and Emily Williams of the IE program in Chapel Hill for their assistance in recruiting students and communications for the program. Continued thanks to Tony Reevy, IE, and Chrissie Greenberg, Emily Williams and the Study Abroad staff at Chapel Hill for their support. We are grateful, finally, for the hospitality shown by residents of the town of Highlands who continue to welcome our students into the community each semester.

Sarah Workman and Jim Costa
IE-Highlands Field Site Directors
December 2016

TABLE OF CONTENTS

COMPARING COMMUNITY DYNAMICS OF OLD-GROWTH HEMLOCK FORESTS IN VARYING STATES OF DECLINE

LAURENCE ALEXANDRA CECIL

Abstract. The eastern hemlock (*Tsuga canadensis*) was once a dominant species of high-elevation slopes in the southern Appalachians. Following the introduction of the invasive hemlock woolly adelgid (*Adelges tsugae*), many hemlock forests across the Eastern United States are experiencing extensive decline. In this study, species assemblages associated with hemlock were compared between three properties managed by the Highlands-Cashiers Land Trust with variably affected *T. canadensis* populations in an effort to compare the structure of communities between surviving and altered communities following infestation. Ordination did not differentiate sites when woody stem diameter measurements of canopy and subcanopy species were compared, but did when shrubs, herbaceous species, and seedlings were included. Ordination incorporating pre-infestation assemblage data from nearby hemlock forests external to the study did not support a change in current forest composition in response to the presence of the invasive pest. The small sample size of the study and a seasonal delay in surveying necessitate further research on the subject to draw firmer conclusions.

Key words: species assemblage; community structure; indicator value; dominance; ordination; Carolina vegetation survey; Tsuga canadensis; Adelges tsugae

INTRODUCTION

The eastern hemlock [*Tsuga candensis* (L.) Carr.] is a slow-growing evergreen tree that once occupied a significant portion of moist, high elevation-slopes in the southern Appalachian Mountains. As a foundation species in its associated community, the dominance of *T. canadensis* in a forest modifies and maintains unique environmental conditions by limiting light penetration and decreasing the pH of substrates through the gradual input of primarily acidic organic matter (Ellison et al. 2005, Martin and Goebel 2013). Hemlock-dominated forests provide a unique habitat that supports many associated organisms including a proportionally greater diversity of avian, mammalian, and insect species as compared to adjacent hardwood forests (Yamasaki et al. 2000, Snyder et al. 2002, Tingley et al. 2002, Coots et al. 2012).

Following the arrival of the hemlock woolly adelgid (HWA; *Adelges tsugae*) to eastern North America in the mid-twentieth century, hemlock populations across the Appalachian Mountains have experienced significant increases in mortality rates (Orwig and Foster 1998, Small et al. 2005). The adelgid effectively defoliates hemlocks by attacking and siphoning liquids from the needles of the tree, obstructing the transport of water and nutrients to and from the photosynthesizing bodies of the tree and ultimately causing its desiccation and death (Orwig and Foster 1998). The presence of HWA and subsequent loss of the eastern hemlock in the forests it once dominated has resulted in a measurable change to many valuable ecosystem functions (Ford et al. 2011, Krapfl et al. 2011)

While most eastern hemlocks show little resistance to HWA, there is some evidence that particular environmental conditions, geography, and forest structure dynamics promote or obstruct the impact of the pest and its associated mortality, though the particularities of this phenomenon are not well known (Montgomery et al. 2009). The conditions under which hemlocks appear to exhibit some natural resistance to HWA are of critical importance to the employment of ecosystem management practices in affected areas. Given the speed of the progression of the adelgid and the intensity and invasiveness of current treatment options

(Dilling et al. 2009), the ability to target the most susceptible trees for treatment will reduce unnecessary landscape disturbance and resource expenditure.

In the fall of 2016, I established permanent vegetative sampling plots in three hemlock-dominated old-growth forests protected under the management of the Highlands-Cashiers Land Trust, based in Highlands, North Carolina. Each study area was variably affected by HWA, providing a spectrum of conditions for analysis. The objectives of this project were to (1) quantify differences in community structure between local study plots, (2) compare the results to survey data collected from similar sites nearby in Macon County prior to the arrival of the HWA to examine possible changes over time in local hemlock forest conditions in response to the invasive pest, and (3) lay the grounds for long-term research in vegetative diversity to monitor forest and community structure over time.

METHODS

Location selection

In this study, I explored botanical differences between unlogged, hemlock-dominated forests in different states of decline in response to the HWA. I worked with the Highlands-Cashiers Land Trust (HCLT) and conducted my work in three Macon County areas under their protection: (1) the intermediately affected Brushy Face site where hemlocks share canopy space with deciduous cove species, (2) the Valley of the Giants where the hemlock population has been largely extirpated, and (3) the Henry Wright Preserve, an area that remains predominantly unaffected by *A. tsugae* (table 1).

TABLE 1. The locations of permanent plots at each site, and dates of vegetative surveys. Coordinates have been abbreviated for the Valley of the Giants and the Henry Wright Preserve to protect sensitive communities with privately owned accesses routes.

Site	Observation date(s)	Latitude	Longitude
Brushy Face (BF)	10/7/16; 10/13/16	35.035917	-83.201646
Valley of the Giants (VG)	10/20/16	35.04	-83.18
Henry Wright Preserve (HW)	10/28/16	35.08	-83.18

The Brushy Face Preserve is an 11.7-acre plot that came under the protection of the HCLT in 2008. It consists of a broadly sloped cove that follows a stream paralleling a section of North Carolina Highway 28. Hemlock growth is concentrated along the banks of the stream. Evidence of dieback in the form of fallen branches is clear when navigating the site.

The Valley of the Giants is a 74-acre tract of a former hemlock forest that contains very few surviving hemlocks. Massive standing dead trees bracketed by ancient, multi-stemmed *Magnolia fraseri* provide a glimpse into what was once a thick evergreen-dominated forest, now mostly consisting of open patches of woody debris from fallen trunks and canopy. A stream also runs through the property at its lowest point.

Finally, standing at 22 acres as one of the best examples of old-growth hemlock forest in the Eastern United States, the Henry Wright Preserve is a fragment of a formerly contiguous ecosystem that was partially logged and developed in the 1950s. Its eastern hemlock population is concentrated at the base of a steep incline along a stream, which is lined with boulders and contains more exposed bedrock than the other two sites. Hemlocks in this plot actively receive pesticide treatments to prevent HWA infestation.

Plot establishment

At the study locations, I set up permanent rectangular plots around living hemlocks with one axis initiated in a creek when possible. This eased the establishment of the axis while typically allowing the plot to capture a greater range in microhabitat conditions from wet bog to drier ridge.

I laid out survey plots according to Carolina Vegetation Survey (CVS) protocol as pioneered by Peet, Wentworth, and White (1998). Using a 50 m survey tape, I measured out a 20 m by 50 m (1000 m^2) quadrat, partitioned into 10m stretches marked with pin flags. I attempted to keep lines as straight as possible by using a compass to shoot a bearing, accepting minor deviations from the line as reasonable error due to the dense and obstructive rhododendron understory.

Each plot consisted of ten 100 m^2 modules, four of which contained "intensive corners" designed to capture vegetation at diminishing scale through the construction of two $\log_{10}$ series of non-permanent interior subquadrats (Peet et al. 1998; figs. 1 & 2). I laid out these smaller, non-permanent sample areas with a 1 m by 1 m PVC framework marked at relevant intervals at the time of surveying.

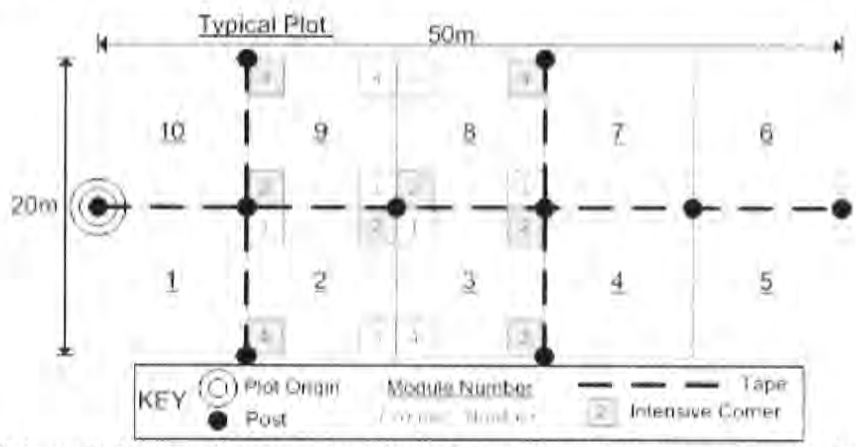


FIG 1. Schematic layout of survey plots from Peet et al. (1998), illustrating standardized location and scaling of the intensive corners of nested subquadrats. Though CVS protocol allows for flexibility of the arrangement of these intensive plots, basic locations shaded in gray were retained for this study.

Vegetative Surveys

I conducted a vegetative survey at each site to determine community species assemblages. Survey methods also followed standard CVS protocol (Peet et al. 1998), with slight modifications. I recorded all species using a 6-letter abbreviated code consisting of the first three letters of genus and species when identifiable to this degree; abbreviated record names of specimens that could only be identified to genus consisted of the first four letters of the aforementioned genus followed by 'SP' to represent unknown species. A list of species abbreviations and corresponding Latin names may be found in Appendix II.

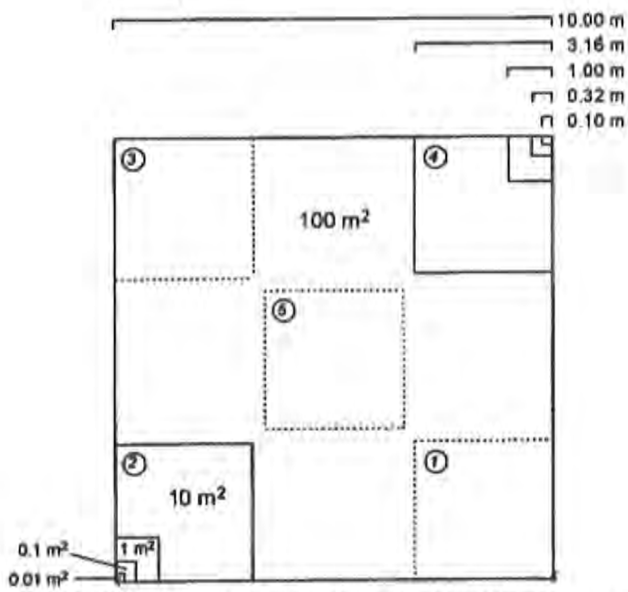


FIG 2. Graduated scale of nested subquadrats, from Lee et al. (2008); these smaller plots were established in two of the four corners illustrated here, varying between intensive modules to capture all possible combinations. The center possibility was not considered due to the increased difficulty involved in laying it out accurately in space. The locations used in this study are illustrated in Figure 1.

The vegetative survey consisted of three distinct parts. I first conducted a blanket search for canopy and subcanopy tree species in each subplot. I measured woody stems of all living trees and standing dead hemlocks greater than 2.54 cm in diameter at breast height (DBH), four feet from the upland forest floor. Given the extreme abundance of *Rhododendron maximum* (rosebay rhododendron) and *Kalmia latifolia* (mountain laurel) in the quadrats and the difficulty of navigating their so-called 'hells', I did not record DBH for these species even when the shrubs reached traditional 'subcanopy' tree heights, deviating from typical CVS protocol. The abundance of these species was well represented in other survey elements outlined below.

I then surveyed the nested quadrats in the intensive modules for all rooted species, beginning at the smallest sub-section (.01 m^2) and scaling outwards to the largest sub-section (3.16 m^2). Once surveys from both sets of nested quadrats within the module were complete, I also examined the whole 10 m^2 plot as the largest intensive scale. I recorded percent cover for each species in the overall module. These measurements were sorted by community level to allow for the overlap of stratified species. I classified species and growth forms into five groups: canopy (the upper cover layer of tree coverage within a forest), subcanopy (saplings and small trees of less than 5 m tall), shrubs (multi-stemmed, sprawling species of varying heights), herbs (non-woody, ground-covering species and the seedlings of other groups), and vines (climbing species rooted within the ground of the plot).

Finally, I surveyed the six remaining modules cumulatively for any species not represented in the intensive modules. I determined overall percent cover of each species by community structure level across the full extent of the 1000 m^2 survey plot. Species that were not rooted in the total study plot but contributed canopy cover were included at this survey level. A cover class was assigned to each species according to standardized ranges described in the CVS protocol (Lee et al. 2008; table 2).

TABLE 2. Ranges of percent cover as grouped into discrete cover classes, as described in Lee et al. (2008). This manner of classification reduces the effects of the randomness of user-determined cover, allowing greater comparison of species and plots.

Carolina Vegetation Survey Cover Classes			
1	Trace (<0.1%)	6	10-25%
2	0-1%	7	25-50%
3	1-2%	8	50-75%
4	2-5%	9	75-95%
5	5-10%	10	95-100%

Analysis

I compared the contents of the three study plots to determine if there was significant difference in community structure between sites of varying post-HWA health. In an attempt to contextualize my results, I also compared data I collected to those drawn from prior CVS data collected in hemlock-dominated forests in nearby Macon County.

The indicator value (IV) for woody species within each study site quadrat was calculated to characterize community dynamics and determine dominant species in each plot. These indices were run through PC-Ord (McCune 1999), an ecological community analysis program, according to the methodology of Dufrêne and Legendre (1997). Their equation involves the multiplication of relative abundance and relative frequency, A and F, together and then by 100, as in

$$IV = A * F * 100$$

where A refers to the ratio of a particular species to the total number of organisms in a plot and F is the percent of modules out of the total sampled that contains the species in question.

Once again using PC-Ord (McCune 1999), multidimensional scaling ordinations were created for three groups of data. Ordination is a statistical technique that represents portions of ecological communities as points in a coordinate plane. PC-Ord utilizes a dissimilarity matrix through non-metric multidimensional scaling (MNS) to most accurately display overlap in species composition between sites. Ordinations are basally structured as Cartesian coordinate systems where the axes have no independent value and serve only to illustrate distance between graphed elements according to a Sørensen dissimilarity index. For this reason, ordinations are most valuable as visual representations of data; further information, such as p-values to test for true correlations, cannot be drawn from the product without risk of stretching the results (Pielou 1984).

The first ordination compared canopy species through the DBH measurements of all study plots, while the second compared species assemblages with the cover classes of all species in the intensive study plots. The final ordination compared the overall cover classes for all species in the three study quadrats to compatible data from six additional plots from nearby sites in Macon County (Peet et al. 2012; table 3). These external plots were within ten miles of the study sites and contained significant hemlock populations with a cover class greater than 4 (table

2). DBH measurements were not available for all pre-HWA surveys, so percent cover was used as the point of comparison between external and study data.

TABLE 3. The locations of six additional CVS plots in Macon County near the Town of Highlands, NC. Coordinates have been abbreviated here for data security, but links to full online references can be found in Appendix I (Peet et al. 2012).

CVS observation code	Observation date	Latitude	Longitude
013-0K-0057	8/10/1991	35.00	-83.14
021-01-0063	7/13/1994	35.08	-83.18
021-01-0070	7/25/1994	35.08	-83.17
023-01-0040	1/1/1996	35.03	-83.17
023-01-0006	1/1/1996	35.03	-83.23
022-01-0375	7/12/1997	35.04	-83.18

RESULTS

Woody Stem Diameter

Woody stem data was most useful in characterizing the forest type of each site. Canopy species frequently typifies forests, as specific tree assemblages are associated with more intricate factors such as moisture level and soil type (Prentice et al. 1992). The raw data has been presented in Tables 4 - 6. Figures 3 and 4 compare cumulative DBH for each species between the three sites and display this data in two different configurations.

TABLE 4. DBH measurements (in meters) for trees in Brushy Face modules 1-10. Cumulative values represent total woody stem presence across the overall quadrat. See Appendix II for key to species name abbreviations.

	Species									
Module	Acerub	Betlen	Lirtul	Magfra	Sasalb	Tsucan	Hamvir	Ilemon	Amearb	Cleacu
BF1	0	0.1812	0	0	0	0.853	0	0	0	0
BF2	0	0	0	0	0	0.631	0	0	0	0.0894
BF3	0	0	0	0	0	0	0	0.1791	0	0
BF4	0.468	0.5589	0	0	0.4025	0.198	0	0.302	0	0
BF5	0.484	0.412	0	0.5108	0	0	0	0	0	0
BF6	0.8536	0	0	0.145	0	0.8762	0.3954	0	0.033	0
BF7	0	0	0	0	0	0	0	0.9865	0.0995	0
BF8	0	0.559	0	0	0	0.584	0	0	0	0
BF9	0	0	0	0	0	1.1953	0.2645	0	0	0
BF10	0	0.1276	0.3885	0	0	0	0.4567	0	0	0
Cumulative	1.8056	1.8387	0.3885	0.6558	0.4025	4.3375	1.1166	1.4676	0.1325	0.0894

TABLE 5. DBH measurements (in meters) for trees in Valley of the Giant modules 1-10; plots lacking trees were excluded. Cumulative values represent total woody stem presence across the overall quadrat. The majority of 'Tsucan' (*Tsuga canadensis)* measurements were taken from standing dead wood, or trunks that remained upright but possessed no living canopy; few living hemlocks remained in the plots. See Appendix II for key to further species name abbreviations.

	Species			
Module	Betlen	Magfra	Tsucan	Ilemon
VG2	0	0	0.8505	0
VG3	0.09	0	1.289	0.5782
VG4	0.4645	0	0.8567	0
VG5	0	1.1108	0.7015	0
VG6	1.0226	0.6232	0.1888	0.0424
VG7	0	0.523	1.121	0.9203
VG8	0.1735	0	0	0
VG10	0	0	0.945	0
Cumulative	1.7506	2.257	5.9525	1.5409

TABLE 6. DBH measurements (in meters) for trees in the Henry Wright Preserve, modules 1-10; plots lacking trees have been excluded. Cumulative values represent total woody stem presence in the overall quadrat. See Appendix II for key to species name abbreviations.

	Species						
Module	Acerub	Betlen	Lirtul	Magfra	Tsucan	Halcar	Ilemon
HW1	0	0	0	0	0	0.183	0.0965
HW2	0	0	0	0	0.461	0	0
HW3	0	0	0	0	1.246	0	0
HW4	0	0	0	0	1.7055	0	0
HW5	0.097	0.1865	0	0	0.556	0	0
HW7	0	0	0	0	1.006	0	0
HW9	0.665	0	0.9475	0	0.276	0	0
HW10	0	0	0	0.449	0	0	0
Cumulative	0.762	0.1865	0.9475	0.449	5.2505	0.183	0.0965

All quadrats showed greatest cumulative diameter measurements for *T. canadensis*, though the degree to which these trees surpassed other canopy species in this regard differed between locations. The study plot at Brushy Face yielded the smallest hemlock diameter measurements, while the Valley of the Giants still maintained the greatest dominance of the species even though nearly all measured trees were classified as 'standing dead' wood. The overall density of trees within each site is also notable. Brushy Face contained the greatest number of trees over the 1000 m^2 sample quadrat, while the Henry Wright preserve was the least densely wooded location (fig. 3).

To compare dominance among other trees across the three plots, I weighted the data represented in Figure 3 against total DBH values of all species, resulting in percentages of overall diameter for each species. Figure 4 illustrates that hemlocks were most dominant at the Henry Wright Preserve and least so at the Brushy Face site, where *Tsuga canadensis* shared dominance with *Acer rubrum, Betula lenta,* and a number of subcanopy hardwood species not found at the other two sites.

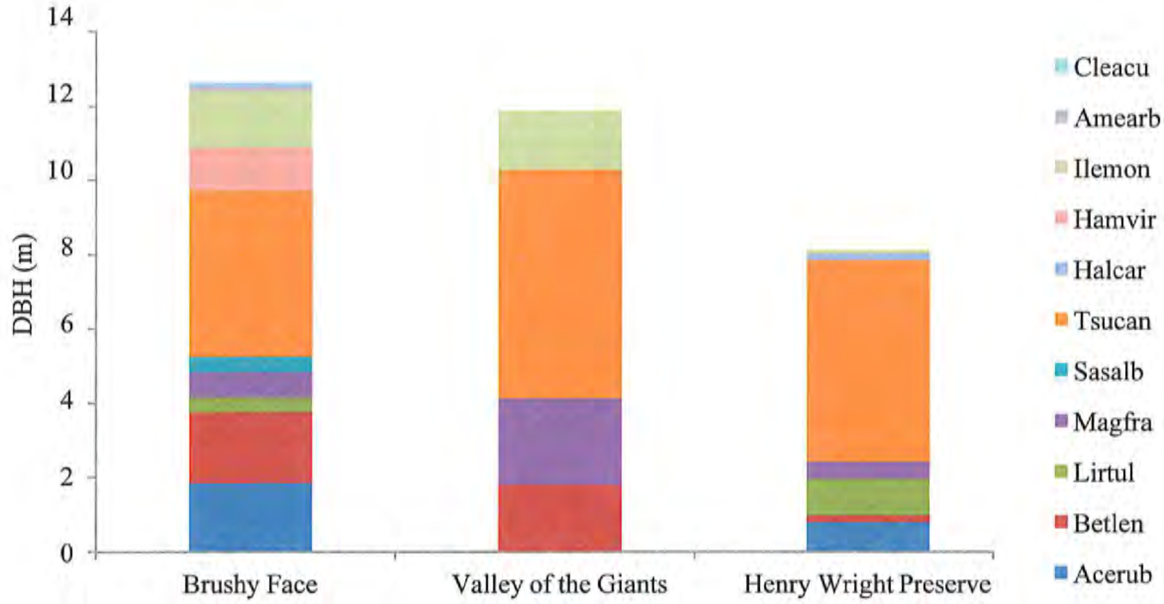


FIG 3. Total DBH values for canopy and subcanopy species in quadrats of each site. This figure illustrates the volume of *Tsuga canadensis* and co-occurring species at each site, as well as the overall density of trees present. See Appendix II for key to species name abbreviations.

FIG 4. DBH percentages for canopy and subcanopy species in quadrats of each site. This figure illustrates the diameter volume out of 100% of each species in the study areas, more accurately reflecting the dominance of *T. canadensis* at the Henry Wright Preserve. See Appendix II for key to species name abbreviations.

Indicator Values

Indicator values (IV) are often used in ecological research to characterize and classify habitat types. They allow the relative weighting of ecosystem elements to essentially determine the species of greatest significance within an area or region. Indicator species identified by these values have been found to be representative of the habitat conditions under which they have risen to dominance, allowing for easier comparison between locations, based primarily on species composition (Dufrêne and Legendre 1997). The values displayed in Table 7 are unitless, but illustrate compositional distance between the three locations.

I compared the highest-ranked species between sites as another test of species dominance within sites, displayed in Figure 5. Indicator values suggest that the sites are quite different in terms of the species that characterize them. Brushy Face is represented by a wide variety of trees, while the Valley of the Giants has more of an even split in dominance between *T. canadensis*, *Betula lenta*, and *Magnolia fraseri*. In contrast, the Henry Wright Preserve shows near exclusive IV dominance of *T. canadensis*.

TABLE 7. Indicator values (%) calculated by summing the relative abundance and relative frequency of individual species within overall quadrats. See Appendix II for key to species name abbreviations.

	Relative Abundance			Relative Frequency			Indicator Values		
Species	BF	VG	HW	BF	VG	HW	BF	VG	HW
Acerub	65	0	35	30	0	25	20	0	9
Betlen	43	51	5	50	50	13	22	26	1
Lirtul	25	0	75	10	0	13	2	0	9
Magfra	16	70	14	20	38	13	3	26	2
Sasalb	100	0	0	10	0	0	10	0	0
Tsucan	24	41	36	60	88	75	14	35	27
Halcar	0	0	100	0	0	13	0	0	13
Hamvir	100	0	0	30	0	0	30	0	0
Ilemon	42	55	3	30	38	13	13	21	0
Amearb	100	0	0	20	0	0	20	0	0
Cleacu	100	0	0	10	10	0	10	0	0

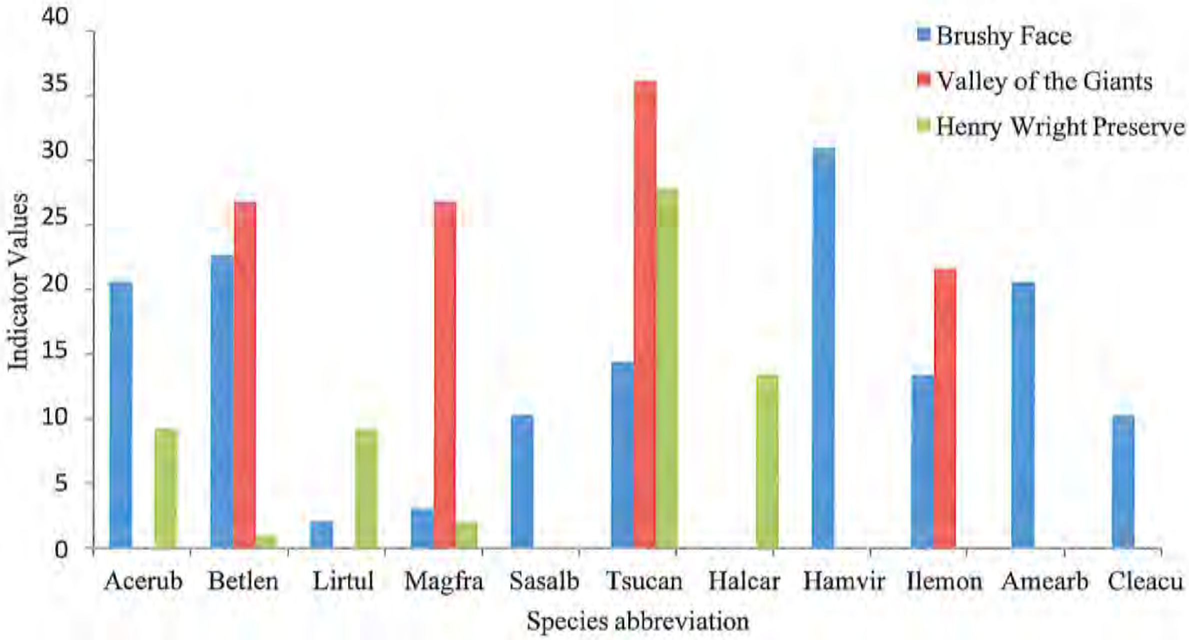


FIG 5. A comparison of indicator values for all woody species across all site quadrats, excluding *Rhododendron maximum* (rosebay rhododendron) and *Kalmia latifolia* (mountain laurel) due to difficulty of measuring all stems of these plants in thick stands. Standing dead wood was measured and included, and comprises a large portion of 'Tsucan' (*Tsuga canadensis*) results. Species listed to the right of 'Tsucan' are subcanopy/shrubby plants that experience dominance on a different plane than the other illustrated canopy species, but are no less important indicators of ecosystem type. See Appendix II for key to further species name abbreviations.

Percent Cover

Percent cover provided a secondary examination of canopy density (table 8) and captured shrubs and groundcover species within plots (table 9). Species assemblages differed in a broader sense when examined at the herbaceous level, where specific microhabitat conditions allow for a greater range of adaptation and diversity than the canopy can accommodate (Prentice et al. 1992). It is important to note that cover classes were also determined for species that were not rooted in the plots but contributed to canopy cover.

Figure 6 illustrates the differences in species assemblages across all community levels. Botanical biodiversity, represented here by the sections within the charts, was highest at Brushy Face and lowest in the Henry Wright Preserve. Figure 6 also serves as another reflection of dominance, though herbaceous species experienced obscured representation due to the inclusion of the much broader extent of canopy species. In terms of canopy cover in particular, *Tsuga candensis* in the Brushy Face plot shared dominance with *Betula lenta*, *Acer rubrum*, and *Sassafras albidum*. In the Valley of the Giants, *B. lenta* and *Magnolia fraseri* completely surpassed *T. canadensis* in cover dominance. *T. canadensis* retained cover dominance in the Henry Wright Preserve, but coexisted with a number of deciduous hardwoods including *A. rubrum*, *B. lenta*, *Liriodendron tulipifera*, *M. fraseri*, and *Quercus rubra* at the plot margins.

TABLE 8. Cover class for canopy species in complete (overall) quadrats. See Appendix II for key to species name abbreviations.

	Species							
Module	Acerub	Betlen	Lirtul	Magfra	Oxyarb	Querub	Sasalb	Tsucan

BF	4	6	0	2	3	3	4	6
VG	0	7	0	7	0	0	0	4
HW	6	4	6	4	0	4	0	8

TABLE 9. Cover class values for subcanopy and shrub species in complete (overall) quadrats. See Appendix II for key to species name abbreviations.

	Species								
Module	Halcar	Hamvir	Ilemon	Amearb	Cleacu	Kallat	Leufon	Rhomax	VaccSp
BF	0	4	4	2	2	6	0	8	0
VG	0	0	5	0	0	4	0	8	2
HW	2	0	1	0	0	0	5	9	0

TABLE 10. Cover class values for woody seedlings, herbaceous species, and vines in complete (overall) quadrats. See Appendix II for key to species name abbreviations.

Species	Module		
	BF	VG	HW
Acerub	1	1	1
Aritri	1	0	0
Aspmon	0	0	1
Betlen	1	1	1
BrasSp	1	0	0
CareSp	1	0	0
Chimac	1	0	0
Cleacu	2	2	0
Denpun	0	1	0
Dryint	1	1	1
Gaybac	2	1	1
Galurt	2	1	0
Goopub	1	1	0
Hamvir	1	0	0
Hexshu	1	0	0
Hydarb	0	0	1
Ilemon	1	1	1
Impbif	1	0	0
Kallat	1	1	0
Lirtul	1	1	1
Magfra	1	1	0
Medvir	1	1	0
Nyssyl	1	0	0
Oxastr	1	0	0
PileSp	1	0	0
Pinstro	1	0	0
Polacr	0	0	1
Polper	1	0	0
Quemon	1	0	1
Querub	0	1	1
Rhomax	1	1	0
Ruball	1	4	1
ViolSp	1	0	1
Smilax	1	1	2
Bryoph	2	2	4

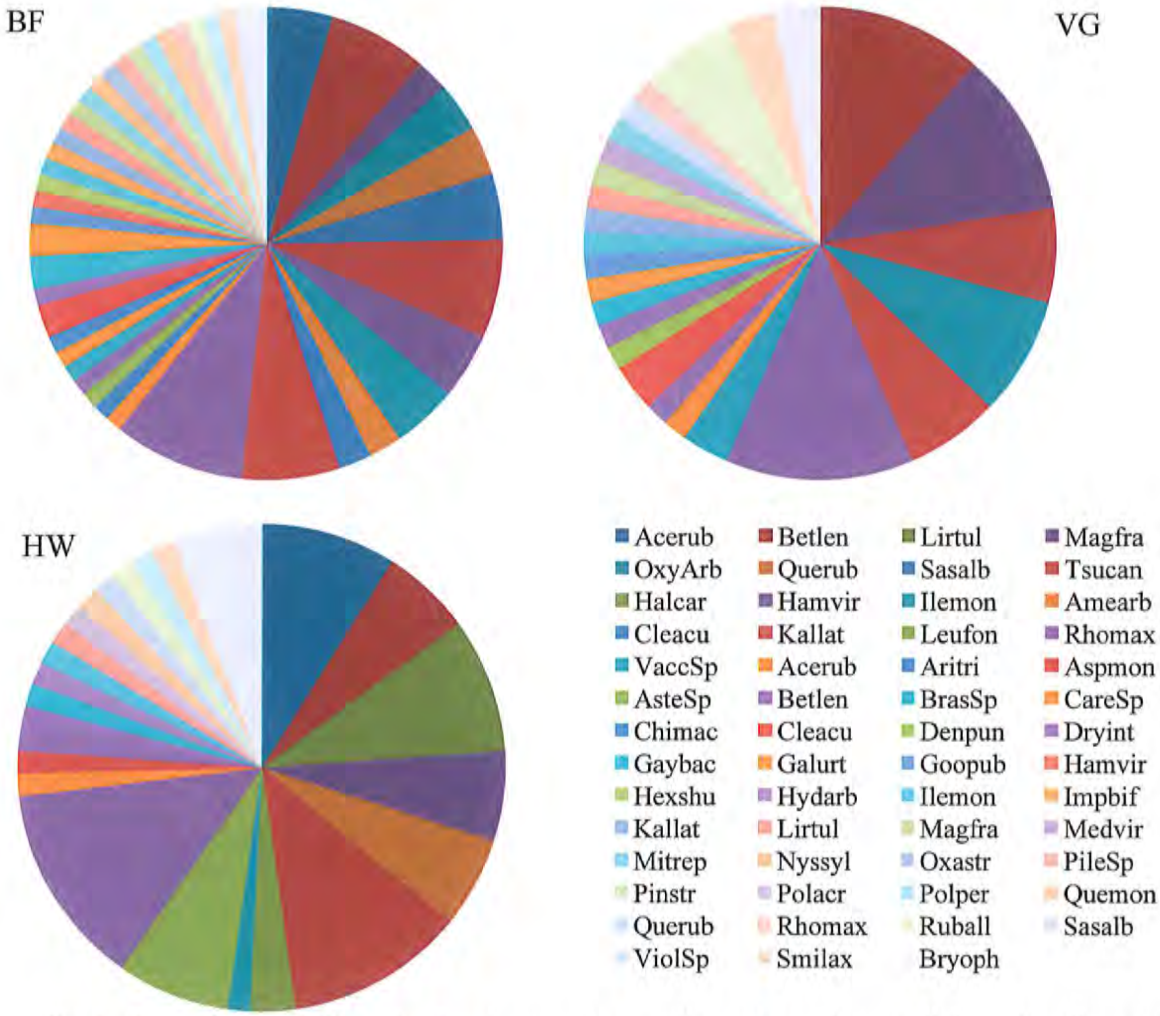


FIG 6. Cover classes of all species found across the overall sample quadrats at all three sites. From left to right, the top two figures represent species assemblage by cover class for Brushy Face and the Valley of the Giants; the bottom left figure therefore represents the Henry Wright Preserve. See Appendix II for key to species name abbreviations. Second occurrences of woody species within the legend refer to seedlings.

Ordination

Ordination of the DBH measurements from all wooded modules across sites did not reveal much similarity within or between the different locations (fig. 7). However, when cover classes of *Rhododendron maximum, Kalmia latifolia,* herbaceous species, seedlings, and vines were included in addition to other woody species in an ordination, the intensive modules of the different locations began to cluster together in separate regions of the plane. A three-dimensional analysis best revealed these groups (fig. 8). This second ordination also showed that the internal modules of the plots were more similar to one another, illustrating that the individual habitats of the local conditions of each site were generally associated with specific assemblages.

Ordination comparing study sites to pre-HWA Macon County survey data showed them to be significantly distinct from four of the external surveys, but quite similar to the remaining two (fig. 9). While interesting, this does not reflect a defensible or notable difference in species assemblages over time and space.

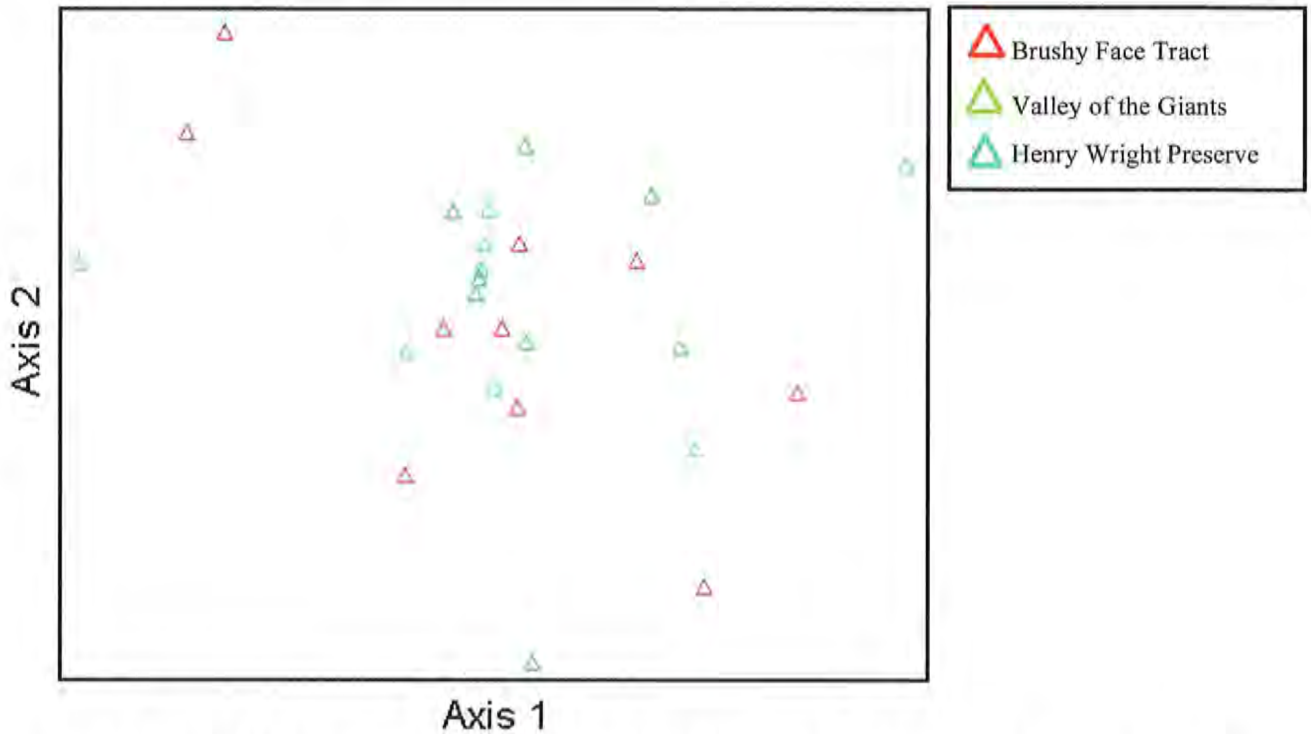


FIG 7. PC-Ord MNS ordination of woody stem data (DBH) from all plots containing canopy/subcanopy species, displayed in two dimensions. Axes represent non-dimensional distance between plot elements, rather than physical space.

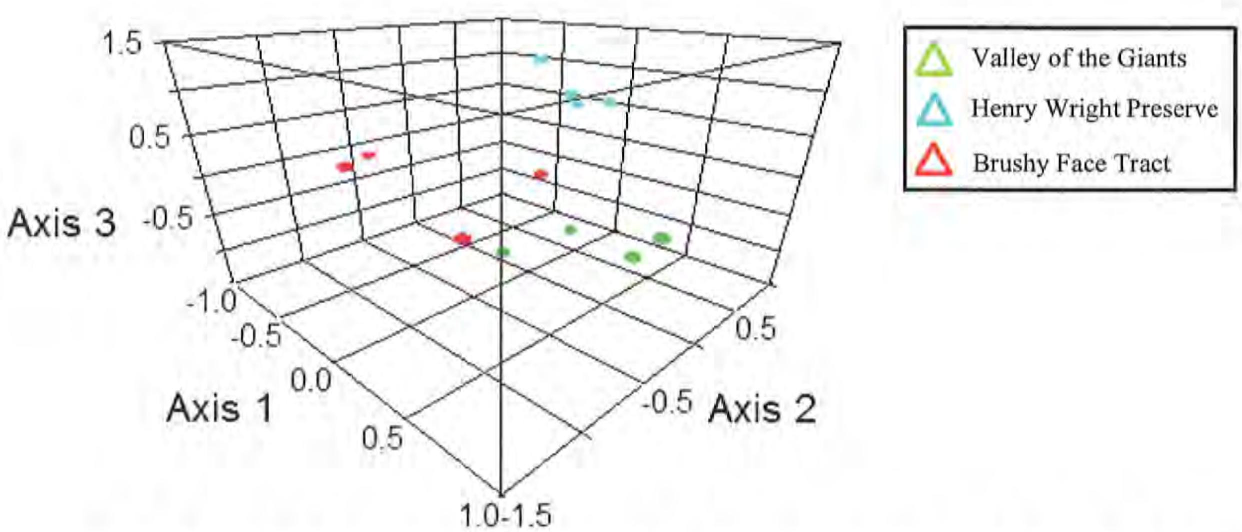


FIG 8. PC-Ord MNS ordination of cover class for all four intensive plots in the three site quadrats, displayed in three dimensions to better illustrate the clustering of points. Axes represent non-dimensional distance between plot elements, rather than physical space.

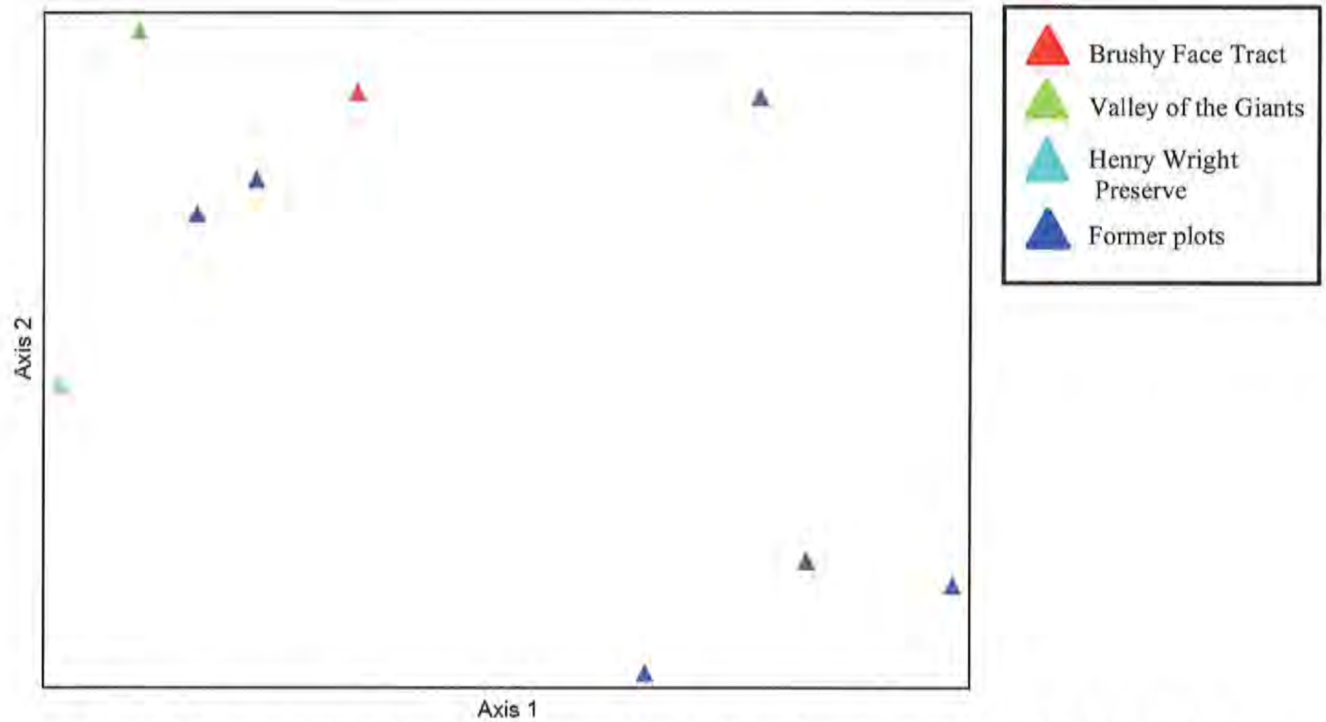


FIG 9. PC-Ord MNS ordination of species assemblage by cover class from the three study plots and six pre-HWA CVS surveys also conducted in Macon County, North Carolina.

DISCUSSION AND CONCLUSIONS

The overall survival of hemlocks at Brushy Face and the Henry Wright Preserve is atypical in the Southern Appalachians. A study conducted in New England found 80% hemlock mortality 15 years after HWA arrival (Small et al. 2005), while long-term research plots at the Coweeta Hydrologic Laboratory in Otto, North Carolina, showed 80% mortality within six years of infestation (Ford et al. 2011). Southern Appalachian conditions such as heavy rainfall and mild seasons favor the spread and success of the HWA, but also the growth of *Tsuga canadensis* (Ford et al., 2011, Krapfl et al. 2011). A dense abundance of trees in favorable habitat facilitates the spread of crawling HWA larvae and advances infestations (Ford et al. 2011).

DBH measurements, IV results, and cover class data all provided insight to the significance of tree species at each location. *T. canadensis* presented the greatest cumulative woody stem diameter at all three sites (fig. 4), but indicator values suggested that hemlocks did not significantly characterize the forest type at Brushy Face (fig. 5). Cover data showed that *T. canadensis* occupied similar canopy classes at Brushy Face and the Valley of the Giants (fig. 6); however, hemlocks at the Brushy Face site shared woody stem dominance with other hardwood species while those in the Valley of the Giants were the primary canopy species by volume (fig. 4).

After HWA infestations reach the branches of mature hemlocks, significant defoliation may occur within a few years (Small et al. 2005, Ford et al. 2011, Krapfl et al. 2011). The resulting increase in canopy openness and light penetration has cascading impacts on the microclimate and growth patterns in the overall forest (Small et al. 2005). This ultimately results in rapid growth for shade-intolerant canopy competitors like *Acer rubrum, Betula lenta,* and

Liriodendron tulipifera (Ford et al. 2011), leading to a species assemblage that more closely resembles that of the Brushy Face cove hardwood forest in primary composition (figs. 3 & 6). However, shrub expansion and herbaceous recruitment occur in open and disturbed areas of transitioning cover, resulting in initial local increases in plant biodiversity where light reaches the forest floor (Small et al. 2005). Herbaceous diversity was highest at Brushy Face and lowest in the Henry Wright Preserve (table 10; fig. 6). Herbaceous species and woody seedlings grew on nurse logs and in open forest patches at Brushy Face and the Valley of the Giants, while those at the Henry Wright Preserve occurred nearly exclusively in HWA treatment clearings around hemlocks; disturbance was a common factor in herbaceous development across sites.

Ordinations visually captured the dissimilarity of species assemblages at the three locations. Sites examined in this study were distinct from one another when forest cover and understory species were included in analysis (fig. 8), but not when interpretation was restricted to the density of canopy and subcanopy trunks (fig. 7). Due to the longevity of their growth patterns, tree density remains relatively constant even after a significant change in microclimate (Ellison et al. 2005, Small et al. 2005). Contrastingly, shrubs, herbaceous plants, and woody seedlings are quick to respond to disturbance events in local conditions, and better characterize a forest in a state of transition (Orwig and Foster 1998, Small et al. 2005, Ford et al. 2011).

Exterior ordinations indicated that the three study sites were distinct from four out of six pre-HWA survey plots, though the final two appear to be indistinguishable from modern site data (fi. 9). With the majority of historic surveys appearing as different from current assemblage data, an assumption could be made that hemlock forests in Macon County have changed as a whole from pre-HWA conditions. However, the sample size of both historic and modern data sets was too small to draw significant conclusions suggesting a local shift in community ecology; further research on this subject would require a much broader range of material for study.

Some notable sources of error likely contributed to differences in species assemblage when study sites were compared against one another and the external pre-HWA data through ordinations. An abnormally extended drought over the course of the study resulted in early dieback of herbaceous species and abnormal growth patterns for hydric species. Due to the lengthy process of establishing plots, I was unable to conduct surveys until October; traditional CVS protocol requires that all survey data be collected between June and September to maximize species diversity and capture the full extent of the herbaceous layer of a forest habitat (Lee et al. 2008). In addition, I surveyed the plots during the full month of October (table 1), over the course of which there were notable differences in canopy cover and herbaceous abundance with annual leaf drop and abscission. This affected not only comparisons within the study, but also those made to external data; pre-HWA sites were surveyed in July, August, and curiously, January (table 2). Species assemblages differ throughout the year, amounting to an inaccurate comparison between the sets of sites when data result from different timeframes.

Environmental parameters such as soil moisture, litter depth, elevation, slope, aspect, and proximity to water were not taken into consideration in this study. These measurements would have provided further insight into the conditions that made sites and associated species assemblages unique (Kincaid 2007). Additionally, little was known about the environment of pre-HWA sites compared to study plots in the final ordination. I selected external sites based on proximity to my research plots, but mountain habitats are sufficiently variable that wholly different environmental conditions persist within a single square mile. One site in particular had a significant population of *Tsuga caroliniana,* a Southern Appalachian endemic generally restricted to rocky gorge walls (Weakley 2015). While the two species of hemlock sometimes co-occur in open forests, it is also possible that this site was a rocky ledge community. Studies found that hemlock mortality was more severe in rocky and dry regions than in the more mesic montane coves where study plots were established in this study, potentially skewing the results towards exaggerated distinction.

A major component of this project was the establishment of permanent sample plots at the three study sites. It is the hope of the Highlands-Cashiers Land Trust, the Highlands Biological Station, and myself that these plots be utilized in future years to monitor changes in the species assemblage of these locations. This preliminary study revealed that significant differences do occur at herbaceous scales at these sites. Perhaps future study following this project could expand plots and surveys to a wider array of properties and corroborate these results, or draw new conclusions related to change in hemlock community dynamics over broader spatial and temporal scales.

ACKNOWLEDGEMENTS

I owe thanks first and foremost to my mentor and the executive director of the Highlands-Cashiers Land Trust, Dr. Gary Wein, who helped me navigate the confusing depths of rhododendron hells and scientific papers alike. I would still be in those hells without Kyle Pursel who cleared the way for me, literally when the underbrush was too dense and figuratively during the development of this project. Thanks is also due to Dr. Beverly Collins of Western Carolina University who kindly assisted me with data analysis to produce ordinations, and taught me and my classmates everything we know about community ecology. I would have been lost without the help of Dr. Robert Peet of the University of North Carolina at Chapel Hill, who provided me with a wealth of information and endured my constant stream of questions with patience and kindness. Finally, I must thank Dr. James Costa, Dr. Sarah Workman, and Ms. Alyssa Fuller of the Highlands Biological Station, who led my classmates and me through an excellent semester in the field.

LITERATURE CITED

Coots, C., P. Lambdin, J. Grant, R. Rhea, and E. Mockford. 2012. Pscopteran species associated with eastern hemlock in Southern Appalachians. Florida Entomologist **95** :224-227.

Dilling, C., P. Lambdin, J. Grant, and R. Rhea. 2009. Community response of insects associated with eastern hemlock to Imidacloprid and horticultural oil treatments. Environmental Entomology **38**: 53-66.

Dufrêne, M. and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs **67**: 345-366.

Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliot, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, G. M. Lovett, J. Mohan, D. A. Orwig, N. L. Rodenhouse, W. V. Sobczak, K. A. Stinson, J. K. Stone, C. M. Swan, J. Thompson, B. V. Holle, J. R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment **3**: 479-486.

Ford, C. R., K. J. Elliot, B. D. Clinton, B. D. Kloeppel, and J. M. Vose. 2011. Forest dynamics following eastern hemlock mortality in the southern Appalachians. Oikos **121**: 523-536.

Kincaid, J. A. 2007. Compositional and environmental characteristics of *Tsuga canadensis* (L.) Carr. forests in the Southern Appalachian Mountains, USA. Journal of the Torrey Botanical Society **134**: 479-488.

Krapfl, K., E. Holzmueller, and M. Jenkins. 2011. Early impacts of hemlock woolly adelgid in Tsuga canadensis forest communities of the southern Appalachian Mountains. Journal of the Torrey Botanical Society **138**: 93-106.

Lee, M. T., R. K. Peet, S. D. Roberts, and T. R. Wentworth. 2008. CVS-EEP Protocol for Recording Vegetation, Version 4.2. Biology, University of North Carolina, Chapel Hill.

Martin, K. L. and P. C. Goebel. 2013. The foundation species influence of eastern hemlock (*Tsuga canadensis)* on biodiversity and ecosystem function on the Unglaciated Allegheny Plateau. Forest Ecology and Management **289**: 143-152.

McCune, B. and M. J. Mefford. 1999. PC-ORD. Multivariate analysis of ecological data, version 4.0 for Windows.

Montgomery, M. E., S. E. Bentz, and R. T. Olsen. 2009. Evaluation of hemlock (*Tsuga*) species and hybrids for resistance to *Adelges tsugae* (Hemiptera: Adelgidae) using artificial infestation. Journal of Economic Entomology **102**: 1247-1254.

Orwig, D. A. and D. R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. Journal of the Torrey Botanical Society **125**: 60-73.

Peet, R. K., T. R. Wentworth, and P. S. White. 1998. A flexible, multipurpose method for recording vegetation composition and structure. Castanea **63**: 262-274.

Peet, R.K., M.T. Lee, M.D. Jennings, & D. Faber-Langendoen. 2012. VegBank – a permanent, open-access archive for vegetation-plot data. Biodiversity and Ecology **4**: 233-241.

Pielou, E. C. 1984. The interpretation of Ecological Data: A Primer on Classification and Ordination. Wiley, New York.

Prentice, I. C., S. P. Harrison, R. Leemans, R. A. Monserud, and A. M. Solomon. 1992. A global biome model base on plant physiology and dominance, soil properties and climate. Journal of Biogeography **19**: 117-134.

Small, M. J., C. J. Small, and G. D. Dreyer. 2005. Changes in a hemlock-dominated forest following woolly adelgid infestation in southern New England. Journal of the Torrey Botanical Society **132**: 458-470.

Snyder, C., J. A. Young, D. P. Lemarie, and D. R. Smith. 2002. Influence of eastern hemlock (*Tsuga canadensis*) on aquatic invertebrate assemblages in headwater streams. Canadian Journal of Fisheries and Aquatic Sciences. **59**: 262-275.

Tingley, M. W., D. A. Orwig, R. Field, and G. Motzkin. 2002. Avian response to removal of a forest dominant: consequences of hemlock woolly adelgid infestations. Journal of Biogeography **29**: 1505-1516.

Weakley, A.S. 2015. Flora of the southern and mid-Atlantic states, working draft of May 2015. University of North Carolina Herbarium, North Carolina Botanical Garden, Chapel Hill, NC.

Yamasaki, M., R. M. DeGraaf, and J. W. Lanier. 2000. Wildlife Habitat Associations in Eastern Hemlock – Birds, Smaller Mammals, and Forest Carnivores. In: McManus, Katherine A.; Shields, Kathleen S.; Souto, Dennis R., eds. Proceedings: Symposium on sustainable management of hemlock ecosystems in eastern North America. Gen. Tech. Rep. NE-267. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. p.135-143.

Appendix I

Online VegBank entries for referenced external CVS Plots

CVS observation code	Reference Link
013-0K-0057	http://vegbank.org/cite/urn:lsid:cvs.bio.unc.edu:observation:2410-{E0AF9B45-9D29-4F0D-8612-52CBBAB78A07}
021-01-0063	http://vegbank.org/cite/urn:lsid:cvs.bio.unc.edu:observation:3086-{E440B812-1318-4A06-8C39-5D3A9CB50137}
021-01-0070	http://vegbank.org/cite/urn:lsid:cvs.bio.unc.edu:observation:3093-{56294409-707C-4D41-8E52-B515E87B1AB3}
023-01-0040	http://vegbank.org/cite/urn:lsid:cvs.bio.unc.edu:observation:3231-{795317BA-E634-4C09-AD57-84EC57817F06}
023-01-0006	http://vegbank.org/cite/urn:lsid:cvs.bio.unc.edu:observation:3197-{16A52868-B3E4-411D-B8E8-1E67A08218BF}
022-01-0375	http://vegbank.org/cite/urn:lsid:cvs.bio.unc.edu:observation:3099-{55DA59E2-58D4-4F8E-954A-9D1835D38628}

Appendix II

Species name abbreviations

Reference code	Latin name
Acerub	Acer rubrum
Amearb	Amelanchier arborea
Aritri	Arisaema triphyllum
Aspmon	Aslplenium montanum
Betlen	Betula lenta
BrasSp	Brassica sp.
CareSp	Carex sp.
Chimac	Chimaphila maculata
Cleacu	Clethra acuminata
Denpun	Dennstaedtia punctilobula
Dryint	Dryopteris intermedia
Gaybac	Gaylussacia baccata
Galurt	Galax urceolata
Goopub	Goodyera pubescens
Halcar	Halesia carolina
Hamvir	Hamamelis virginiana
Hexshu	Hexastylis shuttleworthii
Hydarb	Hydrangea arborescens
Ilemon	Ilex montana
Impbif	Impatiens biflora
Kallat	Kalmia latifolia
Leufon	Leucothoe fontanesiana
Lirtul	Liriodendron tulipifera
Magfra	Magnolia fraseri

Medvir	Medeola virginiana
Mitrep	Mitchella repens
Nyssyl	Nyssa sylvatica
Oxyarb	Oxydendrum arboreum
Oxastr	Oxalis stricta
PileSp	Pilea sp.
Pinstro	Pinus strobus
Polacr	Polystichum acrostichoides
Polper	Polygonum persicaria
Quemon	Quercus montana
Querub	Quercus rubra
Rhomax	Rhododendron maximum
Ruball	Rubus alleghaniensis
Sasalb	Sassafras albidum
Smilax	Smilax sp.
Tscucan	Tsuga canadensis
ViolSp	Viola sp.
Bryoph	Bryophyte

THE EFFECTS OF OFF-HIGHWAY VEHICLES ON STREAM SEDIMENTATION IN THE NORTH FORK OF THE BROAD RIVER BASIN

LAREN K. EVERAGE

Abstract. This paper reports the preliminary results of research conducted in the Chattahoochee National Forest to assess the impacts of off-highway vehicles (OHVs) on stream sedimentation. Research focused specifically on the effects of the Locust Stake OHV trail system, located in Habersham County, GA, on sedimentation in the North Fork of the Broad River. To examine whether trails caused increased levels of total suspended solids (TSS) and turbidity within stream channels, sedimentation effects were compared between two treatment sites in watersheds with OHV trails (Locust Stake and Upper Locust Stake) and one sampling site in a control watershed (Kimbell Creek). Within the treatment sites, sedimentation was studied during periods of trail closure and open, active use. Analysis indicated that watersheds with OHV trails experienced higher levels of both TSS and turbidity. Furthermore, rate of increase in TSS compared to flow was greater within treatment watersheds. The same results were observed when comparing open OHV trails to closed trail systems. Paired watershed analysis indicated that the Locust Stake OHV trail system has significant negative impacts on sedimentation in the North Fork of the Broad River, but more research is needed to quantify the magnitude to which open OHV trail systems affect stream sedimentation.

Key words: Chattahoochee National Forest; Locust Stake; off-highway vehicles; stream sedimentation; total suspended solids; turbidity.

INTRODUCTION

Off-highway vehicle (OHV) use is an increasingly popular outdoor recreational activity in the United States. For this study, OHVs are defined as: (i) four-wheel drive automobiles, including jeeps, pickup trucks, and sport utility vehicles; (ii) cross-country motorcycles; (iii) all-terrain vehicles (ATVs); and (iv) other specially designed or modified off-road motor vehicles. Between 1993 and 2003, the estimated number of OHVs within the United States increased by 174 percent, surpassing eight million (8,010,000) vehicles by the end of 2003 (Cordell et al. 2008). The number of persons above the age of 16 years participating in recreational OHV use also steadily increased after 1994, and by spring 2016, 11.09 million households owned at least one OHV (Statista 2016). OHV use peaked in 2008 with 13.27 million users participating in the activity and gradually declined to present day numbers (Statista 2016).

Since 2004, OHV users have accounted for over ten million visits to national forests and grasslands annually (USDA 2005). Of the 154 national forests in the United States, 112 are accessible to OHV users, opening a substantial portion of the 158,000 miles of managed trails to OHV traffic (USDA 2009, USDA 2013, USDA 2016). These trails are particularly important recreational areas within the southern United States and are widely used in Georgia and its surrounding areas. In 2007, 33.7% of all OHV users (76,997,300) were residents of the southern United States, and 3.1% of all participants (1,319,400) came from Georgia alone (Cordell et al. 2008). By 2012, 44% of all OHV sales were concentrated in the southern United States (Imlay 2014). A significant portion of these OHV users, including those in states other than Georgia, utilize the Locust Stake trail system for recreational purposes.

Balancing the desires of OHV users for more recreational opportunities with national directives to prevent undesirable environmental impacts introduces new challenges to public-land managers. OHV use leads to a wide variety of environmental impacts, including accelerated compaction and erosion of soils, decreased soil infiltration, altered magnitude and timing of stream discharge, modified stream channel structure, and, more generally, habitat degradation (Chin et al.

2004). This study examined the potential stream sedimentation responses of channels near OHV trails located within the Locust Stake OHV Trail System in Habersham County, GA.

The Locust Stake trail system is a designated OHV use area located within the Chattahoochee National Forest. The trails are open from March through December of each year but have the potential to close if more than 1.25 inches of rain fall within a 24-hour period. The trail system was developed in the mid-1980s from existing logging roads, and undesignated OHV use has since added additional trails to the system. Since the mid-1990s, concerns have risen regarding the potential sedimentation impacts from repeated trail use. The trails remained open until 2012 when they were closed to assess environmental damage. During this maintenance period, a series of silt fences were installed to attempt to control erosion. Loops 1-5 were reopened in 2014, offering five miles of useable trail. These loops were closed again in December 2014 for routine maintenance and remained closed until July 28, 2016. Five more miles of trail (including the entirety of Loop 6) were permanently closed in 2014 to mitigate the severe erosion associated with OHV use. Before 2014, an estimated 3,500-4,500 users per year utilized the trail system with hundreds more using the trail illegally. From its reopening on July 28, 2016 through October 31, 2016, 446 paid users (and an estimated 100-150 unpaid users) utilized the OHV trail system.

Water Quality Standards

The Georgia Department of Natural Resources (1974) has designated the North Fork of the Broad River as an important river for drinking water, supplying over one million people with clean water in 25 neighboring municipalities. Under this classification, the river must meet both state and federal water quality standards for drinking water. The U.S. Environmental Protection Agency (EPA) established limits to TSS levels in streams, which may not exceed a 30-day average of 30 mg/L or a seven-day average of 45 mg/L (USEPA 2000). Surface waters in excess of these limits are in violation of the provisions set forth under the Clean Water Act (USEPA 2011). Georgia also has its own set of standards for rivers supplying drinking water, but there are no quantitative specifications regarding allowable levels of either turbidity or TSS. These provisions rely solely on qualitative visual data to assess water clarity, stating simply that "all waters must be free from turbidity which results in a substantial visual contrast in a water body due to a man-made activity" (Georgia DNR 1974).

Previous Studies

Previous studies show that OHV trails, and more generally, all road or trail crossings, pose extensive threats to the geomorphology and hydrology of forest stream systems. OHV sites exhibit considerable soil loss within trail segments. Eroded soils deposit within stream channels, causing increased mud coating, sediment plug formation, embeddedness, and fine sediment deposition, and decreased pool depths and volumes (Chin et al. 2004, Marion et al. 2014). Increases in both TSS and turbidity can be attributed to erosional impacts from OHV use (Chin et al. 2004). Eroded sediments from trails are transported to nearby streams during storm events where they are either suspended in the water column or deposited along the stream bed. Several studies converge on the notion that the presence or absence of OHV use, including both past and current OHV use, has a greater effect on stream response than either intensity or recency of OHV usage (Foltz 2006, Marion et al. 2014).

A number of past reports have observed the geomorphic responses to OHV use in humid forested environments similar to the environments considered in this study. These reports found that most sediment that reaches stream channels originated from OHV trail segments located on hillsides with steep grades (Ayala et al. 2005). Marion et al. (2014), in a study conducted on OHV trails in the Ouachita National Forest in Arkansas, determined that individual stream response to OHV use was largely correlated with localized channel and valley geomorphology. Their research indicated that smaller channels are more consistent in their geomorphic responses than larger channels. They further concluded that sediment impacts outweighed runoff impacts in OHV affected areas, as evidenced by increased sediment deposition despite high runoff contributions in streams near trail crossings (Marion et al. 2014).

Few studies have directly examined the impact of OHV trails on levels of total suspended solids (TSS) within stream channels. TSS concentrations are dependent on rate of stream flow and are highly influenced by surrounding land use (Riedel and Vose 2002a;b, Clinton et al. 2010). During storm events, TSS levels are highest due to erosion from surface runoff and the resuspension of bed sediments. The purpose of this research project was to investigate potential sedimentation responses to OHV use within the Locust Stake OHV trail system. This study hypothesized that OHV trail systems had significant negative impacts on stream sedimentation, resulting in higher TSS levels per unit increase in flow as compared to a reference watershed. Specific objectives were to (i) examine the effect that OHV trails have on TSS levels of water samples collected during storm events; (ii) investigate the differences between TSS levels of streams near open versus closed trail systems; and (iii) determine if a correlation exists between stream flow and TSS in each of the treatment and reference watersheds.

STUDY AREA AND METHODS

Study Area

The study area encompasses two watersheds located within the Chattahoochee National Forest. Both sites are confined to locations in Habersham County, Georgia, and managed by the Chattooga River Ranger District (fig. 1). The climate is temperate, with warm, humid summers, mild winters, and year-round precipitation. Precipitation generally averages between 50 and 55 inches per year, mostly in the form of rain (USDOC 2013, USDOC 2016).

The treatment watershed, or the Locust Stake site, is located on a south-facing slope and covers an area of 420 acres with an average elevation of 440 meters (approximately 1,440 feet). A mesic hardwood forest dominates lower elevations and transitions into a mixed pine-hardwood forest at higher elevations. This site includes the Locust Stake OHV trail system with 11 trails encompassing a total length of 9.2 miles. Trail widths average between 5-8 feet while grades range between 0-45%, with most grades between 5-15% (Favro 2012).

The reference watershed, Kimbell Creek, is located south of the treatment watershed. It is located on a south-facing slope with an area of 429 acres and an average elevation of 428 meters (approximately 1,400 feet). Habitats within this watershed are generally sub-mesic with mixed pine-hardwood forests in upland areas. This watershed does not include any OHV trails.

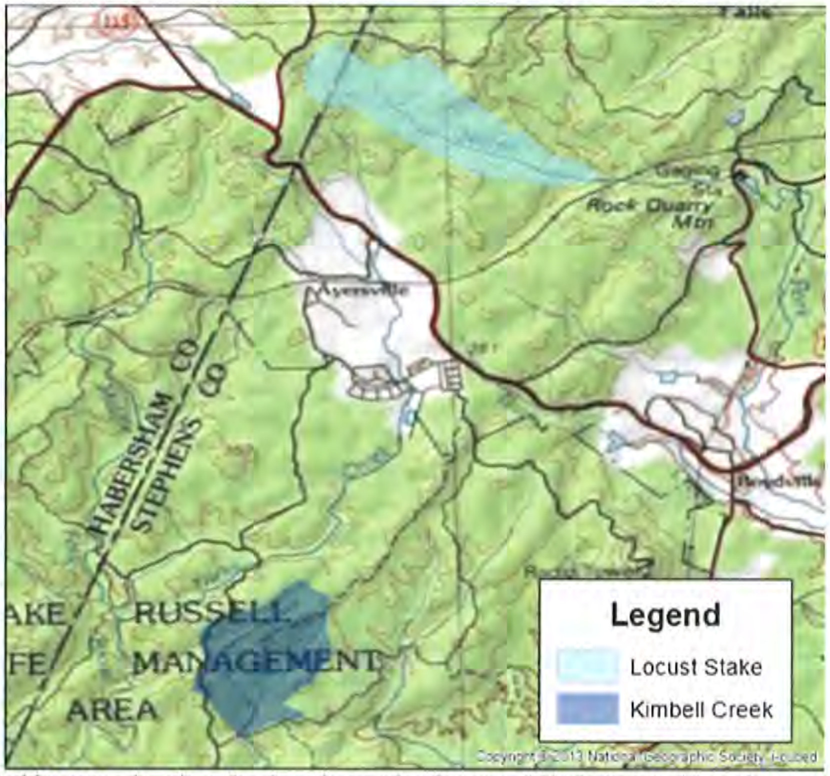


FIG 1. Topographic map showing the locations of reference (Kimbell Creek) and treatment (Locust Stake) watersheds (ESRI 2016).

Experimental Design

This study evaluated stream water quality response to OHV use using a paired watershed approach coupled with an upstream versus downstream approach. In the paired watershed approach, water quality from two closely-located and geomorphically similar watersheds are compared. The two watersheds are similar in area, land cover, aspect, and elevation but differ in their pre-management cover conditions. In this study, the Locust Stake site represented a catchment impacted by OHV use, while the Kimbell Creek site was generally free from such disturbances.

The effects of tributaries unaffected by OHV use on the treatment watershed were evaluated by comparing reaches of the catchment up- and downstream of the confluence with the incoming tributary. This approach minimizes effects of geomorphic and hydrological variation between streams, allowing a relatively unbiased interpretation of tributary impacts on water quality response (Marion et al. 2014). It is important to note that incoming tributaries are not completely undisturbed and may experience slight impacts from preexisting and closely-located OHV trails. Furthermore, this up- versus downstream approach helps to determine the effects that permanently closed OHV trails have on stream sedimentation. The downstream portion of the treatment watershed includes both open and closed trails, while the upstream reaches only encompass trails that are open for most of the year.

Sedimentation impacts from OHV use were evaluated using both a paired watershed regression and upstream versus downstream regression within the treatment watershed. Linear regressions were performed to compare TSS and flow for each site during periods where trails were either open or closed. To perform such regressions, flow was modeled from measured stream stage using WinXSPRO, a channel cross section analyzer published by the United States Forest Service (Hardy et al. 2005). Both TSS levels and slopes of linear regressions were then analyzed using either t-tests or confidence intervals to determine statistical significance.

Field Sampling

Erosion and sediment yields were quantified using an automated sampler (Teledyne, Inc.), which collected flow proportional water samples from both the reference (Kimbell Creek) and treatment (North Fork of the Broad River) sites. Samplers were manually programmed to collect samples during heavy rain events when sediment transport rates were at a maximum. Twenty-four one liter samples were collected during each storm event. The sampler was enabled when the stream level rose 0.04 feet above its baseline depth over a one hour period. Samples were collected at uniform time intervals; all 24 samples were collected at 15 minute intervals. Locations at each site were also equipped with an ISCO sonde to measure in-stream turbidity.

Laboratory Measurements

Samples from each site were retrieved weekly and transported to the Coweeta Hydrologic Laboratory for analysis. A turbidimeter (Hach 2100P) was used to measure the turbidity in nephelometric turbidity units (NTU) of 15 milliliters of each sample. Samples were gently agitated (inverted five to seven times) before measurement in order to recombine sediments evenly throughout the water sample. The sample cell was rinsed with deionized water between each use and wiped down with a lint-free cloth before each measurement to remove any unwanted residue that could potentially skew the readings.

Total suspended solids (TSS) were analyzed for each sample. TSS was determined by measuring the dry weight of each filtered sample as a function of known volume. The filter papers (Whatman GF/C glass 1.5 microfiber, 5.5 cm) were rinsed with distilled, deionized water, placed onto a vacuum pump (Millipore), and washed with 500 ml of deionized water. The filter papers were dried in an oven for 90 minutes at 125°C, and the weight of the dried filter was recorded. The samples were then vigorously agitated (shaken until all sediment was evenly distributed throughout the water sample), and their volumes were measured and recorded. The entire sample was filtered, except if turbidity measurements were high. In the latter case, only 250 ml were filtered. The filters with sediment residue were dried for two and a half hours at 105°C. Each filter was weighed, and the weight was recorded in grams to four decimal places. Four extra filter papers were prepared using the same methods; these served as blanks. The TSS for each sample was determined using the following equation:

$$TSS = [W_2\,(mg) - W_1\,(mg)]\,/\,V\,(ml) \qquad (1)$$

Where, TSS is total suspended solids, W_1 is the weight of the dried empty filter paper, W_2 is the weight of the dried filter with sediment particles, and V is the volume of the filtered sample.

RESULTS

During the research period, each studied site experienced a different number of collection events in response to rise in stream stage (tab. 1). Only those collected samples that occurred as a result of recorded precipitation were analyzed for TSS and turbidity. For Kimbell Creek, the reference watershed, 18 of the 35 samples were collected as a response to storm events and transported off-site for analysis. Locust Stake and Upper Locust Stake samplers collected 36 and 30 total samples, respectively. Of these, only 21 (Locust Stake) and 22 (Upper Locust Stake) samples were analyzed.

TABLE 1. Sample collection information for reference and treatment sites.

Site	Collection Start Date	Collection End Date	Number of Collected Samples	Number of Analyzed Samples
Reference				
Kimbell Creek	27 February 2015	29 September 2016	35	18
Treatment				
Locust Stake	2 April 2015	12 October 2016	36	21
Upper Locust Stake	27 June 2015	12 October 2016	30	22

TSS Comparisons between Sites

For each site, the TSS values (mg/L) for all individual storm events were averaged and standard deviation was calculated (tab. 2). Standard error was then calculated for each site using the following formula:

$$SE_{\bar{x}} = \frac{s}{\sqrt{n}} \tag{2}$$

Where $SE_{\bar{x}}$ is the standard error of the mean, S is the standard deviation of the mean, and n is the number of observations in the sample.

The positive and negative standard error values were then used to create a margin of error around the mean for comparison between sites. If the mean TSS value for the treatment site fell outside of the margin of error for the reference site, then the mean TSS values for the compared sites were considered statistically different (tab. 2).

Mean TSS for Kimbell Creek was 53.36 mg/L with a standard error of ± 1.95 mg/L. Locust Stake had an average TSS of 69.13 mg/L, while Upper Locust Stake had an average of 64.89 mg/L. Mean TSS values for both treatment sites exceeded the maximum value included in the margin of error for Kimbell Creek (fig. 2). Therefore, average TSS values for each treatment site were considered to be statistically greater than those in the reference site.

TABLE 2. Average total suspended solids (TSS) for reference and treatment sites, including standard error. *Indicates significant difference between mean TSS in treatment and reference watersheds.

Site	Average TSS *(mg/L)*	Standard Error *(mg/L)*
Reference		
Kimbell Creek	53.36	± 1.95
Treatment		
Locust Stake	69.13*	± 5.33
Upper Locust Stake	64.89*	± 4.75

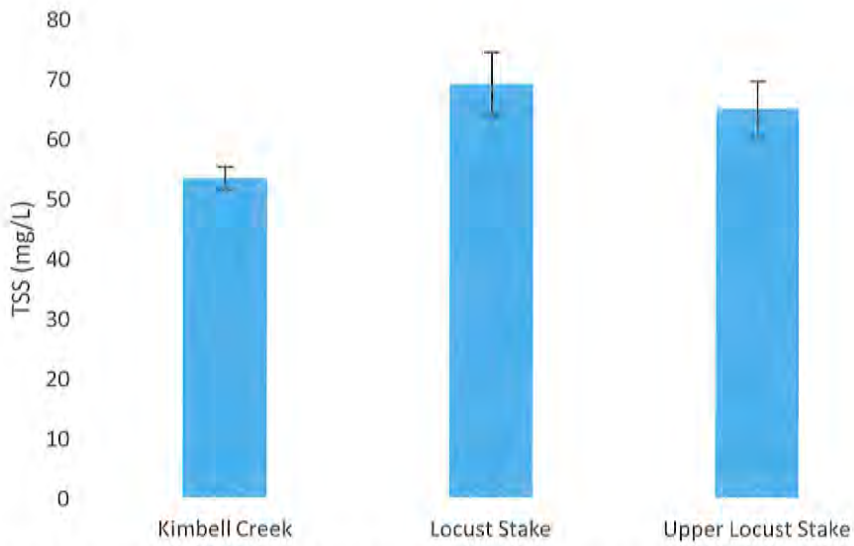
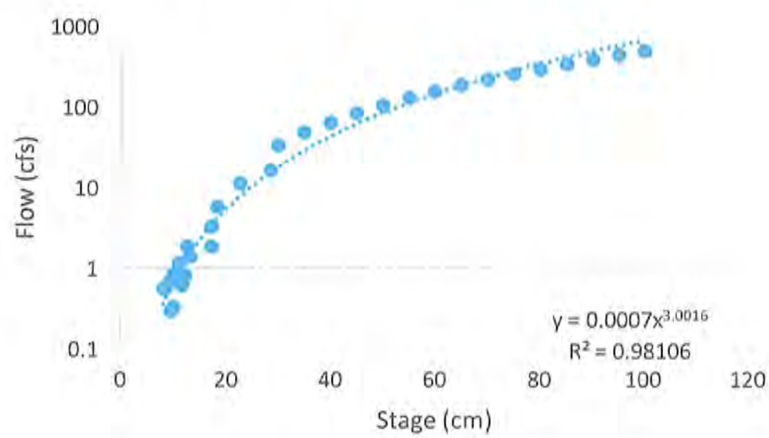
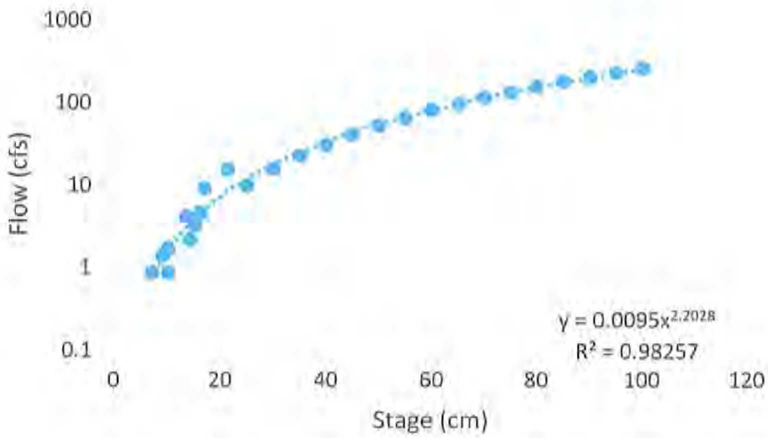


FIG 2. Average total suspended solids (TSS) in mg/L for each reference and treatment site. Vertical bars represent standard error for all included values.

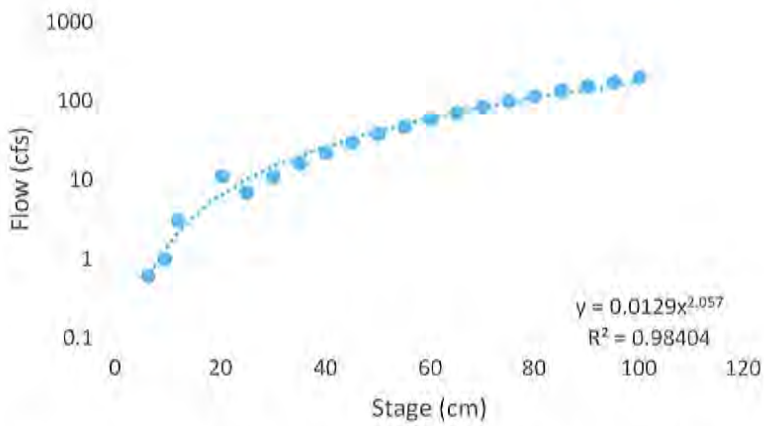
For each storm event, hydrologic stage was recorded using an automated sampler, and flow was modeled from these data (fig. 3). WinXSPRO, a program designed to analyze the dimension of a stream, was used to model flow (cubic feet per second, cfs) from inputted stage (depth in ft) and cross-sectional water slope (ft/ft) data. The output rating curve was used to predict flow for each recorded stage when a water sample was collected.



(a)



(b)



(c)

FIG 3. Rating curves, or graphs of flow (cfs) versus stage (cm), for (a) Kimbell Creek, (b) Locust Stake, and (c) Upper Locust Stake.

Measured TSS values were graphed against modeled flow for individual storms to determine the slope of the linear regression between the variables. Slopes were averaged and standard error was determined (tab. 3). The positive and negative standard error values were then used to create a margin of error around the mean for comparison between sites. If the mean slope for the treatment site fell outside of the margin of error for the reference site, then the mean slopes for the compared sites were considered statistically different. T-tests were also performed to test the difference between reference and treatment sites. T-scores which fell below the alpha value of 0.05 indicated that TSS levels between sites were statistically different.

Both Locust Stake (P=0.030) and Upper Locust Stake (P=0.0049) had significantly higher TSS values than did Kimbell Creek over the entire range of flows (tab. 3). Both t-scores fell below the predetermined alpha value of 0.05 indicating that the slopes within the treatment sites were significantly greater than those of the reference site.

TABLE 3. Average slopes for the linear relationship between total suspended solids (TSS) and flow at each site during storm events, including standard error and *P*-value. *Indicates significant difference between mean slopes of treatment and reference sites.

Site	Average Slope *((mg/L)/cfs)*	Standard Error *((mg/L)/cfs)*	*P*-value
Reference			
Kimbell Creek	3.67	± 0.83	
Treatment			
Locust Stake	14.85*	± 4.53	0.030
Upper Locust Stake	15.32*	± 3.46	0.0049

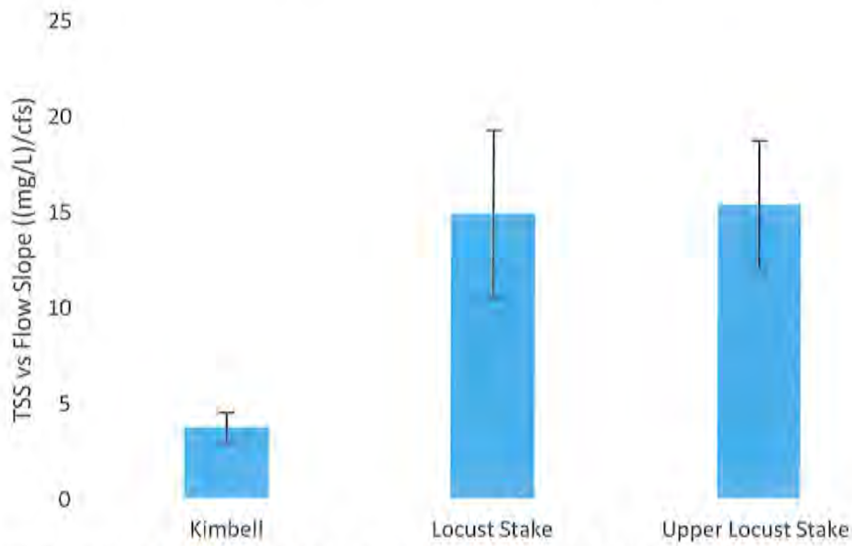
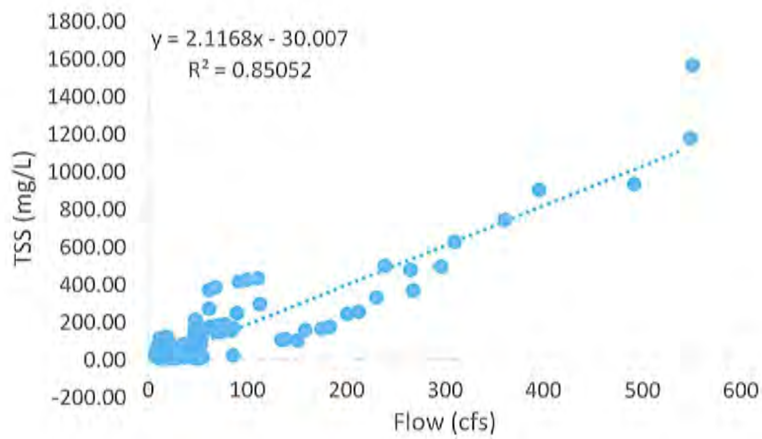
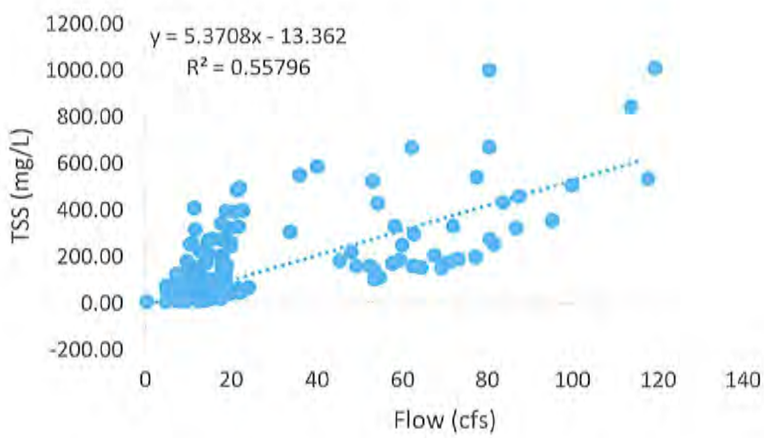


FIG 4. Average slope ((mg/L)/cfs) for the linear relationship between total suspended solids and flow at each site. Vertical bars represent standard error for each site.

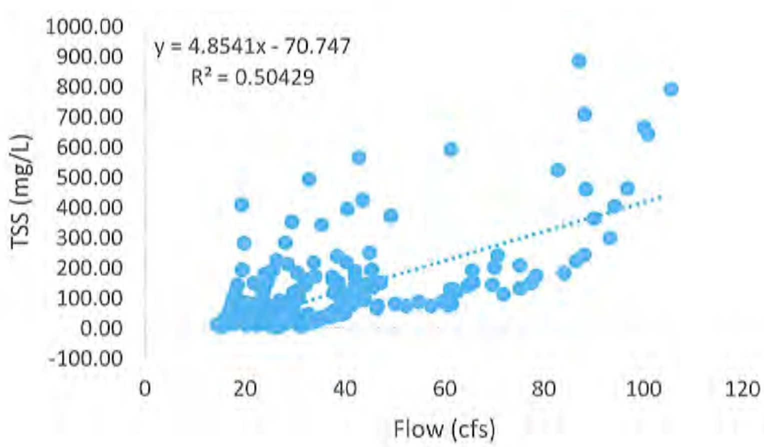
TSS values were then graphed against flow for all storms at each site. From these graphs, a linear regression was performed to determine the rate at which TSS rises per unit increase in flow. TSS rose 2.12 mg/L for every unit increase in flow for Kimbell Creek. Locust Stake experienced a 153% faster rise in TSS with a slope of 5.37 (mg/L)/cfs. Upper Locust Stake also had a faster rate of increase in TSS with a slope of 4.85 (mg/L)/cfs. For any given flow, TSS in Upper Locust Stake was 129% higher than in the reference watershed.


y = 2.1168x - 30.007
R² = 0.85052
TSS (mg/L)
1800.00
1600.00
1400.00
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
-200.00
0
100
200
300
400
500
600
Flow (cfs)

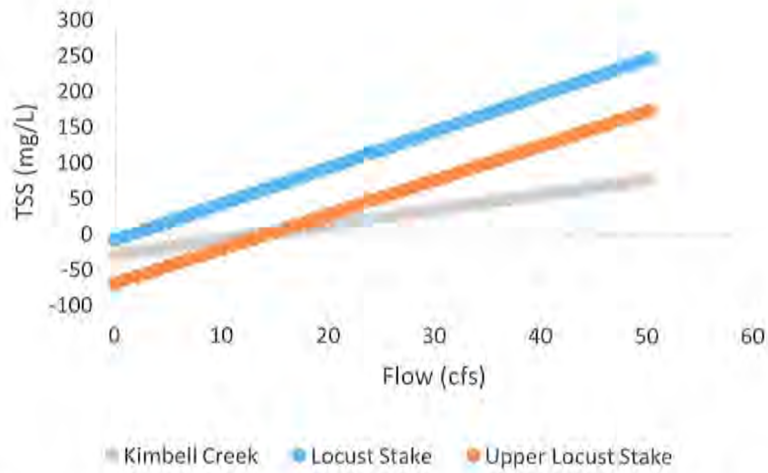
(a)


y = 5.3708x - 13.362
R² = 0.55796
TSS (mg/L)
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
-200.00
0
20
40
60
80
100
120
140
Flow (cfs)

(b)


y = 4.8541x - 70.747
R² = 0.50429
TSS (mg/L)
1000.00
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
-100.00
0
20
40
60
80
100
120
Flow (cfs)

(c)



(d)

FIG 5. Graphs of total suspended solids (mg/L) versus flow (cfs) for all storms in (a) Kimbell Creek, (b) Locust Stake, and (c) Upper Locust Stake. (d) Consolidated linear trend lines for each of the reference and treatment sites.

TSS Comparisons in Treatment Watersheds with Open and Closed OHV Trail Systems

For each treatment site, TSS samples were differentiated between those collected during OHV trail closures and those collected during periods when trails were open for public use (tab. 4). For Locust Stake, 20 samples were collected when trails were closed, and one sample was collected when trails were open. 21 samples were collected in Upper Locust Stake during periods of closure, while only one was collected after trails were opened. Only one storm event occurred after trails were opened that enabled the automated sampler to begin collecting water.

TABLE 4. Sample collection information for treatment watersheds during periods of OHV trail closures and openings.

Site	Trails Closed			Trails Open		
	Start Date	End Date	Samples	Start Date	End Date	Samples
Locust Stake	2 April 2015	27 July 2016	20	28 July 2016	12 October 2016	1
Upper Locust Stake	27 June 2015	27 July 2016	21	28 July 2016	12 October 2016	1

TSS values were graphed against storm flow to determine the slope of the linear relationship between the two variables. Slopes were averaged, and standard deviation and standard error were determined (tab. 5). The standard error values were then used to create a margin of error around the mean for comparison of TSS between times of open and closed OHV trail systems within each treatment site. If the slope for the storm that occurred during trail opening fell outside the margin of error for the storms occurring during trail closure, then the slopes for the different periods were considered statistically different.

The mean linear regression slope for storms occurring when Locust Stake trails were closed was 13.53 (mg/L)/cfs with a standard error of ± 4.55 (mg/L)/cfs. The slope increased to 41.25 after OHV trails were opened. Upper Locust Stake during trail closure experienced an average storm slope of 12.91 (mg/L)/cfs with a standard error of ± 2.60 (mg/L)/cfs. Slope also increased to 65.89 (mg/L)/cfs after trails were opened. Slopes for storm events at both treatment sites that occurred after trail opening extended beyond the determined margins of error for closed trails (fig. 6).

Because these values were beyond the allowable values, linear slopes were considered to be statistically greater in treatment sites with open OHV trail systems than those with closed trail systems.

TABLE 5. Average slopes for the linear relationship between total suspended solids (TSS) and flow at each treatment site during storm events, including standard error. Storms were differentiated between periods of OHV trail openings and closings. *Indicates that average slopes of open trail systems were significantly different than those of closed trail systems.

Site	Average Slope *((mg/L)/cfs)*	Standard Error *((mg/L)/cfs)*
Open		
Locust Stake	41.25*	
Upper Locust Stake	65.89*	
Closed		
Locust Stake	13.53	± 4.55
Upper Locust Stake	12.91	± 2.60

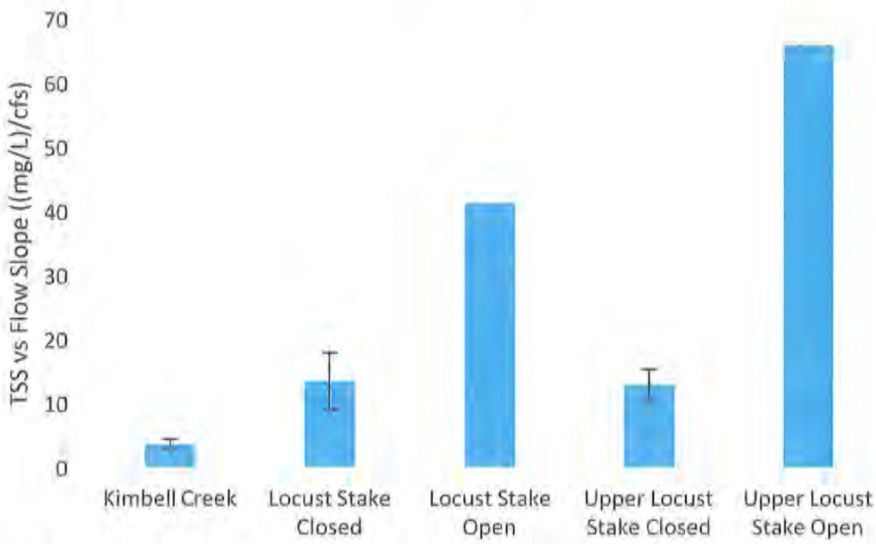
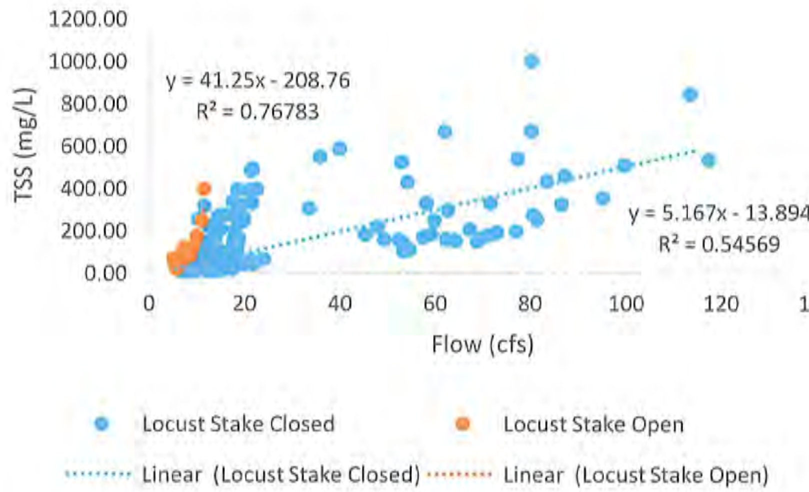


FIG 6. Average slope ((mg/L)/cfs) for the linear relationship between total suspended solids and flow at each site, differentiating between periods of open and closed OHV trails within treatment sites. Vertical bars represent standard error for each site.

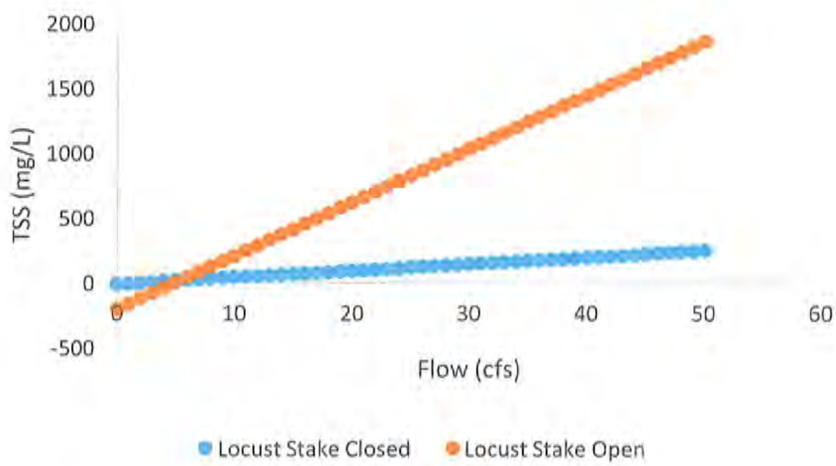
TSS values were plotted against flow for storms at each treatment site during periods where OHV trails were either open or closed (fig. 7). From these graphs, a linear regression was performed to determine the rate at which TSS rises per unit increase in flow. The rate of increase in slopes between open and closed trail periods for each site was then determined (tab. 6). TSS rose 5.17 mg/L per unit rise in cfs in Locust Stake when trails were closed. Rate of increase in TSS rose 698% faster after trails were opened. The same pattern held for Upper Locust Stake. During trail closure, the linear slope between TSS and flow was 4.97 (mg/L)/cfs. Slope rose to 65.89 (mg/L)/cfs after trail opening, a 1219% increase in TSS per any given flow.

TABLE 6. Linear regression slopes ((mg/L)/cfs) for each treatment site before and after OHV trail opening.

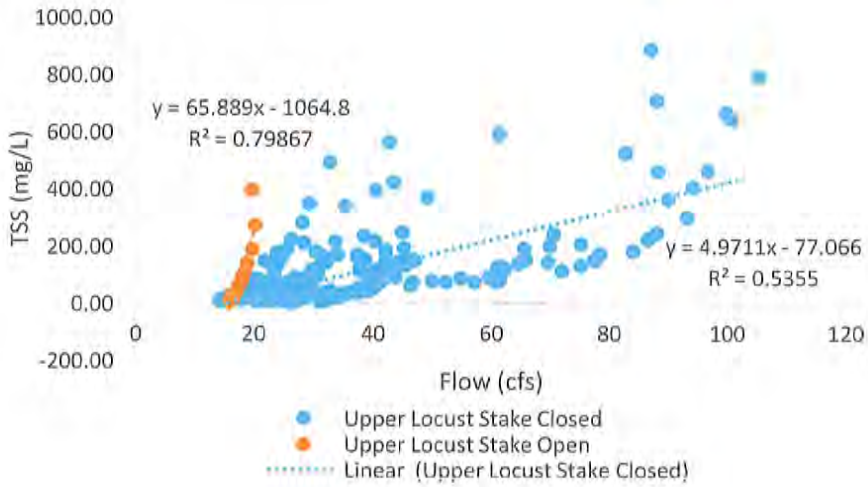
Site	Slope during Trail Closure ((mg/L)/cfs)	Slope after Trail Opening ((mg/L)/cfs)	Rate of Increase
Locust Stake	5.17	41.25	698 %
Upper Locust Stake	4.97	65.89	1219%



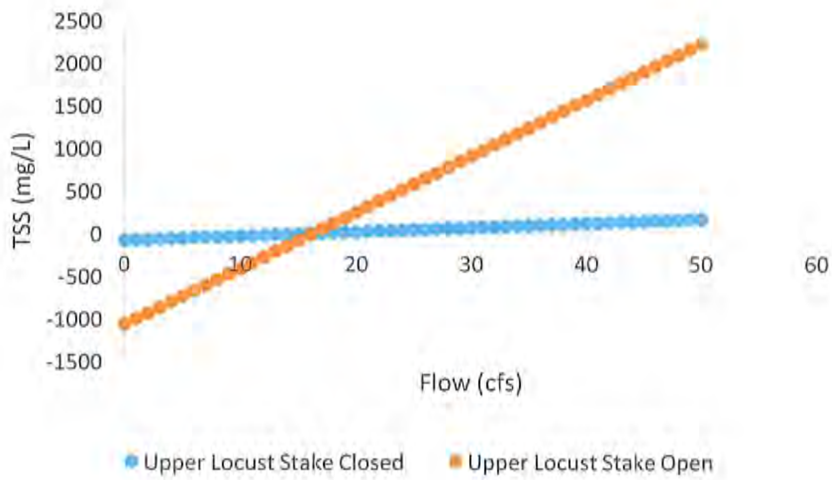
(a)



(b)



(c)



(d)

FIG 7. Graphs of total suspended solids (mg/L) versus flow (cfs) for all storms in (a) Locust Stake (N(closed) = 480; N(open) = 24; 24 samples per storm event) and (c) Upper Locust Stake (N(closed)=504; N(open)=24). Graphs showing only trend line data in (b) Locust Stake and (d) Upper Locust Stake.

Turbidity versus TSS

TSS and turbidity (NTU) were measured for each collected sample, and values were plotted against one another to determine the extent to which they are related (fig. 8). The coefficient of determination for the linear regression between TSS and turbidity was 0.759, indicating that 75.9% of the variance in TSS can be explained by the linear model.

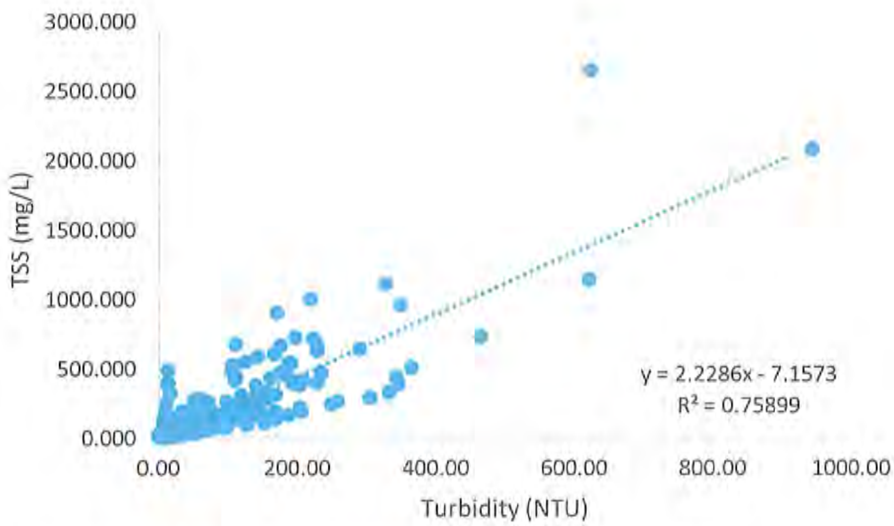
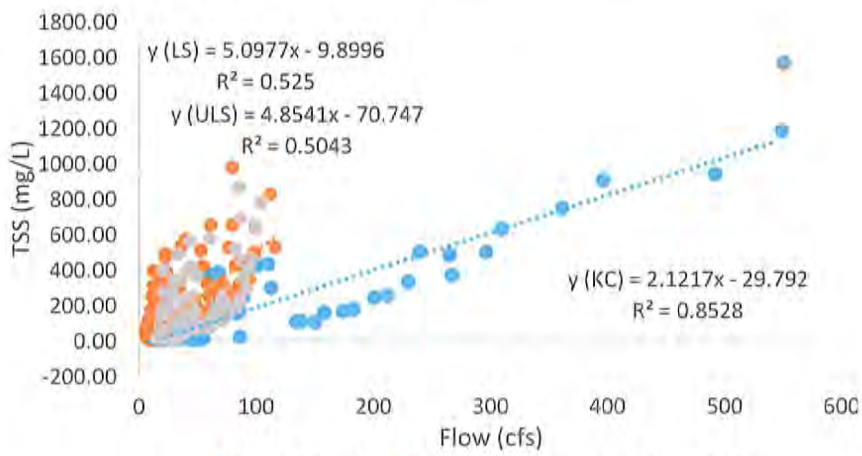
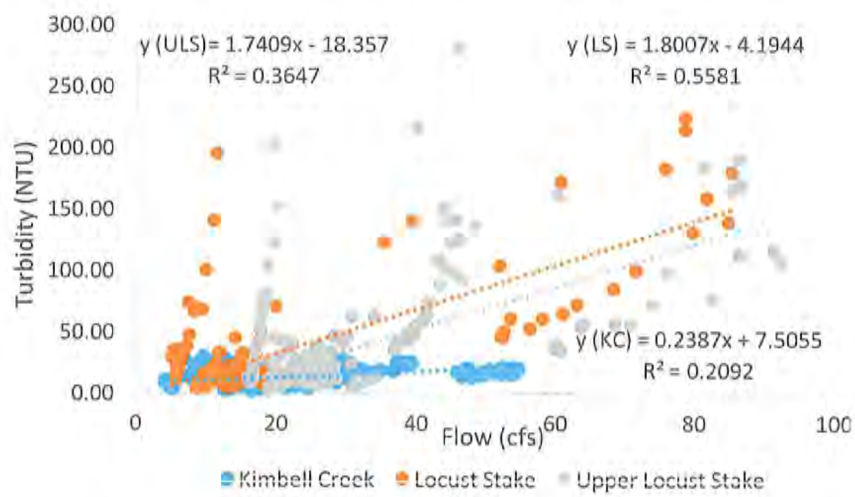


FIG 8. Graph of total suspended solids (mg/L) versus turbidity (NTU) for all sites, including a linear regression and coefficient of determination.

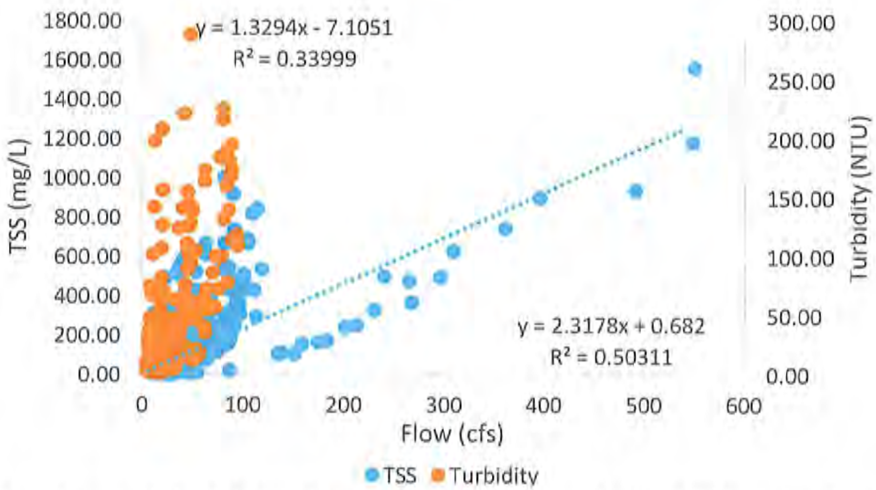
Turbidity and TSS were then plotted against flow, using a linear regression model to determine correlation (fig. 9). The coefficient of determination was used to determine which model, TSS or turbidity, best indicated sedimentation impacts in streams (tab. 7). TSS better predicts stream sedimentation for Kimbell Creek (R^2=0.85) and Upper Locust Stake (R^2=0.50), while turbidity is a better indicator in Locust Stake ($R^{2=}$0.53). Overall, TSS models (R^2=0.51) are better indicators of sedimentation than are turbidity models (R^2=0.34).



(a)



(b)



(c)

FIG 9. Graph of (a) total suspended solids (mg/L) versus flow (cfs), (b) turbidity (NTU) versus flow, (c) TSS and turbidity versus flow. Trend lines and coefficients of determination are included on each graph.

TABLE 7. Coefficients of determination (R^2) for total suspended solids (TSS) and turbidity at each reference and treatment site.

Site	TSS Coefficient of Determination (R^2)	Turbidity Coefficient of Determination (R^2)	Better Model
Reference			
Kimbell Creek	0.85	0.21	TSS
Treatment			
Locust Stake	0.53	0.56	Turbidity
Upper Locust Stake	0.50	0.36	TSS
All	0.51	0.34	TSS

DISCUSSION

The principal objective of the study was to test whether a significant difference in stream sedimentation occurred between watersheds with and without OHV trail systems and treatment watersheds with open or closed OHV trail systems. Based on the results, a significant increase in stream sedimentation was found in watersheds with OHV trails compared to watersheds without such trails (tab. 3). Furthermore, sedimentation was shown to increase significantly in treatment watersheds after trails were opened for public use (tab. 6). This study only considered suspended sediments and cannot provide sufficient conclusions on the impacts of OHV trails on sediment deposition.

Upon examination of differences between turbidity and TSS at each site (fig. 8), TSS was found to be a better indicator of stream sedimentation than turbidity, as evidenced by a greater coefficient of determination when compared to flow (tab. 7). Discrepancies between the two measured values are a direct result of differences in test criterion. TSS measures the dry weight of all suspended solids in the water, including sediment, inorganic materials, and organic materials. Turbidity measures the intensity of the scattering of light by the same suspended particles but is influenced by dyes, which absorbs light rather than scattering it. Turbidity also excludes settleable solids, or sediments that are quick to deposit after agitation, causing lower turbidity readings than the true value (Fondriest Environmental Inc., 2016). Because TSS measures both suspended and settleable solids and is not influenced by the presence of dyes, it can be used to calculate rates of stream sedimentation. It is possible to use stream flow to predict TSS values if the linear regression between the values are known.

Ecological Implications

This study suggests that OHV trails, even during periods of no use, have a significant impact on stream sedimentation within the surrounding watershed. These findings are consistent with previous reports by Foltz (2006) and Marion et al. (2014) which concluded that OHV trails, regardless of current use, have increased sediment deposition near trail crossings. The current study further suggests that recency of OHV use is significant in regards to sedimentation. This result challenges the results of Marion et al. (2014), who concluded that the occurrence of past OHV usage is more important than the recency of use. Rates of increase of TSS compared to flow is significantly greater in recently utilized OHV trails compared to closed trail systems (fig. 7), which may imply that more recent OHV use magnifies sedimentation responses in watersheds where trails are present.

Water Quality Standards

During storm events, both reference and treatment watersheds exceeded the TSS limits set forth under the Clean Water Act (tab. 2). Because this study only examined stream sedimentation during storm events when TSS and turbidity levels were at a maximum, it cannot accurately conclude whether surface waters in the North Fork of the Broad River violate federal water quality standards. Visual assessment of streams within the Locust Stake OHV trail system revealed that waters are severely turbid, which places the river in violation of Georgia water quality standards. Further research must be completed to assess sedimentation during base stream flow, which could be combined with the results of TSS analysis during storm events to determine if the North Fork of the Broad River is in clear violation of published water quality standards.

Sources of Error

One potential source of error within the data arises from the limited number of storm events occurring after trails were opened. During this time period (28 July 2016 to 12 October 2016), only one storm event occurred at each site that enabled the automated samplers to begin collecting water samples. During the months when OHV trails were open, the southeastern portion of the United States was undergoing a prolonged drought. The United States Department of Agriculture classified Habersham County, GA, the location where the study was conducted, as an area that experienced episodes of moderate to exceptional droughts (Heim 2016). Because of the lack of storm events, the resulting conclusion may be insufficient, especially since a single sample was used to represent normal sedimentation levels of streams after trail openings. To strengthen the conclusion made in this study that open trail systems have significantly higher sedimentation levels and rates of increase than closed trail systems, more samples must be taken during storm events occurring when OHV trails are open for use.

Further Research

This study only considered impacts of OHV use on suspended sediments in streams during storm events. Further research needs to be completed to include all potential sedimentation impacts that result from the presence of OHV trail systems. Previous research shows that ATV use leads to increased deposition and decreased pool depth and volume in watersheds with open trail systems (Chin et al. 2004). Further research could be conducted to determine the impacts on bedload sediments by OHV trail systems during periods where trails were both opened and closed when compared to a reference watershed. This potential study could quantify the impacts that both presence and recency of OHV use have on sediment deposition in streams. Other recommendations for further research include quantifying suspended sediment levels outside of storm events to determine baseline sedimentation impacts of OHV trails.

CONCLUSION

Preliminary analysis of paired watersheds indicated that the Locust Stake OHV trail system has significant negative impacts on stream sedimentation in the North Fork of the Broad River. These effects were reflected in increased levels of TSS and turbidity along with faster rates of increase in TSS when compared to reference watersheds. Furthermore, open trail systems were found to have even greater levels of TSS and turbidity when contrasted with closed trail systems. These impacts are reasonable consequences of accelerated erosion and sediment deposition, which have previously been associated with OHV trails. Since the presence and recency in use of OHV trails are the only important differences between the paired watersheds, this study logically concludes that the trail system is the cause of the observed differences. More research is needed to further quantify the magnitude of stream sedimentation in watersheds with open trail systems versus those with closed trails. Results from these studies will have important implications for the creation and management of OHV trails.

Acknowledgments

I would like to thank Dr. Chelcy Ford Miniat and Patsy Clinton at the Coweeta Hydrologic Laboratory for their help with the construction and completion of this project. I would also like to thank Mrs. Cindi Brown for her supervision during the laboratory analysis of the collected samples. Phillip Walrod from the Chattooga River Ranger District was central to the completion of this project; without his efforts, neither samples nor data would have been retrieved. Lastly, I would like to thank Dr. James Costa and Dr. Sarah Workman at the Highlands Biological Station for their editorial comments and assistance with statistical analysis.

Literature Cited

Ayala, R. D., P. Srivastave, C. J. Brodbeck, E. A. Carter, and T. P. McDonald. 2005. Modeling sediment transport from an off-road vehicle trail stream crossing using WEPP model. Proceedings of 2005 ASAE Annual International Meeting. American Society of Agricultural and Biological Engineers, St. Joseph, MO, p. 1-10.

Chin, A., D. M. Rohrer, D. A. Marion, and J. A. Clingenpeel. 2004. Effects of All-Terrain Vehicles on Stream Dynamics. Gen. Tech. Rep. SRS-74. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. p. 292-306.

Clinton, B. D., J. M. Vose, and D. L. Fowler. 2010. Flat Branch monitoring project: stream water temperature and sediment responses to forest cutting in the riparian zone. Res. Pap. SRS–51. Asheville, NC: USDA Forest Service, Southern Research Station. 8 p.

Cordell, H. K., C. J. Betz, G. Green, and M. Owens. 2005. Off-highway vehicle recreation in the United States, regions, and states: a national report from the survey on recreation and the environment (NSRE). Washington, DC: USDA, Forest Service. p. 1-47.

Environmental Systems Research Institute (ESRI). 2016. ArcGIS Desktop: 10.4. Redlands, California, USA.

Favro, J. 2012. Locust Stake ATV Trail System Inventory, Assessment and Management Plan. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. See USDA 2016 and https://fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5382439.pdf.

Foltz, R. B. 2006. Erosion from All Terrain Vehicle (ATV) Trails on National Forest Lands. Proceedings of 2006 ASABE Annual International Meeting. American Society of Agricultural and Biological Engineers, St. Joseph, MO. p. 1-10.

Fondriest Environmental Inc. 2016. Turbidity, total suspended solids and water clarity – environmental measurement systems. 2 December 2016. Web.

Georgia Department of Natural Resources (DNR). 1974. Ga. Comp. R. & Regs. R. 391-3-6-.03. Atlanta, GA: Georgia Department of Natural Resources: Environmental Protection Division. <http://epd.georgia.gov/sites/epd.georgia.gov/files/related_files/site_page/391-3-6-.03%20Triennial%2013%20Final%20Edits.pdf>.

Hardy, T., P. Panja, and D. Mathias. 2005. WinXSPRO, A Channel Cross Section Analyzer, User's Manual, Version 3.0. General Technical Report RMRS-GTR-147. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Washington DC, USA.

Heim, R. 2016. U.S. Drought Monitor – Southeast. Lincoln, NE: US Department of Agriculture, The National Drought Mitigation Center. 2 December 2016. Web.

Imlay, M. 2014. Utility Task Vehicles by the Numbers. Specialty Equipment Market Association. 30 November 2016. Web.

Marion, D. A., J. D. Phillips, C. Yocum, and S. H. Mehlhope. 2014. Stream channel responses and soil loss at off-highway vehicle stream crossings in the Ouachita National Forest. Geomorphology **216**: 40-52.

Riedel, M. S., and J. M. Vose. 2002a. Forest Road Erosion, Sediment Transport, and Model Validation in the Southern Appalachians. Proceedings of Second Federal Interagency Hydrologic Modeling Conference, July 28-August 1, 2002, Riviera Hotel, Las Vegas, Nevada.

Riedel, M. S. and J. M. Vose. 2002b. The dynamic nature of sediment and organic constituents in TSS. Proceedings of 2002 National Water Quality Monitoring Conference, United States Advisory Committee on Water Information – National Water Quality Monitoring Council, Madison, WI, May 20^{th} – 23^{rd}.

Statista. 2016. Number of people living in households that own an ATV (all-terrain vehicle) in the United States from spring 2008 to spring 2016 (in millions). The Statistics Portal. 30 November 2016. Web.

US Department of Agriculture (USDA). 2016. Locust Stake OHV Trail System. Washington, DC: US Forest Service: Chattahoochee-Oconee National Forests. 24 September 2016.

<http://www.fs.usda.gov/recarea/conf/recarea/?recid=10513>.
US Department of Agriculture (USDA). 2009. 36 CFR 212, 251, 261, and 295 - Travel Management; Designated Routes and Areas for Motor Vehicle Use. Code of Federal Regulations. Washington, DC: US Government Publishing Office. 24 September 2016. <http://www.fs.fed.us/recreation/programs/ohv/OHVrule.pdf>.
US Department of Commerce (USDOC), NOAA, Community Collaborative Rain, Hail, & Snow Network (CoCoRaHS). 2016. Station Report Summary. 24 September 2016. Web.
US Department of Commerce (USDOC), NOAA, National Weather Service. 2013. What's Typical in North and Central Georgia? 24 September 2016. Web.
US Environmental Protection Agency (USEPA). 2011. 40 CFR 133 – Secondary Treatment Regulation. Code of Federal Regulations. Washington, DC: US Government Publishing Office.
US Environmental Protection Agency (USEPA). 2000. Total Maximum Daily Load (TMDL) Development for Sediment in the North Fork of the Broad River Watershed. San Francisco, CA.

SURVEY OF *ANEIDES AENEUS* IN MACON COUNTY, NORTH CAROLINA

WILL HAMILTON AND JORDAN IDDINGS

Abstract. The green salamander, *Aneides aeneus*, is an arboreal amphibian, and it is endangered largely as a result of habitat destruction. Due to its habitat specificity and detectability issues, it is difficult to locate populations and therefore difficult for conservation groups to protect the salamanders' habitat. A model was created with ArcGIS to determine areas of possible green salamander occupation, and we set out to ground truth areas in the Chattooga River Basin highlighted by that model (Hardman 2014, ESRI 2015). We surveyed sites in Macon County, NC, marking areas with suitable habitat. During our survey, we discovered eleven sites occupied by green salamanders, some of which were previously undocumented, and extended their known range westward by four miles. After running Kendall's rank correlation coefficient, we found that the model does not effectively predict green salamander habitat in our survey area. This raises the need for an improved model that accurately predicts presence of green salamander habitat that can be used to direct conservation efforts for this endangered and elusive species.

Key words: Aneides aeneus, *ArcGIS, Blue Ridge Escarpment, green salamanders, habitat, model, Plethodontidae.*

INTRODUCTION

The green salamander (*Aneides aeneus)* is a rare, arboreal species in the lungless salamander family, Plethodontidae, found in the Southern Appalachians (Brodman 2004). They are relatively small, reaching about 7.5-14 cm (3-5.5 inches) in length from snout to tail tip, and are dark brown to black in color with bright green markings on their backs that resemble lichens. Like other members of the Plethodontidae family, *Aneides aeneus* have flattened naso-oral and dorso-ventral regions (Petranka 1998, Davis 2004). During the mating season, males display a circular yellow mental gland on the ventral surface of their lower jaw (Waldron and Pauley 2007). Green salamanders are specialists that are especially suited to conditions typically too dry for other types of salamanders. They nest in moist rock crevices, but leave at night to forage for food in tree canopies and under bark. Their prey includes small insects and insect larvae, spiders, mites, and skin shed by other salamanders (Lee and Norden 1973).

Several studies focusing on green salamander behavior indicate the sedentary nature of the species. Movement is largely restricted to home rock crevices, with an increase in activity from dusk until 11pm (Gordon 1961). Although earlier studies typically considered arboreal activity to be minimal, it later became evident that green salamanders seasonally change between arboreal habitats and rock crevices (Waldron and Humphries 2005). Green salamanders also express a strong preference for deeper crevices over shallow ones, and express territorial behavior when intruders enter a residential salamander's preferred crevice (Cupp 1980, Armstrong 2010). Armstrong (2010) found that green salamanders respond less strongly to invading salamanders of their own species as opposed to other species.

Green salamanders range from southern Pennsylvania and Ohio down into northern Alabama and Mississippi in the larger Cumberland Plateau region, with two disjunct groups in the southern Appalachians along the Blue Ridge Escarpment (Corser 2001). There have been some recent findings of disjunct populations within the ridge and valley provinces of northern Georgia and eastern Tennessee as well (K. Pursel, personal communication). While their overall range spans multiple states, the dispersal rate of these salamanders is considered to be relatively low (Johnson 2002, Smith and Green 2005). The species, nevertheless, retains a metapopulation dynamic where subpopulations interact and colonize new or previously occupied areas (Corser

2001). Johnson (2002) provides evidence that different subpopulations can experience gene flow, yet genetic drift increases as distance between subpopulations increases above one kilometer. There is some evidence that green salamanders can colonize new habitats, such as rock outcrops exposed by road cuts (E. Corey, personal communication).

Populations in the Blue Ridge region of the green salamander range experienced crashes during the 1970s (Snyder 1991, Corser 2001). Corser (2001) further notes another crash that occurred in the late 90s. Several factors may contribute to decline in *A. aeneus* populations; Daszak et al. (1999) describes pathogens such as Ranavirus that affect the salamanders. Snyder (1991) points out environmental degradation as a potential cause for decline since the permeable skin of green salamanders makes them especially vulnerable to contaminants. Many studies, however, deem habitat destruction as the most significant threat due to fragmentation of subpopulations paired with relatively low dispersal rates of green salamanders (Larson et al. 1984, Petranka et al. 1993, Blaustein et al. 1994, Corser 2001). Once-robust populations within the Highlands, NC, area have become extirpated after nearby residential development (K. Pursel, personal communication). Green salamander numbers are still low today, and while this species is not federally protected, it is a candidate endangered species under the Federal Endangered Species Act and is currently listed as endangered in North Carolina (Norman n.d.).

We conducted our survey on the Macon County side of the Highlands Plateau. Our study area also included the Gulf drainage in the Cullasaja and Little Tennessee River basins. While extensively surveyed and studied elsewhere green salamander habitat is largely understudied in western North Carolina. Our study area is also known for having more exposed granitic dome mountains and sandier soils than other areas in the Blue Ridge region (Schwartzman 2010).

A statewide model designed to locate possible habitats for green salamanders was created by Rebecca Hardman (2014), but ground truthing efforts in the western portions of the Blue Ridge Escarpment have been limited to date. This study investigated to what degree Hardman's model functions as a predictor of green salamander presence in the Macon County area using the mapping program ArcGIS to obtain population data about green salamanders (ESRI 2015). The study will help improve the survey effort for the Upper Chattooga River Basin. We predict that Hardman's model will function poorly as a predictor for this study area in the western part of NC because the model was predominantly designed to cover areas in the eastern Blue Ridge Escarpment, and does not account for the difference in bedrock. This survey will, nevertheless, give us a better idea of the extent of the range of *A. aeneus* in the western Blue Ridge region, and allow future models more data points to improve predictability.

Methods & Materials

We used previously generated information about areas with known green salamander populations and areas with potential populations to determine the sites we would survey. Known areas were obtained from Lori Williams (personal communication), based upon past surveys conducted by the North Carolina Wildlife Resources Commission. Potential new areas were identified using the model created by Hardman (2014) and general surveys for suitable potential habitat in southeastern Macon County, NC. The habitat model includes soil moisture, canopy cover, and elevation as factors to predict areas of potential green salamander habitat. The model, however, is imperfect in that it does not incorporate current site conditions, and marks areas, such as open fields, that would not sustain a green salamander population. Aerial photography, as a result, helped us to determine which areas highlighted by the model were of sufficient potential. We then downloaded points of interest onto a Garmin GPS device and traveled to the survey points.

All of the potential habitat sites were located on land owned by the U.S. Forest Service or the Highlands-Cashiers Land Trust. At each site, we traversed as much ground as possible looking for rock face suitable for green salamanders. Upon finding exposed rock face, we continued searching for more rock outcrops on the same general elevation contour since topographical features generally recur on a given contour line. We used GPS to mark locations of potential habitat. While examining outcrops, we used headlamps to illuminate cracks in the rock because salamanders are often found deep within rock cracks (Armstrong 2010). We took care not to come into direct contact with the green salamanders, as an endangered species handling permit would be required to do so. For each rock outcrop we recorded number of green salamanders found from each age class (adults, juveniles, and hatchlings). We also recorded the presence or absence of other salamanders and organisms such as crickets and spiders, and we assessed the quality of the outcrop as habitat. A quantitative ground truth rating was given on a scale of 1-4, with 1 being almost unsuitable habitat, 2 being marginal habitat, 3 being seemingly suitable habitat, and 4 being a rock with deep horizontal cracks in which green salamanders were found (highly suitable habitat).

We used ArcMap (ESRI 2015) to show the general areas in which green salamanders were found. Since the exact location of green salamander habitat is classified to prevent poaching, we illustrated broad spans of land where habitat was found as opposed to specific points. Maps we created were analyzed by the Highlands-Cashiers Land Trust to ensure ample ambiguity with the documented points to avoid making the exact location of the points determinable since this is an endangered species susceptible to poaching.

The overall model consists of two different sub-models to predict habitat presence — the first uses several variables including elevation, forest presence, and soil types (all variables), and the second only uses soil data such as soil type and bedrock depth (soil only) (Hardman 2014). To test the model, we generated scatter plots comparing both sub-model rankings for a given site to our ground truth rankings. A strong agreement between these ranking would support the model as a strong predictor for *A. aeneus* habitat. Kendall's rank correlation coefficient was used to quantify the level of agreement between the model and ground truth ranks (Gottfried 1971). The Kendall coefficient ranges from -1 to 1, where -1 means there is complete disagreement between rankings and 1 demonstrates strong agreement. Due to the fact that the model operates on a 0 to 1 scale, we converted ground truth ranks to a 0 to 1 scale for equivalent comparison.

RESULTS

Eleven occupied sites and a total of 41 individual *A. aeneus* were found (table 1). The most fruitful site contained five adults, five juveniles, and four hatchlings, and the model rankings for that site were 0.0387 for soils and 0.0441 for all variables. Model rankings display the likelihood, on a 0-1 scale, that green salamander habitat can be found at a given point. We found 60 potential habitat sites; the points are displayed over both sub-models: all variables (fig. 1) and soil only (fig. 2). Most of the suitable (rank 3) and occupied (rank 4) sites shared similar characteristics: most were above the mid-level of the slope in exposed rock outcroppings with other nearby occupied or seemingly suitable sites. Evergreen ericaceous plants such as mountain laurel (*Kalmia latifolia*) and rhododendrons (*Rhododendron maximum, R. carolinianum*) also tended to be present in higher ranking sites. Other animals noted living on the rocks and amongst the cracks of occupied rocks included: gray-cheeked salamanders (*Plethodon montanus)*, southern Appalachian salamander (*Plethodon teyahalee*), crickets, spiders, and mud dauber wasps. Sites with spiders and crickets did not generally have green salamanders occupying them. This is consistent with previous findings on green salamanders (K. Pursel personal communication). Some of the occupied and suitable sites were outside the known range of green salamanders in North Carolina, including some within the Little Tennessee River drainage.

TABLE 1. Number of *A. aeneus* salamanders of each age class (adult, juvenile, hatchling) found in 11 sites. Rankings from each model are also included; rankings are not included where a site is outside of the range of the model (Hardman 2014). Ground truth rankings are also included; all the rankings are 4 because these sites were occupied.

Ground truth rank	Adults	Juveniles	Hatchlings	Soil only	All Variables
4	5	5	4	0.0387	0.0441
4		1		0.0387	0.0555
4	2	1		0.0387	0.0330
4		5		0.0387	0.0427
4	5				0.1749
4	1				0.1227
4		1		0.0513	0.1528
4	1	2		0.0513	0.2152
4		1		0.0176	0.1261
4	1			0.0176	0.0631
4	4	2		0.0176	0.0752

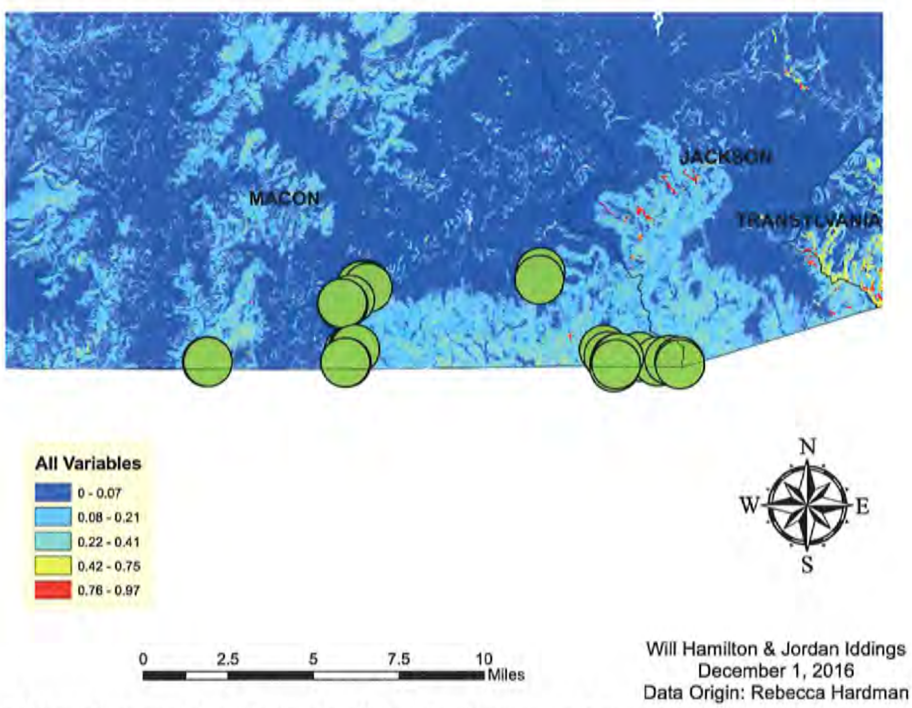


FIG. 1. All variables model with site locations. Each green spot represents a potential green salamander habitat site. Sixty sites were found, and green salamanders were found at 11 of those sites. The model takes all factors into account, and displays the likelihood that a given area will have green salamander habitat with red being highly likely and blue being minimal probability (Hardman 2014).

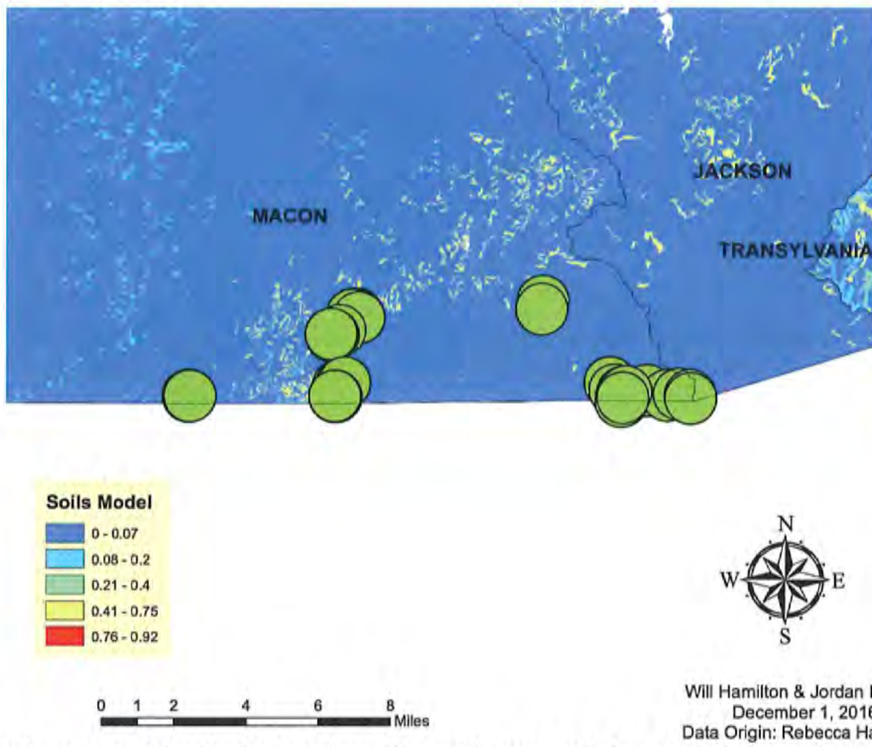


FIG. 2. Soils only model with site locations. The model uses soil data to predict the likelihood that a given area will have green salamander habitat with red being highly likely and blue being minimal probability (Hardman 2014). Each green spot represents a potential green salamander habitat site. Sixty sites were found, and green salamanders were found at 11 of those sites.

Scatter plots of field (ground truth) ranking versus the model ranking for all variables (fig. 3a) and soils data (fig. 3b), demonstrate little agreement between the two ranking systems. The Kendall rank correlation coefficient is 0.133 for the all variable model and -0.0011 for the soils model, indicating little to no agreement between the field and model ranking systems since a coefficient of 1 shows complete agreement and -1 demonstrates complete disagreement. This lack of agreement indicates poor predictive value of the existing model in southwestern North Carolina.

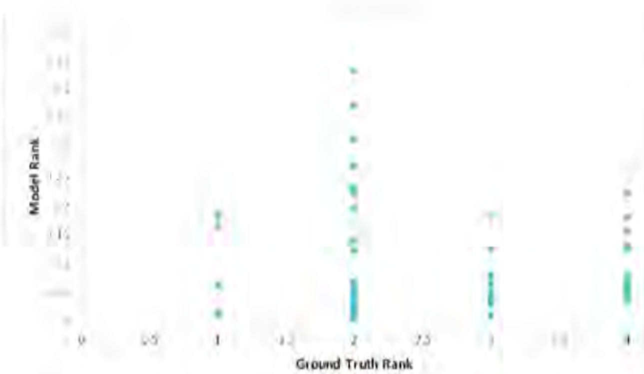


FIG. 3a. The "all variables" model (y-axis) versus the rank assigned in the field (x-axis). For both systems, a higher ranking represents a higher probability of green salamander presence.

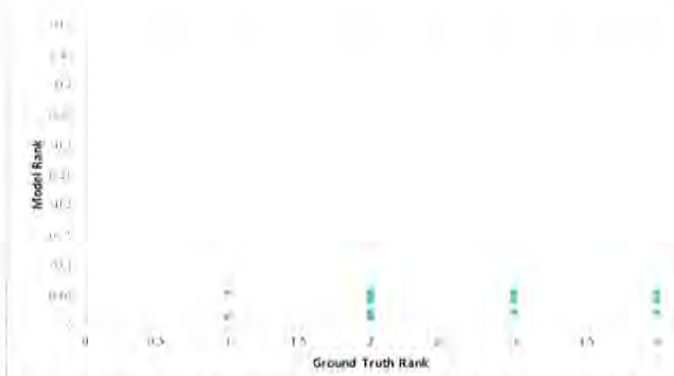


FIG. 3b. The "soils data" model (y-axis) versus the rank assigned in the field (x-axis). For both systems, a higher ranking represents a higher probability of green salamander presence.

DISCUSSION

The low Kendall coefficient values (0.133 for all variables and -0.0011 for soils data) indicates that Hardman's (2014) model did not have predictive value for southwestern North Carolina. While the model was designed to incorporate the whole of the Blue Ridge range, the data from the Chattooga River watershed were underrepresented during the creation of the original model. The Chattooga Basin has more granitic domes and outcrops than do other, more typical, parts of the Blue Ridge area. For these reasons, it is likely that the model is biased towards eastern parts of the range, leaving the Chattooga area inadequately represented. Hardman (2014) points out that her model cannot reliably predict whether there is truly a rock outcrop in a given area.

It is also worth noting that our observations on similar characteristics between high-ranking sites such as surrounding vegetation types and landscape characteristics may be worth further study. Notes on the presence of other species of salamanders or organisms may also be useful in future studies about how these factors affect and correlate with green salamander presence and possible site suitability. There is evidence that terrestrial salamander distributions are strongly linked to changes in landscape categories that can be modeled (Keyser et al. 2011). Our data alone, however, are not extensive enough to draw any definitive conclusions about these factors. Further research is needed to make the existing model more accurate for the surveyed area.

Sources of error in our data stem from the difficulty in fully surveying an area projected to have green salamanders. We may have not seen certain rock outcrops during our surveys due to cliffs or rhododendron thickets obstructing our view. Some outcrops were too unsafe for us to access. We were unable to reach some cracks in rock outcrops where green salamanders might have been found. Oftentimes salamanders, green or otherwise, were too deep in the rock cracks to identify with certainty. It is possible that we did not see green salamanders housed in rocks that we surveyed. Williams (personal communication) found that at least three visits to potential sites are needed to confirm green salamander presence or absence in a given season, which is defined as the fall (October to mid-November) when green salamanders establish themselves in rock crevices. Severe drought and wildfires occurred in the Blue Ridge Escarpment in 2016, making conditions poor for finding salamanders that would likely spend more time deeper within the crevices.

In addition to making improvements on the model, further exploration could be done in ground truthing in the Chattooga basin. While we explored much area that was previously unsurveyed, more data on the occurrence of green salamanders in the Blue Ridge Escarpment would be useful. Discoveries of green salamander habitat could also be made by revisiting potential sites and looking in areas we did not reach. One of the sites surveyed for this study where green salamanders were found extended the known range of the species westward by nearly 6.5 km (4 miles) and into the Little Tennessee River drainage in North Carolina, demonstrating that surveying beyond the known extent of their range could prove fruitful. Climate change may also impact this species, so knowing the extent of their range may allow for better monitoring of the species as well as determine potential corridors linking widely separated habitat. Surveying green salamanders to better understand their range, thus, aides in conservation efforts towards preserving the species.

In conclusion, the results we found are consistent with previously published research on green salamanders. We found green salamanders in typical rock face habitats and deep in rock cracks with relatively small openings (Rossell et al. 2009). Our study provides additional data on the range of green salamanders and provides evidence that Hardman's model is ineffective for the study area. There are, however, evident commonalities in the distribution tendencies of green salamanders that point to certain landscape features being better suited to large populations than others. Thus, a model could still prove useful for remotely locating *A. aeneus* in the Chattooga Basin so long as the proper landscape elements are used to generate that model (Keyser et al. 2011). A well-designed model could be useful to conservation organizations seeking to locate green salamanders in order to preserve their population.

Acknowledgements

Thank you to Kyle Pursel for actively mentoring throughout our project. Both Kyle Pursel and Gary Wein provided technological support in working with ArcGIS (ESRI 2015). Sarah Workman and Jim Costa contributed advice in synthesizing scientific papers. Amanda Mullikin also provided thoughtful edits during the production of this paper.

Literature Cited

Armstrong, P. F. 2010. Behavioral interactions and crevice depth preferences in the green salamander *Aneides aeneus* (Order No. 1488727). Available from ProQuest Dissertations & Theses Global. (852626311). 10-11-2016. Web.

Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology **8**: 60–71.

Brodman, R. 2004. Green salamander, *Aneides* aeneus in the eastern region of the U.S. Forest Service District 9 (populations in Indiana, Ohio, Pennsylvania, Maryland and West Virginia). US Department of Agriculture. 11-11-2016. Web.

Corey, E. Personal communication. Interview. 2016.

Corser, J. D. 2001. Decline of disjunct green salamander (*Aneides aeneus*) populations in the southern Appalachians. Biological Conservation **97**: 119–126.

Cupp, P. C. 1980. Territoriality in the green salamander, *Aneides aeneus*. Copeia **1980:** 463–468.

Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases **5**: 735–738.

Davis, T.M. 2004. *Aneides aeneus* (green salamander). Herpetological Review **35**:51–52.

Environmental Systems Research Institute (ESRI). 2015. ArcGIS Desktop: Build 5524 (10.4). Redlands, California, USA.

Gordon, R. E. 1961. The Movement of Displaced Green Salamanders. Ecology **42**: 200–202.

Gottfried, N. 1971. Randomness, independence, and rank correlation. Pages 192-207. Introduction to statistics: a nonparametric approach. Houghton Mifflin Company, Boston, Massachusetts, United States.

Hardman, R. H. 2014. Modeling occurrence of the green salamander, *Aneides aeneus*, in the Blue Ridge Escarpment (Order No. 1570497). Available from ProQuest Dissertations & Theses.

Johnson, A. N. 2002. Determining the genetic distances between sub-populations of *Aneides aeneus* in the Westvāco Wildlife and Ecological Research Forest. Theses, Dissertations and Capstones. Paper 670.

Keyser, P. D., L. A. Williams, K. M. Kelly, C. A. Haas, V. L. Ford, and T. B. Wigley. 2011. An assessment of distribution patterns of terrestrial salamanders in the central Appalachians using two landscape models. The American Midland Naturalist **166**: 194–210.

Larson, A., D. B. Wake, and K. P. Yanev. 1984. Measuring gene flow among population having high levels of genetic fragmentation. Genetics **106**: 293–308.

Lee, D. A. and A.W. Norden. 1973. A food study of the green salamander, *Aneides aeneus*. Journal of Herpetology **7**: 53–54.

Norman, J. (n.d.). Green Salamander *(Aneides aeneus)*. Savannah River Ecology Laboratory. University of Georgia. 09-11-2016. Retrieved from http://srelherp.uga.edu/salamanders/aneaen.htm

Petranka, J. W., M. E. Eldridge, and K. E. Haley. 1993. Effects of timber harvesting on southern Appalachian salamanders. Biological Conservation **7**: 363–370.

Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Smithsonian Institution, Washington, USA. 587pp.

Pursel, K. Personal communication. Interview. 2016.

Rossell Jr, C. R., J. Hicks, L. A. Williams, and S. C. Patch. 2009. Attributes of Rock Crevices Selected by Green Salamanders, *Aneides aeneus*, on the Blue Ridge Escarpment. *Herpetological Review* **40**: 151–153.

Schwartzman, E. 2010. An inventory of the natural areas of Macon County, North Carolina. North Carolina Natural Heritage Program. 09-12-2016. Web.

Smith, A. M. and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography **28**: 110–128.

Snyder, D. H. 1991. The green salamander (*Aneides aeneus*) in Tennessee and Kentucky, with comments on the Carolinas' Blue Ridge populations. Journal of the Tennessee Academy of Science **66**: 165–169.

Waldron, J. L. and W. J. Humphries. 2005. Arboreal habitat use by the green salamander, *Aneides aeneus*, in South Carolina. Journal of Herpetology **393**: 486–492.

Waldron, J.L., and T.K. Pauley. 2007. Green salamander (*Aneides aeneus*) growth and age at reproductive maturity. Journal of Herpetology **41**: 638–644.

Williams, L. A. Personal communication. E-mail. 2016.

DISTRIBUTION OF *CRYPHONECTRIA PARASITICA* AMONG AMERICAN CHESTNUT TREES IN THE SOUTHERN APPALACHIAN MOUNTAINS

ALEXANDER W. KELLOGG

Abstract. The American chestnut (*Castanea dentata*) was once an abundantand tall-growing tree species thriving in the eastern forests of the United States of America. In the first decade of the twentieth century, the fungus *Cryphonectria parasitica* was brought from Asia to North America on a shipment of Chinese chestnut trees (*Castanea mollissima*) and rapidly spread throughout the forests of the eastern U.S., infecting nearly all American chestnut trees it came in contact with and rendering the species functionally extinct. The cultural and economic losses sustained by the species' demise have spurred research into how the American chestnut might one day be restored to its former prominence and stature by gaining a level of tolerance to the blight. In order to reach this goal, it is necessary to understand the conditions under which chestnut blight thrives and to alter them to better suit the resurgence of the American chestnut. In this study, I mapped American chestnut coppices at three different locations in Macon County, North Carolina, and used ArcGIS to evaluate mean elevation, slope, aspect, integrated moisture index (IMI), and forest type to determine if any of these factors correlated with chestnut blight distribution. I found that most factors were not significantly different between infected and uninfected coppices of the same site, suggesting that these factors had a limited influence on chestnut blight distribution. I suggest that future research include coppices found at greater ranges of elevation, slope, aspect, IMI, and forest types to better determine how these factors impact blight distribution.

Key Words: American chestnut; aspect; Castanea dentata; *chestnut blight;* Cryphonectria parasitica; *distribution; elevation; forest type; IMI; slope.*

INTRODUCTION

The American chestnut (*Castanea dentata*) was once among the dominant tree species inhabiting the forests of the Appalachian mountain chain from Alabama to Maine, comprising as much as 25% of the forest (Burnham 1988). For centuries, it was heavily relied upon by the many human inhabitants of the Appalachian Mountains as a source of food, firewood and valuable timber, as well as by countless forest dwelling species that fed off of its mast and relied on it as an integral part of their habitat. In 1904, an import of Chinese chestnut trees (*Castanea mollissima)* arrived in New York City from Asia, carrying the fungus *Cryphonectria parasitica*. After spreading to and killing most if not all of the American chestnuts in and around New York City by 1910, the "chestnut blight" rapidly spread south along the Appalachian Mountain range, where it infected all mature American chestnut trees it came into contact with, killing most of them by 1950 (Burnham 1988). The American chestnut was able to avoid extinction due to its ability to resprout from the root collar of dead trees, forming a group of clumped saplings known collectively as a "coppice." However, since the introduction of the blight, American chestnuts have become functionally extinct as they are no longer able to flower or bear fruit, and are able to grow only as high as the mid-canopy, at most, before succumbing to the blight (Bolgiano and Novak 2007).

C. parasitica infiltrates American chestnut trees most commonly through open wounds in the bark caused by wood-boring insects, tempestuous weather, birds or small mammals, such as squirrels. Once it is able to get past the bark, the fungus sends out mycelia that girdle the stem or trunk of the tree, cutting off the flow of xylem and phloem that transport nutrients and water from the roots to the branches and leaves, eventually killing the tree. The chestnut blight is identified by orange pustules, called stromata, that grow under the bark and break through to the surface as well as by cankers that form on the surface of the bark as the tree reacts to and attempts to isolate the infection. The stomata produce both asexual spores, known as conidia, and sexual spores, known as ascospores, as a means of efficiently propagating the fungus. The single-celled conidia can be carried long distances on the feet, fur, or feathers of organisms that come in contact with it, as well as via water, while the multicellular ascospores tend to travel via air currents (Bolgiano and Novak 2007).

A variety of methods for improving American chestnut resistance to the chestnut blight, such as cross breeding *C. dentata* with the blight tolerant *C. mollissima*, (Baraket et. al. 2009) and employing biological controls to counter the blight (Heiniger and Rigling 1994, Cortesi and Milgroom 2004), have been largely unsuccessful. Despite these failures, the American Chestnut Foundation, founded in 1983 to explore ways to restore the American chestnut, continues to experiment with crossbreeding *C. dentata* and *C. mollissima* to form viable, blight resistant hybrids (Hebard 2005). This study aims to assist in future restoration efforts of hybrid American chestnuts by identifying which, if any, geographical components of the American chestnut's habitat, such as elevation, slope, aspect, integrated moisture index (IMI), and surrounding forest type, have a significant impact upon the distribution of chestnut blight. Such data may be utilized in future restoration efforts to determine which sites in the southern Appalachian Mountains would be most conducive to the survival of the hybrid species.

Methods

From August to October of 2016, I collected data from American chestnut coppices at three sites in the Nantahala National Forest of Macon County, North Carolina, two of which were located at Coweeta Hydrologic Laboratory in Otto, North Carolina (Reynolds Gap and Watershed 7). The third site was located on Yellow Mountain, northeast of Highlands, North Carolina (Yellow Mountain). I randomly tagged 170 chestnut coppices at Reynolds Gap (RG) and Watershed 7 (WS), and 169 points at Yellow Mountain (YM), then plotted each coppice point onto a Garmin Montana 650t GPS for a total of 509 data points. I then collected bark samples from coppices suspected to be infected with the chestnut blight and with Alex Tribo cultured these samples to determine if they were indeed infected with the blight.

After determining which coppices were infected, I depicted the location of each coppice I sampled on ArcGIS (ESRI 2015) on using information from NC OneMap, distinguishing the ones found to be infected with a red star from those that were found to be healthy with a black dot. Using the Extract Values to Points tool in ArcMap, I extracted elevation, slope, aspect, IMI, and surrounding forest type data for each coppice point, then performed statistical analyses on Excel to determine how significant each of these factors were in facilitating the distribution of *C. parasitica* at each site. IMI is a composite score, on a 0-100 scale, of slope-aspect, cumulative down slope water flow, landscape curvature, and a soil's capacity to carry water. It is essentially a method of measuring the moisture retention of a soil and may have an impact upon chestnut blight distribution within a given area (Iverson et al. 1997). The bark tissue culture samples were visually assessed for blight by Dr. Richard Baird of Mississippi State University. Additionally, aerial photography for Macon County depicted in the ArcGIS maps was provided by Dr. Gary Wein of the Highland Cashiers Land Trust.

Results

Vegetation at each of the three sites ranged from Northern Hardwood Forest to drier oak forest types (table 1). American chestnut coppices were found almost exclusively in Montane Oak Forests at Watershed 7 and Yellow Mountain regardless of infection, while coppices were dispersed throughout more diverse forest types at Reynolds Gap. This corresponds to historical data documenting the traditional presence of American chestnut trees in Central and Southern Montane Oak Forests, preferring acidic and xeric soils (Nature Conservancy n.d.).

TABLE 1. Distribution of Infected and Uninfected Coppices in Surrounding Vegetation Types

Site	Infection	Southern Appalachian Northern Hardwood Forest	Southern Appalachian Oak Forest	Southern and Central Appalachian Cove Forest	Central and Southern Appalachian Montane Oak Forest
Reynolds Gap	No	6	29	51	28
	Yes	5	13	21	17
Watershed 7	No	0	9	4	115
	Yes	0	7	1	33
Yellow Mountain	No	1	0	0	132
	Yes	0	0	0	36

Reynolds Gap had the highest blight infection rate, with 32.9% of sampled coppices infected. Watershed 7 and Yellow Mountain had comparably lower infection rates, with 24.1% of coppices infected at Watershed 7 and 21.3% infected at Yellow Mountain. The distribution of coppices at Reynolds Gap (fig. 1) appeared to conform to a clumped distribution model, while coppice distribution at Watershed 7 (fig. 2) and Yellow Mountain appears to be more widely dispersed (fig. 3). Previous research has suggested that clumped coppice distribution is common for *C. dentata*, though more dispersed distribution is also not uncommon (Heinrich 2014).

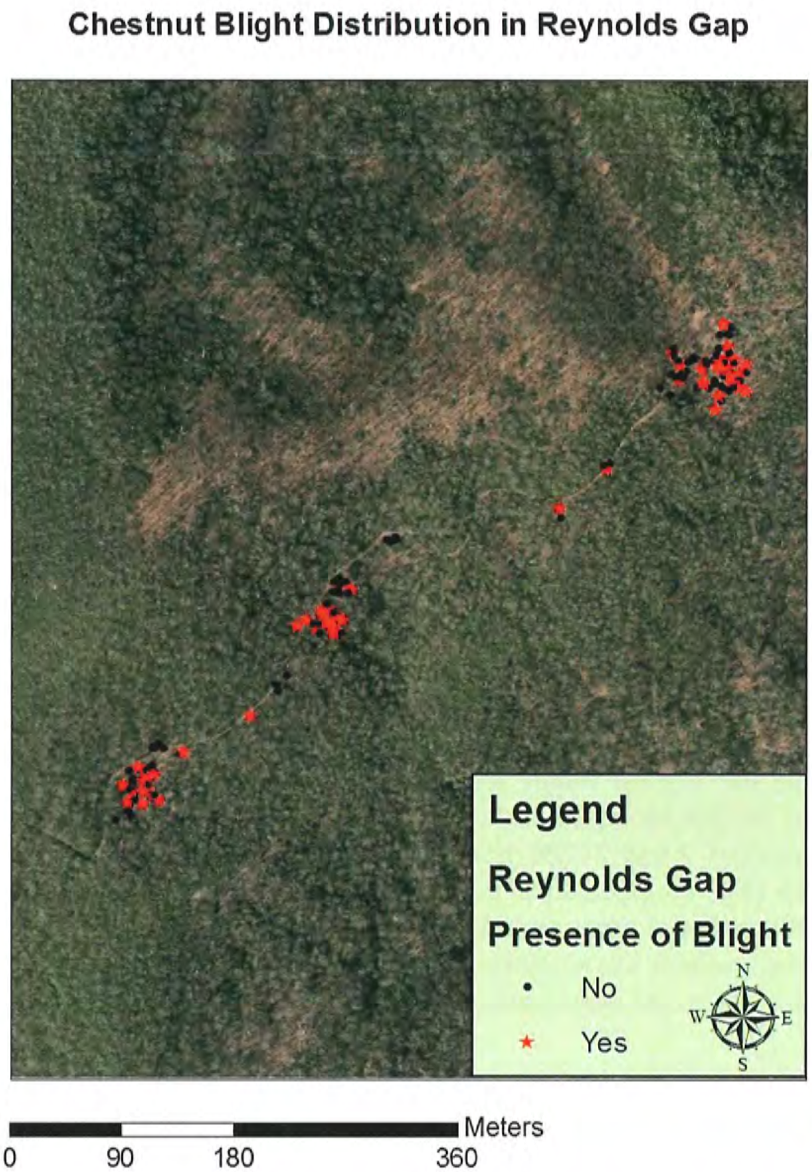


FIG. 1. Chestnut Blight Distribution in Reynolds Gap (ArcMap 10.4, ESRI 2015).

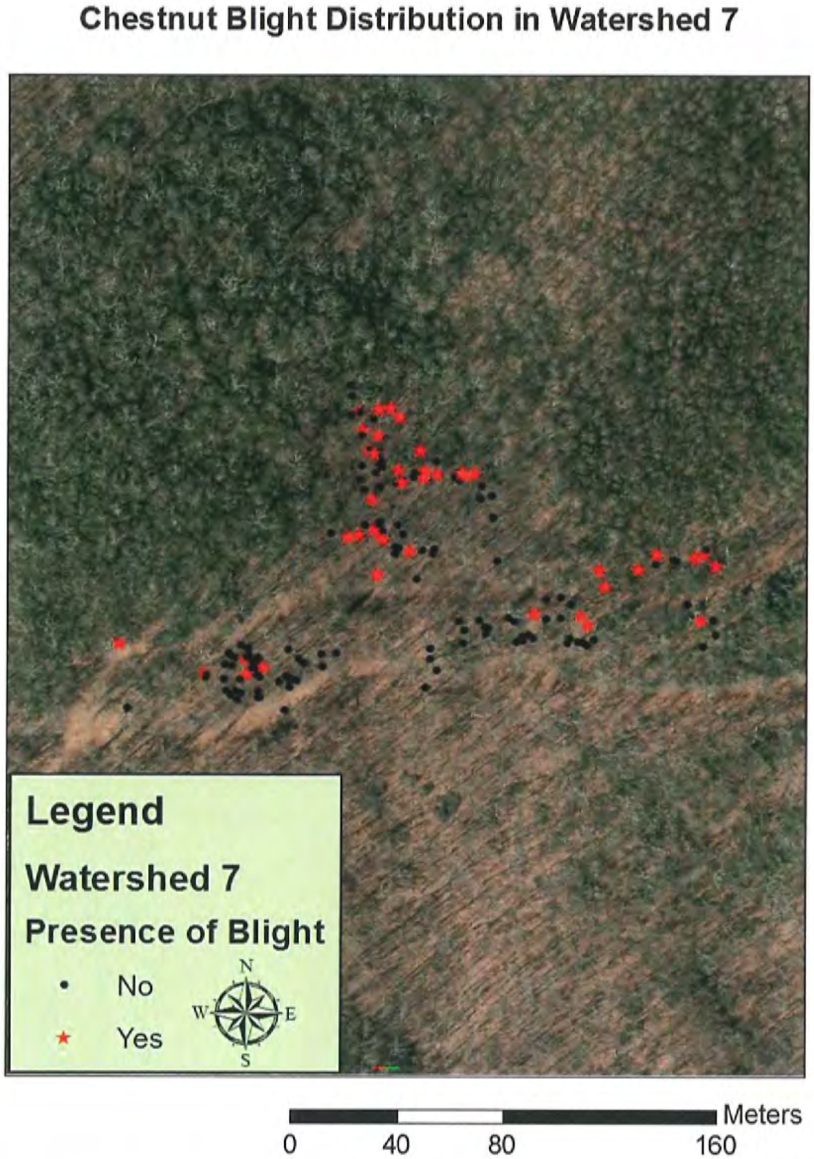


FIG. 2. Chestnut Blight Distribution in Watershed 7 (ArcMap 10.4, ESRI 2015).

FIG. 3. Chestnut Blight Distribution on Yellow Mountain (ArcMap 2014, ESRI 2015).

Mean elevation of infected and uninfected coppices were nearly equal for all three sites. For example, Reynold's Gap coppices were found at a mean elevation of 3803 ft. for infected stems and 3811 ft. for uninfected stems (fig. 4). Standard error revealed that there was a significant difference between the mean elevations of infected and uninfected coppices at Reynolds Gap and Watershed 7, but not Yellow Mountain.

FIG. 4. Distribution of Chestnut Blight Based on Average Elevation. Standard error values: Uninfected (RG: 2.9, WS: 0.1, YM: 2.9). Infected (RG: 4.3, WS: 3.2, YM: 4.3).

Mean slope tended to be higher among uninfected coppices for each site, with the exception of Yellow Mountain. At Reynolds Gap and Yellow Mountain, infected and uninfected coppices did not have significantly different slopes, while uninfected coppices at Watershed 7 were found on significantly greater slope angle than infected coppices (fig. 5). Standard error calculations suggest that slope did not have a great impact upon blight distribution.

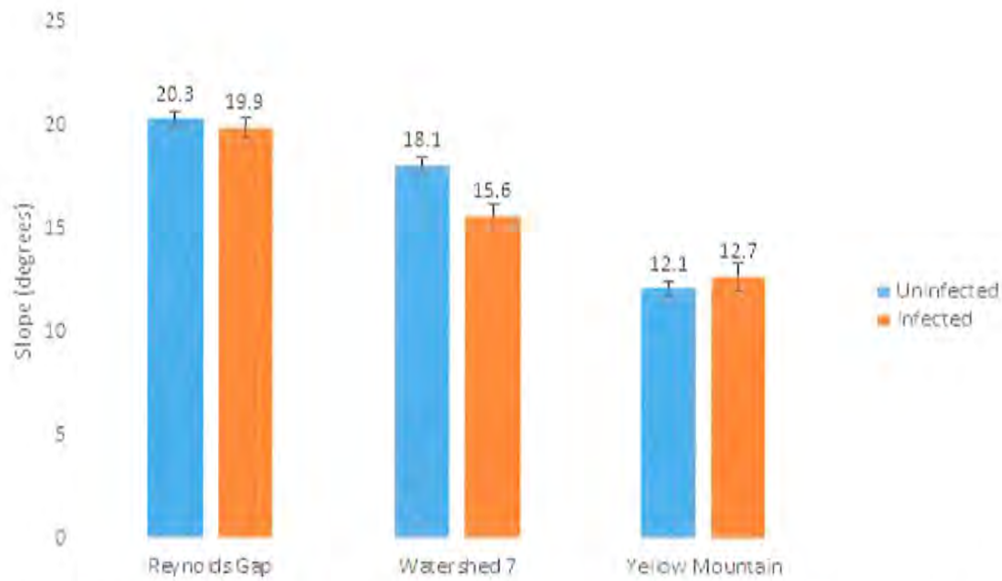


FIG. 5. Distribution of Chestnut Blight Based on Average Slope. Standard error values: In blue, Uninfected (RG: 0.4, WS: 0.4, YM: 0.4). In orange, Infected (RG: 0.5, WS: 0.6, YM: 0.7).

All mean aspects of each site, regardless of infection, were oriented in a southern direction. Aspect did not differ significantly between infected and uninfected coppices at Reynold's Gap and Watershed 7. Aspect did significantly differ between infected and uninfected coppices at Yellow Mountain, with uninfected coppices having a higher mean aspect than infected coppices (fig. 6). Standard error calculations suggest that aspect did not strongly impact blight distribution.

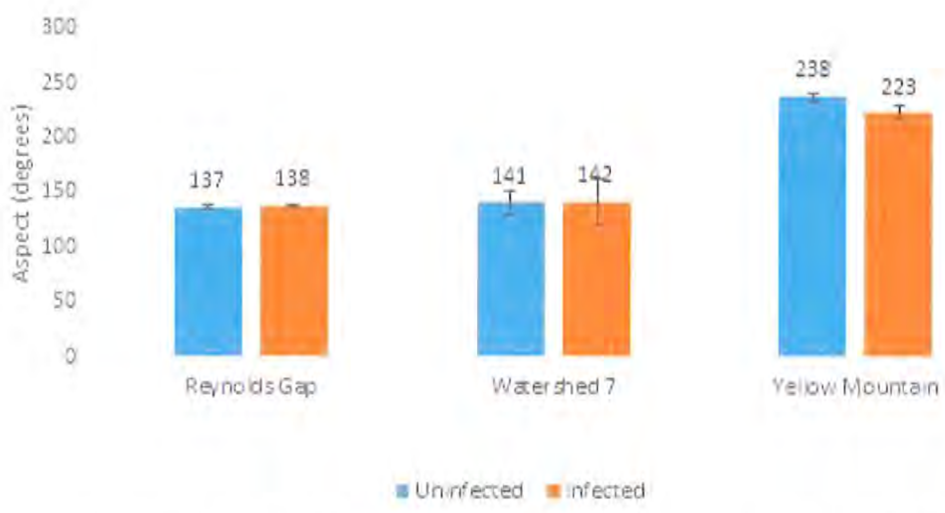


FIG. 6. Distribution of Chestnut Blight Based on Average Aspect. Note: 315°-45° = north, 45°-135° = east, 135°-225° = south, 225°-315°= west. Standard error values: Uninfected (RG: 1.6, WS: 10.8, YM: 3.5). Infected (RG: 1.8, WS: 21.4, YM: 6.1).

The mean IMI score of infected coppices at Reynolds Gap and Watershed 7 were higher than the mean scores of uninfected coppices at each site respectfully, while in contrast, infected coppices at Yellow Mountain had a lower mean IMI score than that site's uninfected coppices (fig. 7). Standard error calculations showed that differences between mean IMI scores of infected and uninfected coppices was only significant at Watershed 7, and was not significant at Reynolds Gap or at Yellow Mountain, suggesting that IMI does not strongly impact blight distribution.

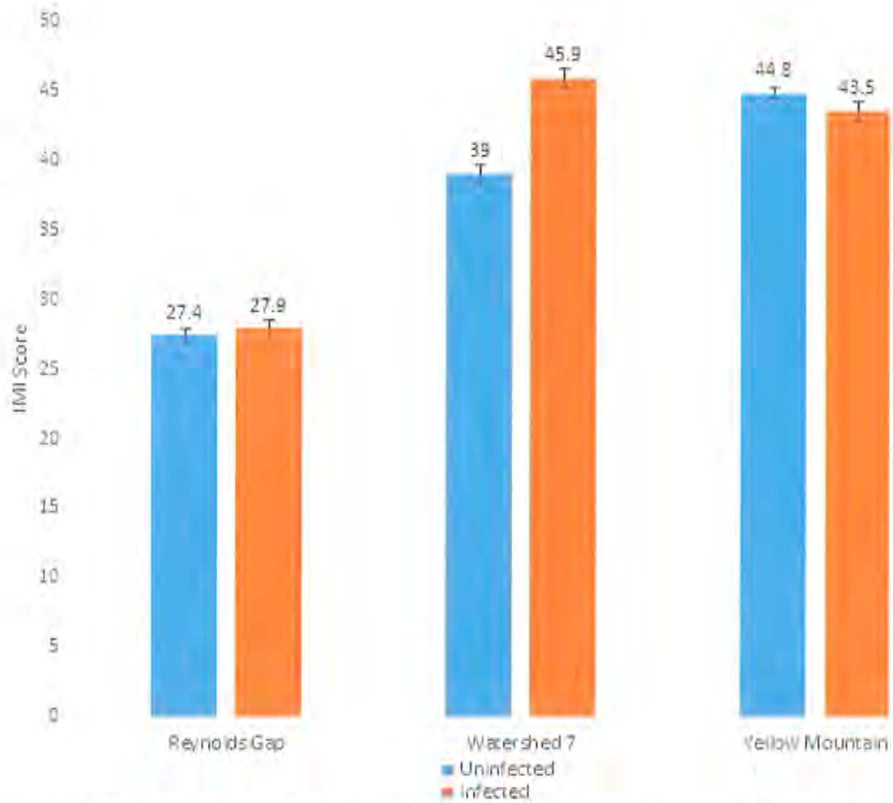


FIG. 7. Distribution of Chestnut Blight Based on IMI Score. Standard error values: Uninfected (RG: 0.4, WS: 0.7, YM: 0.4). Infected: (RG: 0.6, WS: 0.7, YM: 0.7).

DISCUSSION

Standard error calculations showed that there was a significant difference between infected and uninfected coppices based on elevation for two of the three sites surveyed (Reynolds Gap and Watershed 7). On average, uninfected coppices tended to be located a few feet higher than infected coppices (fig. 4). Given that each site was located thousands of feet above sea level and that not all sites saw a meaningful difference of elevation based on presence of blight, there exists no clear trend as to what effect elevation has on blight distribution. Slope was also not significantly different in regards to blight distribution for most sites as the mean slope for infected coppices was marginally steeper than that of uninfected coppices at Yellow Mountain while the opposite was true for Reynolds Gap and Watershed 7 (fig. 5). Watershed 7 did show a more pronounced difference of slope with uninfected coppices growing on slopes that were on average 2.5° steeper than those of the infected coppices (fig. 5). Again, however, no definitive relationship between slope and blight distribution is evident in the data. Mean aspect, despite also not yielding any significant difference between infected and uninfected coppices for two of the three sites (Reynolds Gap and Watershed 7), did reveal that the average aspect of all coppices regardless of infection ranged from the southeast to the southwest (fig. 6). Given that southern facing slopes receive more sunlight than northern facing slopes in the northern hemisphere and that American chestnuts generally only regrow from root collars rather than from seeds, it is possible that, in their reduced state, American chestnuts are only able to sprout on southern facing slopes where more sunlight is available. Further research may have to be done to test this.

Mean IMI scores, though higher among infected coppices at two of the three sites (Reynolds Gap and Watershed 7), was only significantly different between infected and uninfected coppices at Watershed 7 (fig. 7). Coppices at all sites, regardless of infection, favored xeric soils, corresponding to the findings of the surrounding forest type survey showing most coppices growing amid dry Montane Oak forests (table 1). The difference of averages between infected and uninfected groups was more pronounced at Watershed 7, with infected coppices scoring 6.9 points higher than uninfected (fig. 7). This would appear to be more in line with previous research that suggests that *C. parasitica* prefers moister soils given that water is a vector for transmission (Anagnosaikis 2001). However, based on standard error estimates, there is no strong discernable relationship between IMI scores and distribution of chestnut blight.

Conclusions

The results of this research suggest that there exists no significant difference between infected and uninfected coppices across all three sites in regards to elevation, slope, aspect, IMI score, or surrounding forest type. Future research might include taking samples from coppices found at broader ranges of elevation, slope, aspect, IMI, and forest type to more precisely determine if or how these factors impact distribution of *C. parasitica.* Additional research might also look into how or if physical characteristics of individual coppices, such as stem height, diameter, and distance from other coppices, are correlated with infection. This research could be instrumental in discovering ideal locations to reintroduce mature American chestnut trees that have been genetically modified or crossbreed with *C. mollissima* (Chinese chestnut) to achieve a level of tolerance to *C. parasitica.*

Acknowledgements

I would like to first acknowledge and thank my colleague, Ms. Alexandra R. Tribo, for her collaboration in collecting data from American chestnut coppices at our three field sites. I would also like to thank my mentor, Dr. Richard Baird, for his expertise and guidance in seeing this study come to fruition, as well as Dr. Gary Wein, who spent many hours teaching me how to extract, organize, and display my data on ArcGIS. Finally, I would like to thank Dr. Jim Costa and Dr. Sarah Workman for reviewing my paper as it took form and for their work in making the Institute for the Environment program an enjoyable and valuable experience.

Literature Cited

Anagnosaikis, Sandra L. 2001. American chestnut sprout survival with biological control of the chestnut-blight fungus population. Forest Ecology and Management. **152:**225-233.

Baraket, Abdelali et al. 2009. Comparison of the transcriptomes of American chestnut (*Castanea dentata*) and Chinese chestnut (*Castanea mollissima*) in response to the chestnut blight infection. BMC Plant Biology.

Bolgiano, Chris, and Glenn Novak. 2007. Mighty Giants: An American Chestnut Anthology. The American Chestnut Association.

Burnham, Charles R. 1988. The Restoration of the American Chestnut. American Scientist. **76**:478-487.
Cortesi, Paolo, and Michael G. Milgroom. 2004. Biological Control of Chestnut Blight with Hypovirulence: A Critical Analysis. Annual Review of Phytopathology. **42**:311-338.
Central and Southern Appalachian Montane Oak Forest. The Nature Conservancy. Retrieved on 11 Nov 2016. Web. https://www.conservationgateway.org/ConservationByGeography/NorthAmerica/UnitedStates/edc/Documents/HabitatGuides/29.pdf
Environmental Systems Research Institute (ESRI). 2015. ArcGIS Desktop 10.4. Redlands, California, USA.
Hebard, Frederick V. 2005. The Backcross Breeding Program of the American Chestnut Foundation. Journal of the American Chestnut Foundation. **19**:55-78.
Heiniger, Ursula, and Daniel Rigling. 1994. Biological Control of Chestnut Blight in Europe. Annual Review of Phytopathology. **32**:581-599.
Heinrich, Bernd. 2014. American Chestnut Seed Dispersal and Regeneration. Northeastern Naturalist. **21**:619-629.
Iverson, Louis R, Martin E. Dale, Charles E. Scott and Anantha Prasad. 1997. A GIS-derived integrated moisture index to predict forest composition and productivity of Ohio forests (U.S.A.). Landscape Ecology. **12**:331-348.
NC OneMap GeoPortal. Retrieved on 8 Dec 2016. Web. http://data.nconemap.com/geoportal/catalog/raster/download.page

EVALUATION OF STEWARTIA OVATA HABITATS IN THE CHATTOOGA RIVER DRAINAGE: SOIL FERTILITY AND MICROVERTEBRATE ASSOCIATIONS

AMANDA MULLIKIN

Abstract. *Stewartia ovata* is a species of camellia with a scarcely understood range in the southern Appalachian Mountains. I conducted an evaluation of selected S. ovata sites in the Chattooga River drainage to help better characterize the ecology of S. ovata sites, potentially shedding light on why the range of S. ovata is so limited. Study sites located in Rabun County, Georgia and Oconee County, South Carolina were studied in this research, including the upper and lower reaches of Sarah's Creek, Woodall Shoals, Overflow Creek, and the Chattooga River access off of Earl's Ford. Environmental factors such as soil nutrients, soil organic matter, and soil microinvertebrates were examined in order to better understand S. ovata microhabitat and aid cultivation and preservation of this uncommon plant.

Key Words: S. ovata, *Mountain Camellia, soil fertility, Jackson County, Rabun County, soil microinvertebrates, uncommon plant, soil biology, Southern Appalachian Mountains, Overflow Creek, Woodall Shoals, Chattooga River, Sarah's Creek*

INTRODUCTION

The southern Appalachian Mountains are a mountain range in the eastern United States that form a conglomeration of unique ecosystem communities comprised of fragmented groups of flora and fauna, which have consequently adapted to one of Earth's oldest geomorphic formations. Many of the ecosystem communities in the southern Appalachians are home to a variety of endemic species (Barry 1980) which is a likely result of the elaborate orogenic and climatic history of these ancient mountains. The clastic events that took place during the Ordovician period, about 488 million years ago created the rock foundation which has weathered over time into the mosaic of landscapes that evident today. The region was likely also a refugium for northerly species during peri-glacial periods. The mosaic landscape is therefore integrated with of a web of biologic interactions (Peterson 2001) in which, the mountain camellia finds its niche.

The mountain camellia (*Stewartia ovata)* is a shrub of the family Theaceae, which includes teas and related genera. Mountain camellias reach sexual maturity at about three meters tall, and exhibit finely stripped bark with thin zig-zagging branches. The leaves of *S. ovata* are bright green, deeply grooved, ovate and ciliated along the margins, with a long and broad axis. These deciduous shrubs lose their leaves, typically in late October. The flowers of *S. ovata* bloom in late June for a period of about two weeks. The flowers are a little under eight centimeters across and have five petals with golden or purple stamens at the center (Wofford 1989). The large flowers, pleasant aroma, and intricate bark stripes make the mountain camellia a desirable plant for gardeners, landscapers, and conservationists alike (Curtis 1996). *S. ovata* grow in drainage areas are well adapted to cold winters, but are sensitive to disturbances like fire or drought. (Wofford 1989)

S. ovata are an opportunistic sub-canopy ravine species that prefers acidic cove, and mesic forests. Their habitat is generally defined by acidic soils, high moisture availability, and high levels of sunlight (Johnston, pers. comm.). Cove forests tend to have tightly packed canopies, which limit the growth *S. ovata* as it has been recorded to need a generous amount of sun-light to germinate (Jack Johnston personal communication).

Plant distribution patterns depend on the available of nutrients, which are controlled by a variety of biotic and abiotic factors. The complexity of ecosystem relationships make it difficult to say why exactly a species is adapted to a specific set of environmental factors, and the same is true of the range of *S. ovata*. I studied a few aspects of soil biology in *S. ovata* communities, an often-neglected aspect of plant ecology, to try to shed some light on the unique distribution of *S. ovata*. I looked at soil nutrient levels, soil organic matter, and soil microinvertebrates, to see if there were any correlations between these factors.

Materials and Methods

Area of Study

For my evaluation on the causative factors that limit that range of *S. ovata* Jack Johnston, a naturalist and well known rare plant cultivator, showed me several sites previously located by systematically walking through the woods looking for vegetation and landscape abnormalities. We conducted several reconnaissance walks through the woods so that he could teach me where to look for *S. ovata*, and other plant species. Taking these walks, I was able to ascertain habitat qualities of *S. ovata*, such that they almost always grow on a north facing slopes of narrow sighted creek valleys near rivers or streams. We located several known populations of *S. ovata* in Georgia and South Carolina, and sought new populations in the Overflow Creek drainage area of Georgia (table 1). One of my objectives of this study was not only to investigate potential causes of *S. ovata* distribution, but to also document new populations so that others may be able to study this plant. Accordingly, Figure 1 shows the location of *S. ovata* populations in my study area, some of which had not previously documented in Rabun County, Georgia.

TABLE 1. GPS coordinates of study sites where soil, leaf, and microinvertebrate samples were collected: Sarah's Creek, Earl's Ford, and Overflow Creek in Rabun County, GA, and Woodall Shoals in Oconee County, SC.

	Upper Sarah's Creek	Lower Sarah's Creek	Earl's Ford	Overflow Creek	Woodall Shoals
Latitude	N34°56.182	N 34°55.163	N34°54.114	N34°56.614	N34°59.012
Longitude	W83°16.206	W83°15.685	W83°15.140	W83°15.241	W83°06.403

Soil Types

Using ArcMap software, I recorded the GPS points I used to mark *S. ovata* in the field (fig. 1). I utilized United States Department of Agriculture geographic database to obtain taxonomic soil data for Rabun County, GA so that I could create a frame of reference to relate geologic characteristics of where I found *S. ovata*, to my soil nutrient analysis.

Soil microinvertebrate analysis

To explore characteristics of S. ovata habitat I collected 3 leaf litter samples from 1 x 1 meter quadrats established at each of the five sites, for a total of 15 litter samples taken from beneath S. ovata plants. For each litter sample, I collected the top strata of leaf litter and bagged the area of litter contained in the quadrat and placed the sample into a cooler on site. Back in the laboratory I placed the litter samples into Berlese funnels, which are devices constructed to separate soil arthropods from leaf litter with the use of an incandescent light bulb. The litter samples were spread on a grate covering the thinnest part of the funnel which faced down into a jar of 80% ethyl alcohol. After I put the samples in the funnel, I covered the top with a 40W incandescent light for about 48 hours. The light and heat from the bulb desiccated the sample and drove out the animals within into the collection jar. A compound light microscope was then used to identify specimens down to order, using Dindal (1990) as a reference.

Soil and leaf tissue nutrient and analysis

To understand the nutrient needs of *S ovata*, four soil core samples were taken with a soil corer from the A horizon, at approximately ten centimeters, within a 30 meter range of selected *S. ovata* specimens. The first soil core sample was taken from the highest *S. ovata* on the slope of on which the population was found, and the core samples proceeded down the slope. The soil core samples were then put in plastic bags and into a collection cooler on site. The samples were taken to the laboratory at the Highlands Biological Station, and laid out to dry on paper towels over a week-long period. Samples were then sieved with a two millimeter opening and then with a 0.71 mm opening sieve, then packed in paper bags, and sent to the Soil Analysis Laboratory at the University of Georgia, in Athens, GA. Additionally, I collected one soil organic matter sample within a meter of five different *S. ovata* from each site, for a total of five soil organic matter samples. To collect the samples, I measured ten centimeters into the soil with the soil corer and used a trowel so scoop out a sample that reached the ten centimeter depth. I bagged the samples and placed them in the cooler on site. I then laid the soil organic matter samples out the dry with the soil core samples for a week-long period. I sent my samples to the University of Georgia, where the Meleich method was used to determine the soil nutrient content. Organic Matter was determined by the "loss on ignition" method for 3 hours at 360° C. Results are reported in percent by weight. Soil nutrient samples were processed using the Melich III method of extraction.

In addition to soil samples, I collected four leaves from four separate mid-height branches of five *S. ovata* from three sites. The leaf samples were used to assess the variability in leaf tissue cations to help describe the relationship between *S. ovata* and nutrient uptake. I collected the non-senescent leaves from the branches, and then bagged the samples and placed them into a cooler on site. I then laid the leaves out to dry with the soil core and soil organic matter samples for a week-long period. I then packaged the leaves and sent them to the University of Georgia, where Inductively Coupled Plasma Mass Spectrometry was used to determine the nutrient content in my tissue samples.

RESULTS

Soil Types

From the data I collected using ArcMap, I found that *S. ovata* was found most commonly on coarse-loamy, mixed, mesic Umbric Dystrochepts; and also on Clayey, oxidic, mesic, Typic Hapludults soil types (table 2). So, the most common orders that these soil classifications belonged were entisols, inceptisols, and ulltisols. *S. ovata* was most typically found on inceptisols (epts), followed by utisols (ults), and then inceptisols (epts).

TABLE 2. Soil taxonomic classification in Rabun County, GA for *S. ovata* GPS data points. Each data point does not represent an individual plant, but rather a site where plants are known to grow. Soil Survey-NRCS-USDA.

Soil Taxonomic Classification	Number of *Stewartia ovata*

Coarse-loamy, mixed, mesic Umbric Dystrochrepts	35
Fine-loamy, mixed, mesic Cumulic Haplumbrepts	1
Coarse-loamy, mixed, nonacid, thermic Typic Udifluvents	4
Fine, kaolinitic, mesic Typic Kanhapludults	5
Loamy, mixed, mesic, shallow Typic Hapludults	2
Clayey, oxidic, mesic Typic Hapludults	15
Fine-loamy, mixed, mesic Typic Endoaquults	1

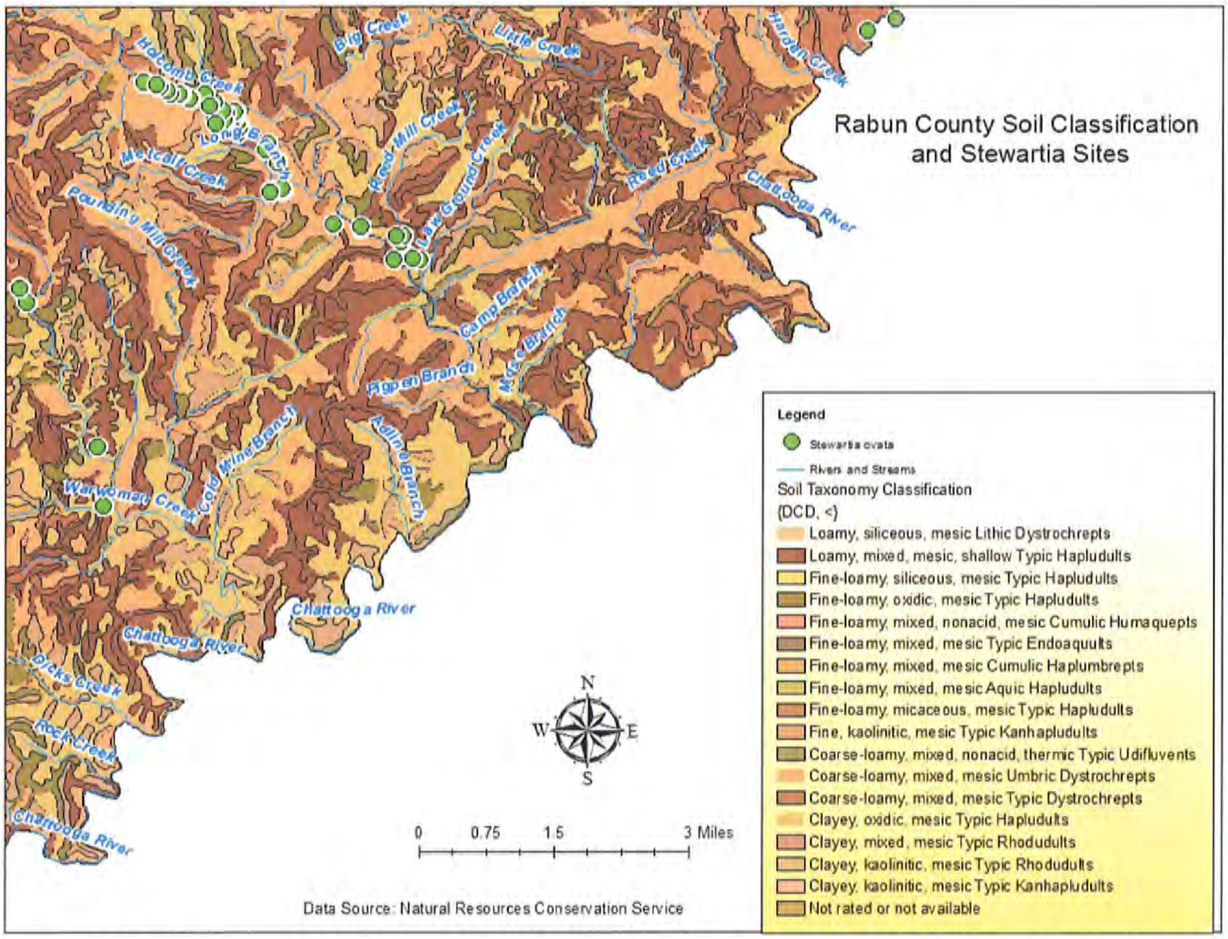


FIG. 1. Documented GPS points of *Stewartia ovata* in Rabun County, Georgia. Soil types where S. ovata was found: Coarse-loamy, mixed, mesic Umbric Dystrochrepts, Tusquitee loam, 10 to 25 percent slopes; Clayey, oxidic, mesic Typic Hapludults Bradson fine sandy loam, 10 to 25 percent slopes; Fine, kaolinitic, mesic Typic Kanhapludults Hayesville fine sandy loam, 10 to 25 percent slopes; Fine-loamy, mixed, mesic Typic Endoaquults, Chatuge loam; Clayey, oxidic, mesic Typic Hapludults, Transylvania-Toxaway complex; Coarse-loamy, mixed, nonacid, thermic Typic Udifluvents, Saluda association, steep.

Stem Count

I took a count of all the *S. ovata* that I found at each site to help get an idea of which sites had higher populations of *S. ovata* (table 3). I did not quantify the size of the area by any pre-determined parameters as I was surveying, I simply counted the specimens that were found.

Overflow Creek had the greatest number of *S. ovata*, and my data suggest that the Upper Sarah's Creek site had the least.

TABLE 3. Field stem count for each study site. Reproductive stems determined if plants reached height of approximately six feet, seedling plants determined as those plants which were under six feet tall.

Site	Reproductive Stems	Seedlings	Total
Lower Sarah's Creek	24	48	72
Upper Sarah's Creek	25	15	40
Earl's Ford	18	35	53
Overflow Creek	73	82	155
Woodall Creek	26	44	70

Microinvertebrates

I counted individual microinvertebrates from all 15 samples of leaf litter (table 4) (three samples from each of the five sites) and identified them down to order. I found that mites (Acari) were by far the most abundant soil microarthropod group in all samples. A fairly consistent diversity of soil microinvertebrates between the sites, but the Overflow Creek had the highest total of soil microinvertebrates and Woodall Shoals had the smallest total number.

TABLE 4. Number of microinvertebrates found at each *Stewartia ovata* site (n=3).

Taxon	Lower Sarah's Creek	Upper Sarah's Creek	Earl's Ford	Overflow Creek	Woodall Shoals
Acari	322	459	379	592	274
Araneae	36	29	13	7	33
Chilopoda	2	1	3	0	4
Collembola	70	69	78	12	77
Coleoptera	1	7	2	5	2
Diplura	5	0	1	7	1
Diptera	7	9	44	2	3
Formicidae	5	1	12	28	13
Gastropoda	2	0	0	0	1
Hemiptera	3	2	1	1	4
Haplotaxida	1	0	0	0	1
Isopoda	17	8	12	25	10
Microcoryphia	4	2	3	3	0
Neuroptera	0	0	1	0	0
Pseudoscorpionida	4	3	7	2	5
Protura	13	25	12	44	13
Thysanoptera	0	17	14	33	24
**Unknown*	11	3	12	9	9

I counted individual microinvertebrates from all 15 samples of leaf litter (table 4) (three samples from each of the five sites) and identified them down to order. I found that mites (Acari) were by far the most abundant soil microarthropod group in all samples. A fairly consistent diversity of soil microinvertebrates between the sites, but the Overflow Creek had the highest total of soil microinvertebrates and Woodall Shoals had the smallest total number.

Tissue Analysis

To determine which soil cations were contained in the leaf tissue of *S. ovata* I sent four leaves from four midlevel branches on five trees from three sites to be analyzed for nutrients (fig. 2 and fig. 3). I present aluminum separately (fig. 4) as the levels were much higher than any of the other nutrients.

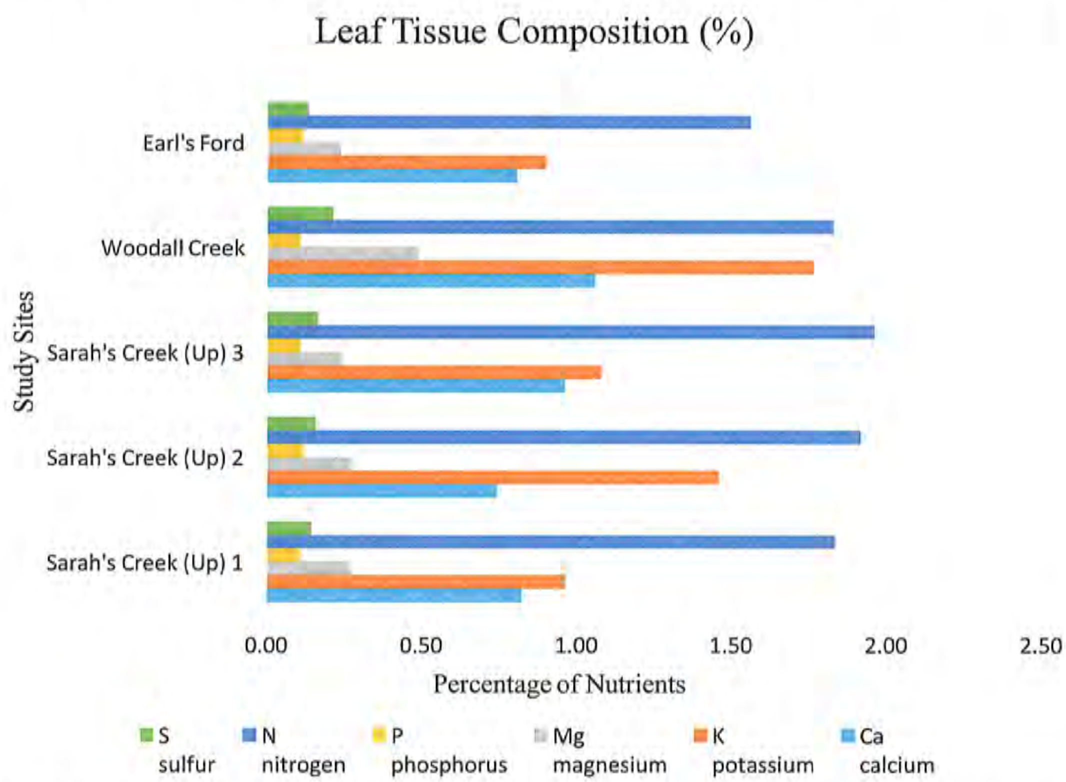


FIG 2: Leaf composition (%) from leaf tissue analysis (ICP) [Soil, Plant, and Water Laboratory, UGA, Athens, GA]. (n=4)

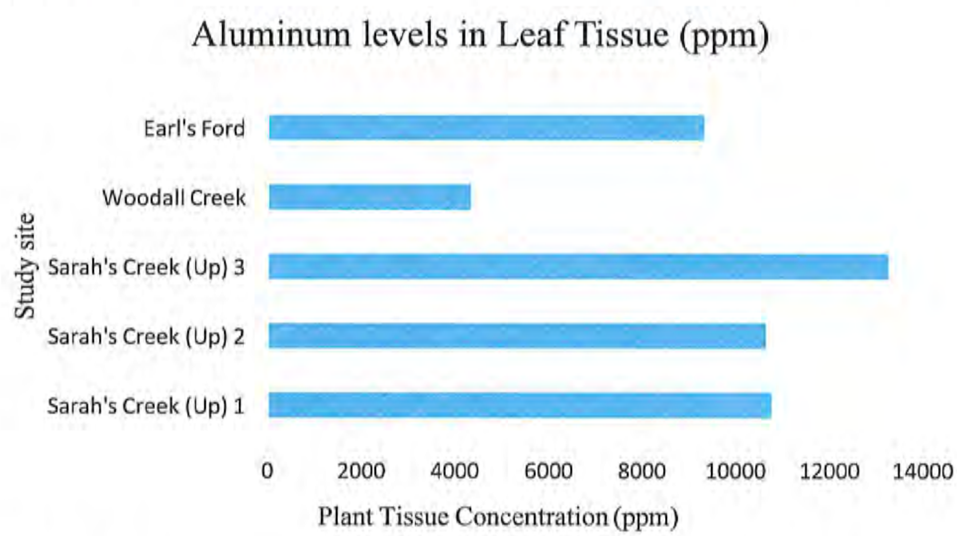


FIG. 3. Leaf tissue aluminum concentration (ppm) (n=1) [Soil, Plant, and Water Laboratory, UGA, Athens, GA].

Soil core nutrient analysis

To represent the nutrient levels of each of the sites, Table 5 contains the average values of major soil cations (mg/kg or ppm) for each of the five sites.

TABLE 5. Mean values of Ca, K, Mg, Mn, P, and Zn in parts per million (ppm) for each of the five study sites. [Soil, Plant, and Water Laboratory, UGA, Athens, GA].

Site	Ca	K	Mg	Mn	P	Zn
Earl's Ford	49.25	35.33	23.70	7.96	2.17	2.29
Lower Sarah's Creek	933.00	122.18	164.45	26.83	3.34	5.31
Overflow Creek	138.50	86.85	54.15	33.86	9.06	2.55
Upper Sarah's Creek	220.25	67.35	46.60	27.82	8.40	2.88
Woodall Shoals	172.75	117.58	44.50	17.07	8.28	5.03

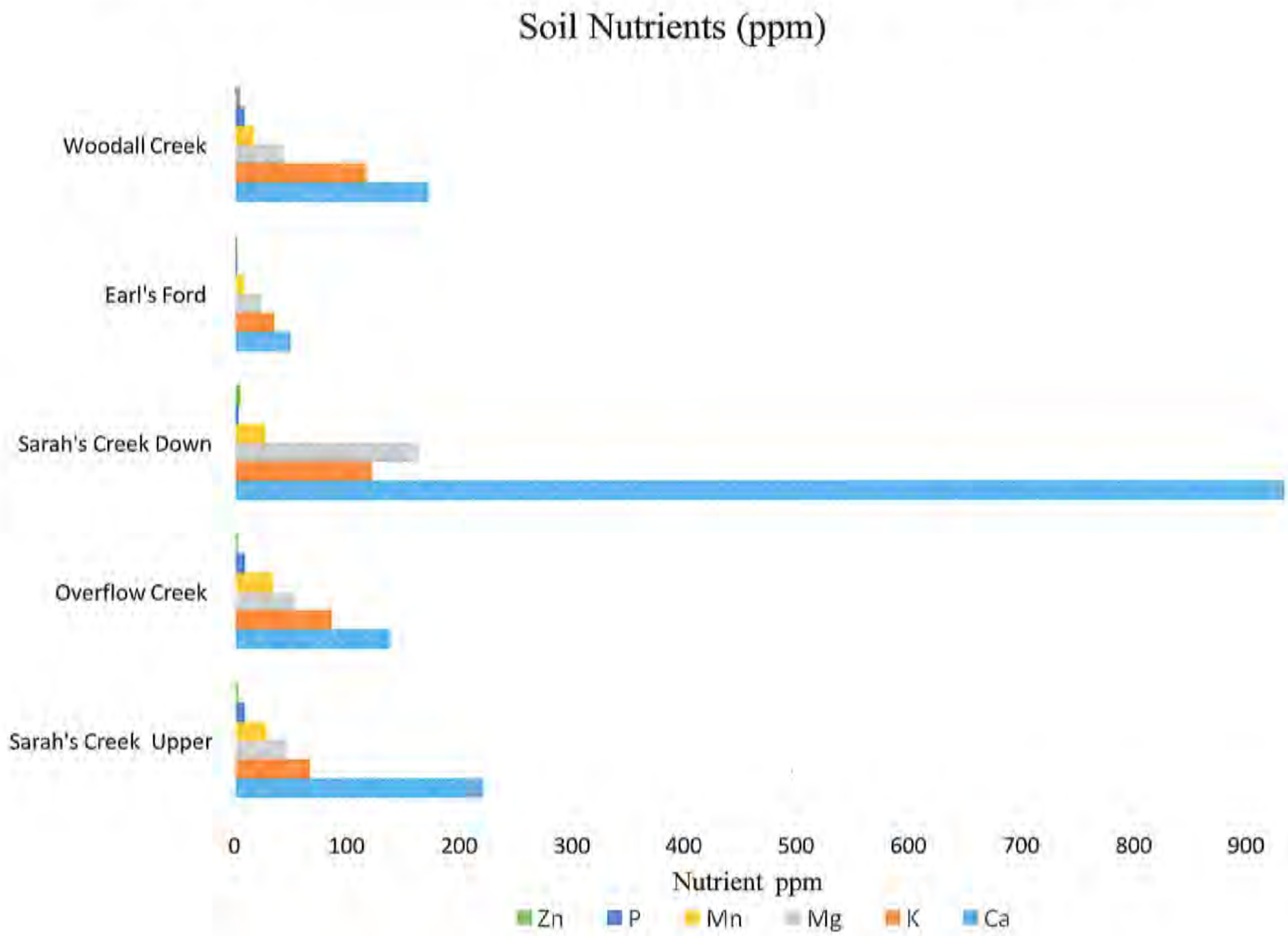


FIG 4. Mean (n=4) soil nutrient levels, in ppm, for the five sites. [Soil, Plant, and Water Laboratory, UGA, Athens, GA].

Soil Organic Matter

Figure 5 represents the soil organic matter percent weight for each of my study site.

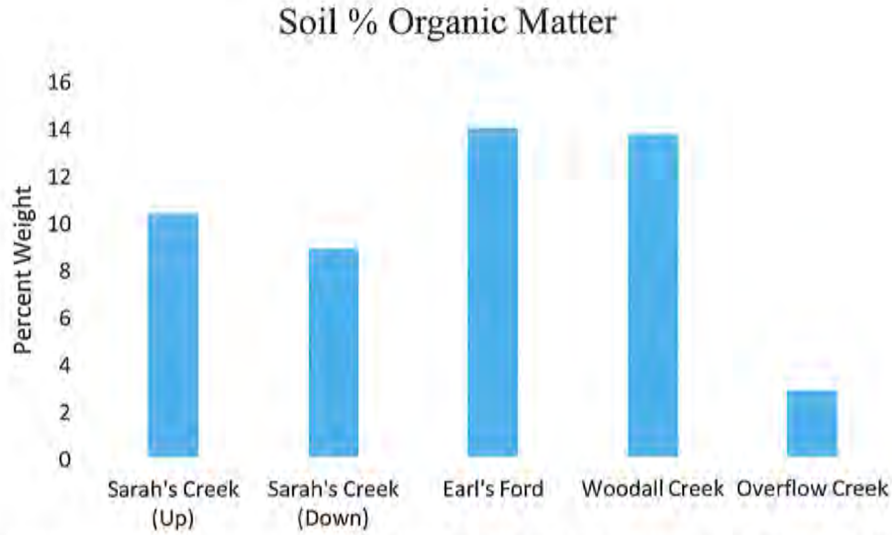


FIG 5. Percent soil organic matter collected from each of the study sites. [Soil, Plant, and Water Laboratory, UGA, Athens, GA].

DISCUSSION

This study approached the characterization of the unique distribution of *S. ovata* in five locations, with several research techniques. For my vegetation surveys and stem counts I did not set a specified area to measure, which leaves my results without much significance. Additionally, because of the small sample sizes, it is difficult to verify any conclusions without further study. This was more of an explorative study where I was looking for significant patterns in soil biology that may encourage future, more organized, studies.

Soil Microinvertebrates

The soil microinvertebrate results indicated that for all five sites the most abundant microinvertebrates were Acari (mites), protura, and collembola. All of these orders feed on decaying vegetation and fungi and for that reason are important ecosystem engineers as they are primarily responsible for breaking down soil organic matter into available nutrients for plants to utilize in cation exchange. Predatory microinvertebrates like Araneae (spiders), psuedoscorpions, and Chilopoda (centipedes) were also found. The ecosystem significance of predatory microinvertebrates in the soil biology associated with S. ovata is that these organisms control populations of herbivorous vegetarian species in the soil, as well as the nutrient processing of the organisms that they consume that are returned to the soil. The other fungal, detrital, and otherwise herbivorous microinvertebrates that were found to be of significance were Isopoda (pill bugs), Formicidae (ants), Thysanoptera (thrips), Microcoryphia (bristletails), haplotaxida Annelida (earth worms), Hempitera (true bugs), Coleoptera (beetles), Diptera (flies), and Gastropoda (snails). I hypothesized that the sites with the largest number of soil microinvertebrates would predictively have had more abundant populations of *S. ovata*, perhaps because the microinvertebrates transform the soil organic matter into useable nutrients for the plants. Alternatively, higher microinvertebrate populations and organic matter and nutrient concentrations might indicate that a longer period has elapsed since disturbance at these sites, reflecting conditions conducive to the buildup and development of larger S. ovata populations. In any case, I did find that the site with the largest population of S. ovata, Overflow Creek, had the largest number of soil microinvertebrates. The Overflow Creek site additionally had the lowest level of soil organic matter, perhaps because the breakdown of soil organic matter by the microinvertebrates leads to greater uptake and mobility.

Soils and leaf nutrients

Cations are positively charged ions contained in soil organic matter. The ability for soils to hold onto cations is called the cation exchange capacity, or CEC (Ketterings et al. 2007). Calcium, magnesium, potassium, sodium, hydrogen, aluminum, iron, manganese, and zinc are the major cations that can bond to negatively charged clay and soil organic matter. Therefore, the more compacted the soil organic matter, the more difficult it is for plants to utilize soil nutrients. The cations that are used in the largest amounts are calcium, magnesium, and potassium (Ketterings et al. 2007), in my linear regression statistical analysis I found that calcium and magnesium levels contributed to the distribution of *S. ovata* (table 5). I calculated the mean cation concentrations per site and found that the P values for calcium and magnesium were (0.04) and (0.04), respectively, for the total stem count which included both seedlings and adult stems. The null model suggested that neither manganese, phosphorus nor zinc were significantly correlated with the total stem count. I conducted an analysis of variance for the soil nutrients for all the sites and I found that all sites varied significantly based on the suite of cations, with the exception of zinc.

I noted that aluminum levels in the leaf tissue were high for all 5 sites (fig. 3), which is interesting because aluminum will replace silica and give soils a more negative charge, and therefore the soils would be more likely to hold on to cations. Aluminum is not considered an essential nutrient, although sometimes low concentrations increase certain plant growth (USGS, 2016) Aluminum has possible toxic effects at high concentrations, which is concerning given that all the *S. ovata* sites exhibited high levels of aluminum. My leaf tissue analysis was conducted on a small sample size, so from the results it is difficult to make any conclusive observations one whether or not the plant uptake reflects soil nutrition. Additionally, due to the time of the year I collected my leaf tissue samples the *S. ovata* samples were beginning to senesce. The time of sample collection likely had an impact on the ICP test results.

Contrary to the results of my linear regression model, I found that the site with the largest population of S. ovata had the highest concentration of available phosphorus. Phosphorus is an essential component of ATP and allows plants to extract nutrients from the soil (Hyland, et al. 2005). The high level of phosphorus at the Overflow Creek site suggests that S. ovata abundance is dependent on the availability and capability to utilize phosphorus to absorb nutrients.

Soil organic matter is the area of the soil which contains plant and animal tissue in various stages of decomposition (Fenton et al. 2008). The soil organic matter levels at my *S. ovata* sites varied slightly, with the lowest level at Overflow Creek as an outlier. Overflow Creek had the largest abundance of *S. ovata,* the highest levels of phosphorus, and largest abundance composition of soil microinvertebrates Because of this relationship, I predict this is why there was a low amount of soil organic matter, and a high amount of phosphorus, but due to the small sample size this should be further studied.

CONCLUSION

This study has shed light on a neglected aspect of the ecology of *S. ovata*, namely the soil microfauna that play an essential role in decomposition and nutrient cycling. Without the existence of soil microinvertebrates, nutrients would take much longer to be broken down and the growth of associated plants species would be much more limited. This study was not intended to identify the main factors determining the scattered distribution of these shrubs; these factors are largely unknown, but I did determine that the largest *S. ovata* population among my study sites (Overflow Creek) was associated with the highest densities of soil invertebrates and high concentration of such crucial nutrients such as phosphorus.

It is not clear if the high soil microinvertebrate populations associated with the Overflow Creek site contribute to the size and healthiness of the *S. ovata* population at that site, or if soil microinvertebrates and *S. ovata* populations stem from an external factor important to both, such as time since last disturbance.

This study furthers our understanding of an important aspect of *S. ovata* ecology. I hope that in so doing, microinvertebrates in the garden are seen less as pests, and more as an important component of the soil community. For gardeners and plants conservationists, seed collectors, and landscapers this study could help guide planting and cultivation projects that may involve *S. ovata*. For example, restoration efforts for *S. ovata* should take into account the preferred soil characteristics and associated microfauna of this species to would a more holistic ecological approach to *S. ovata* restoration.

ACKNOWLEDGEMENTS

I would like to thank Jack Johnston for making this internship a truly enjoyable learning experience, for teaching me how to read a forest, and for sharing his enthusiasm for rare plants. I would like to thank Ms. Alyssa Fuller for sharing her knowledge and excitement for microinvertebrates and spending her time to help me in the lab. I would like to thank Dr. Gary Wien for his help with GIS. I would also like to thank Dr. James Costa and Dr. Sarah Workman for their careful editing, encouragement, and gentle guidance throughout this internship process.

LITERATURE CITED

Barry, J. M. 1980. Natural Vegetation of South Carolina. Columbia, SC: University of South Carolina Press.

Curtis, David L., et al. 1996. Rooting and subsequent overwinter survival of stem cuttings of *Stewartia ovata*. Journal of Environmental Horticulture **14**: 163-166.

Dindal, D. L. 1990. Soil Biology Guide. Toronto, Canada: John Wiley & Sons, Inc.

Fenton, M., C. Albers, and Q. Ketterings. 2008. Soil Organic Matter. Retrieved from http://franklin.cce.cornell.edu/resources/soil-organic-matter-fact-sheet

Hyland, C., Ketterings, Q., Dewing, D., Stockin, K., Czymmek, K., Albrecht, G., & Geohring, L. 2005. Phosphorus Basics – The Phosphorus Cycle. Retrieved from http://nmsp.cals.cornell.edu/publications/factsheets/factsheet12.pdf.

Johnston, J. Personal communication. Interviews. 2016.

Ketterings, Q., S. Reid, and R. Rao. 2007. Cation Exchange Capacity (CEC). Retrieved from http://nmsp.cals.cornell.edu/publications/factsheets/factsheet22.pdf

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Series Classification Database. Available online. Accessed [12/03/2016].

SAPWOOD ANALYSIS OF HARDWOOD TREES IN RESPONSE TO EASTERN HEMLOCK (*TSUGA CANADENSIS*) MORTALITY

BROOKE A. SMITH

Abstract. When a dominant species in an ecosystem dies due to the introduction of an invasive species, consequences of this can affect the balance of the entire ecosystem. Widespread mortality of eastern hemlock (*Tsuga canadensis*) following introduction of hemlock woolly adelgid (HWA, *Adelges tsugae*) began in New England and eventually spread through the Southern Appalachians. The aim of this study was to detect the disturbance caused by hemlock decline and mortality in the growth of hardwood and overstory trees. Tree core extraction was performed on various hardwood and overstory trees in study plots once inhabited by eastern hemlocks in the experimental forest at the Coweeta Hydrologic Lab. Dendrochronology was performed to analyze trends in annual ring width. When examining growth response of species in sites RR6 and RR7, *A. rubrum*, *Betula* spp., *M. fraseri*, and *N. sylvatica* demonstrated responses, and *Quercus* spp. did not demonstrate responses. Regular aerial photography is recommended at Coweeta Hydrologic Lab for each study plot within the watershed in order to test if growth response is related to proximity to hemlock.

Key words: Adelges tsugae; *dendrochronology; hemlock; hemlock woolly adelgid; long-term vegetation change, radial growth analysis; tree-ring analysis;* Tsuga canadensis

INTRODUCTION

Widespread decline of a foundation species in a forest ecosystem has implications for forest recovery and ecological dynamics of the entire ecosystem. In the Southern Appalachian Mountains, the eastern hemlock (*Tsuga canadensis*) is a formerly dominant species that has suffered widespread mortality following infestation of the hemlock woolly adelgid (HWA, *Adelges tsugae*), a small aphid-like insect from southern Japan that spread from New England southeast through the Southern Appalachians, and which likely began causing widespread hemlock mortality in New England in the 1990s (Small et. al. 2005) (Davis et. al. 2007). The HWA does not kill its hosts in Asia because of native host resistance; however, there is no resistance to eastern hemlocks in North America as they evolved without being exposed to it until recent decades and it has no natural enemies in North America (Domec et. al. 2013) (USFS 2015). The HWA attacks *T. canadensis* by laying eggs on the twigs of hemlocks in woolly white clumps. Those clumps are easily dispersed, and when the eggs develop into adult insects, the insects extract nutrients at the base of the needles from the current year's growth, causing the tree to shed needles, stop growth, and eventually starve and die (Small et. al. 2005, Orwig and Foster 1998, Domec et. al. 2013).

Studies have seen oak and mixed hardwoods become the dominant canopy species following eastern hemlock mortality, along with increased shrub growth (Small et. al. 2005), and a study done on such shrub growth found rhododendron (*Rhododendron maximus*) to be a quick colonizer of areas with open canopy cover following eastern hemlock mortality (Ford et. al. 2011). Other data suggests that heavy HWA infestations are accompanied by dramatic changes in stand dynamics that continue long after widespread eastern hemlock deaths from HWA (Orwig 2002).

The aim of this study was to quantify the disturbance caused by hemlock decline and mortality in the growth of hardwood and overstory trees in the selected study site. Dendrochronology was performed to examine degree of radial increased growth trend of hardwoods in stands with hemlock mortality within the Coweeta Basin. In order to average out short-term climatic responses, the dendrochronology analysis requires a 10-year averaging span; this technique is known to capture intermediate-length growth increases, which are associated with changes in canopy cover (Nowacki et. al. 1997).

MATERIALS AND METHODS

Site Description and Experimental Design

The study site is located in the Nantahala mountain range in western North Carolina within the Coweeta Basin of the Coweeta Hydrologic Station in Otto, NC. Some plots contained hardwood trees and rhododendron (*Rhododendron maximum*), while in others the rhododendron and the litter layer had been removed. In two of the plots, the rhododendron were removed and the litter layer was burned (RR7 and RR10), and in the other two, the rhododendron remained undisturbed (RR3 and RR6). All sample plots consisted of various hardwood trees such as red maple (*A. rubrum*), sweet birch (*B. lenta*), black gum (*N. sylvatica*), and oaks (*Quercus* spp.), and dead eastern hemlock (*Tsuga canadensis)*.

Dendrochronology and Field Work

Using an increment borer, tree core samples at breast height were obtained from hardwood trees of various species in the plots. All hardwood trees with a diameter greater than 5 cm were sampled. Two cores were taken from each tree, along with the diameter at breast height (dbh) in cm. The locations of dead hemlocks relative to hardwood trees, canopy gaps, and other field observations were noted at each plot.

One of the two sample cores for each tree was chosen for dendrochronology analysis. These cores were mounted with glue on grooved wooden core mounts and then sanded with increasingly fine grits on a sanding belt (starting with a coarse grit and going progressively finer, from a grit of 400 to around 900). Once the wood appeared almost polished, the cores were examined with a Velmex tree ring measuring device. Annual ring width was measured in micrometers (0.001 mm). The measurements were recorded, organized, and dated with Tellervo software (Brewer 2016). The samples were reconciled with COFECHA software written by Holmes in 1983 to ensure that the dating process was consistent (Altman et. al. 2014, Bunn et. al. 2008, Grissino-Mayer 2001). A master chronology was developed using ring width data from fifteen sample trees. The outmost ring was assumed to correspond to 2016 as all samples were obtained this year. When the correlation of the ring width series with master chronology was strongest with an earlier year and the tip of the sample was broken, the last ring was relabelled to the year nominated by COFECHA.

Detecting Growth Trend: Percent Increase and Absolute Increase

Two methods were used to detect growth trend due to hemlock decline or mortality. A 10-year moving window was used to calculate mean annual ring width, which removes or smooths climatic effects on sapwood growth. Growth was detected when the difference between preceding and subsequent periods exceeded a threshold:

$$\text{Growth}_i = (M_2 - M_1)/M_1$$

where M_1 is the mean annual ring width from the decade excluding year i, and M_2 is the mean annual ring width from the decade including year i.

Using this method, Nowacki and Abrams considered a growth of 25% or greater as significant (1997). Meanwhile, Fraver and White suggested that a fixed growth threshold be used for each species to account for variation in growth response due to species and age (2004). They suggested a value of 1.25 times standard deviation of absolute increase from all years and all trees within that species. Thus, the growth thresholds for the Absolute Increase Method were determined based on 1.25 times standard deviation of absolute increase for four groups of species: *A. rubrum, Betula* spp., *Quercus* spp. and other.

TABLE 1. Sample trees in the plot by species and basal area.

Species	Number of trees	Basal area (m^2)
A. rubrum	6	7258.273
Betula spp.	5	4442.95
F. americana	1	1562.283
M. fraseri	3	2274.144
N. sylvatica	2	2285.234
Quercus spp.	4	5756.576

TABLE 2. Threshold used to determine the growth for the Absolute Increase Method.

Species	Threshold (micrometre)
A. saccharum	285
Betula spp.	252
Quercus spp.	217
Other	448

RESULTS

Study sites RR6 and RR7 were successfully cross-dated, so data analysis was focused on those two plots. The absolute increase and percent increase methods indicated increased annual growth in x number of trees detected from 2004 to 2007 (fig. 1). The absolute increase method also indicated increased growth from 2002, with 7 trees in 2007 demonstrating growth exceeding the threshold.

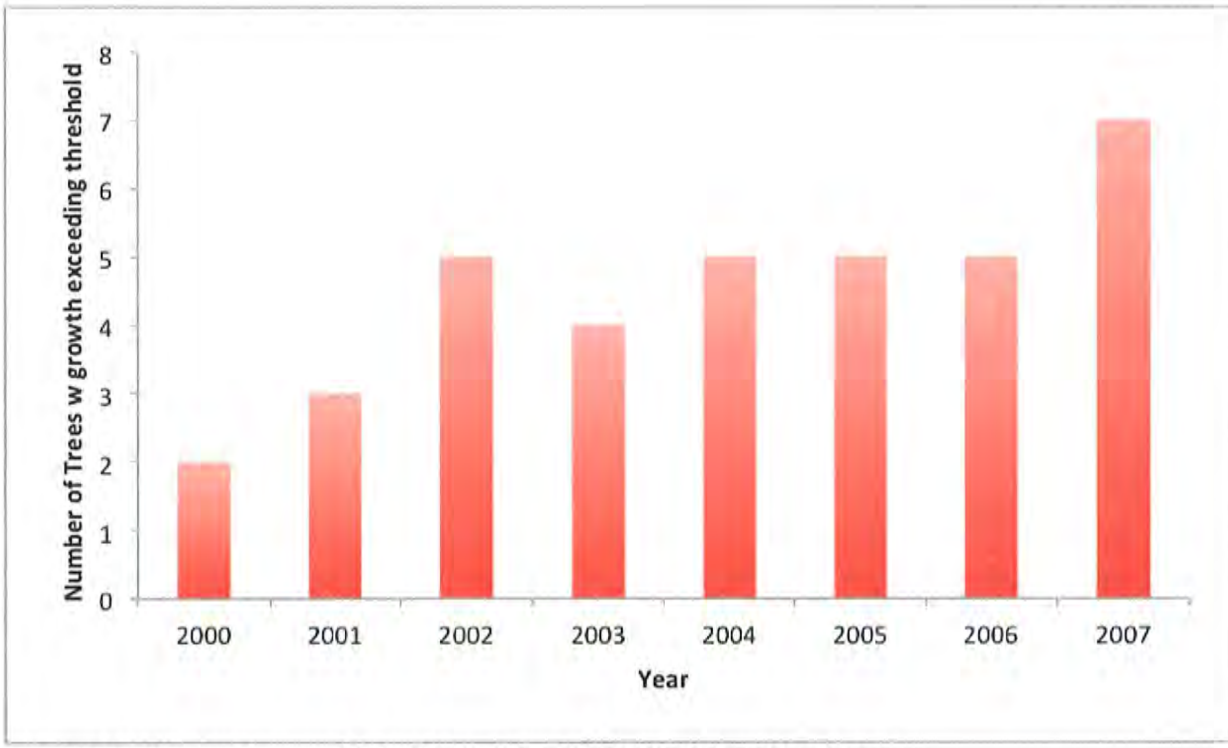


FIG. 1. The Absolute Increase Method (Fraver et. al. 2005) in RR6 and RR7, by year.

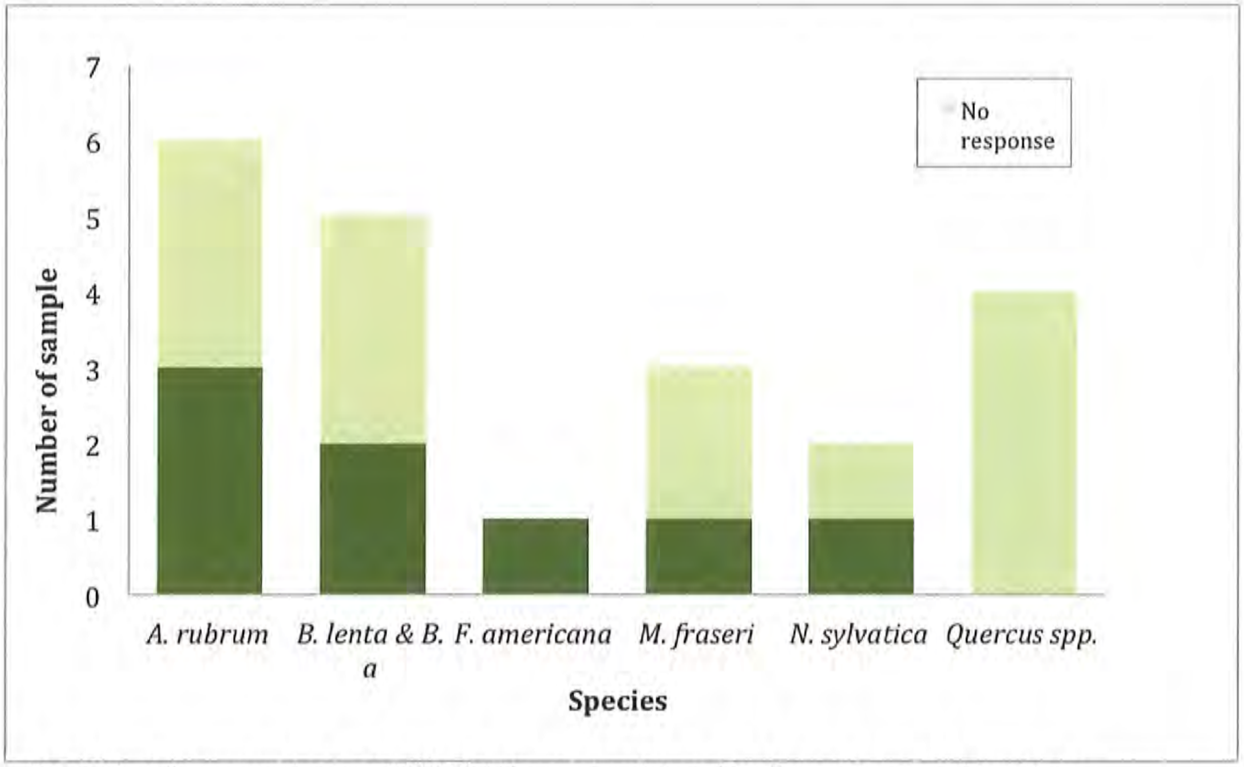


FIG. 2. Growth response between 2000-2007 by species, detected in all species except *Quercus* spp.

Growth response was detected in trees that represent most of the hardwood species in the stands: *A. rubrum, Betula* spp., *F. americana, M. fraseri,* and *N. sylvatica* demonstrated increased annual growth (figs. 3 & 4). No sample trees of *Quercus* spp. showed an increase in annual growth.

Master chronology of sample trees in RR6 and RR7 indicated that positive deviation from the mean ring width (increased growth) occurred in 2009 and 2014. However, the master chronology was not analyzed against climate data so this growth trend cannot be fully attributed as a response to disturbance.

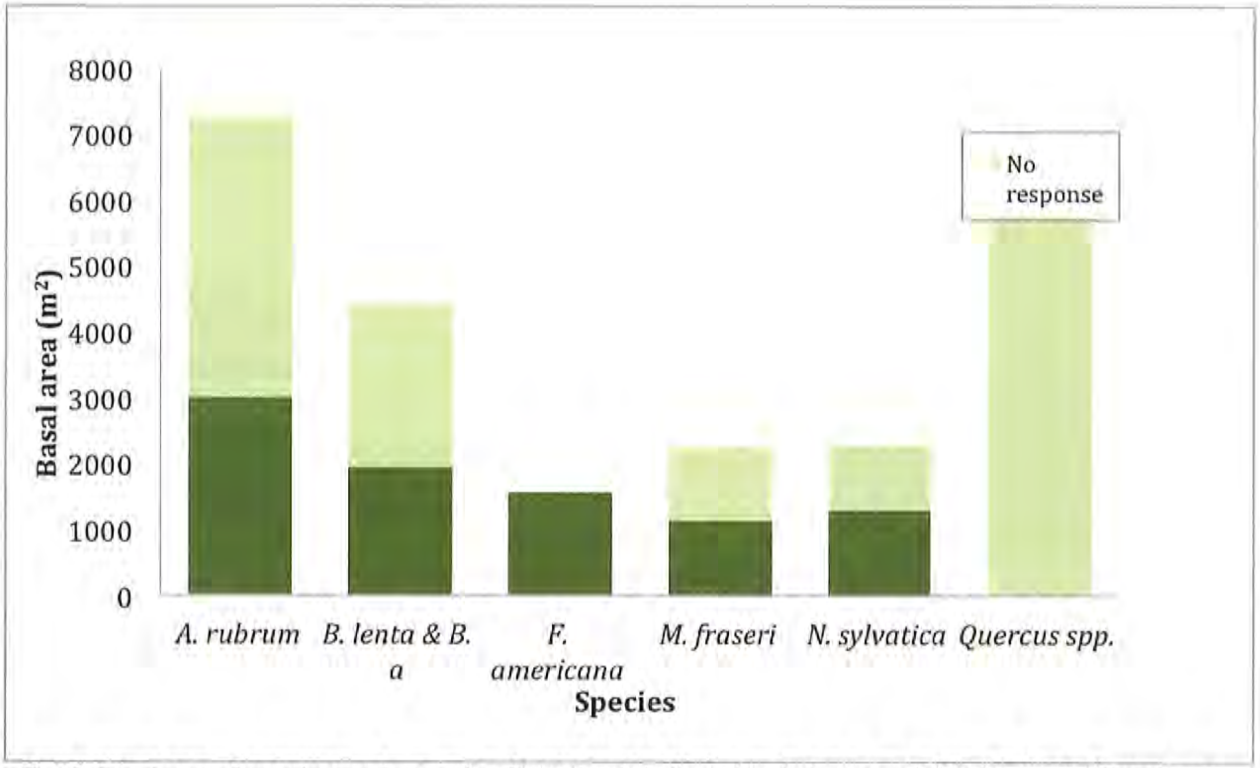


FIG. 3. Basal area of sample trees by species. Detection is indicated in dark green, visible in all but *Quercus* spp.

DISCUSSION

This study utilized dendrochronology to examine the effects of hemlock decline and mortality on hardwood overstory trees in the stands. Increased growth has been detected in some hardwood overstory trees in the stands that experienced hemlock mortality. Both the percent increase and absolute increase methods detected increased growth trend from 2000 onwards, with up to 7 hardwood trees exhibiting increased growth by 2007.

With regard to species-based growth responses, *A. rubrum, Betula* spp., *M. fraseri,* and *N. sylvatica* demonstrated responses, and *Quercus* spp. did not demonstrate responses. The comparative lack of a visible reaction to hemlock mortality in the oaks may be attributable to the age of the oaks. Studies have shown hardwoods to have decreasing growth rates as they age, and with oaks being another dominant species and key member of the ecosystem, and the study plots likely not having been logged since the 1930s, the oaks in the study plots analyzed were likely very old, and exhibiting slow growth rates (Johnson et. al. 2009, Coweeta n.d.). This would imply that the oaks, at a younger age, would exhibit a more measurable growth response to eastern hemlock mortality. Results of studies suggesting dramatic changes in the stand structure indicated specifically by oak and maple trees (Orwig 2002) support the responses indicated by maple (*Acer*) trees in the study plots at Coweeta. Perhaps, when not dominated by rhododendron, and with younger oaks, *Quercus* would be among other hardwoods (*Acer, Betula, Fagus*) in taking up canopy space formerly filled by eastern hemlock, as suggested by Ford et. al. (2011). Further study could be done on hardwoods in several years, perhaps once hardwood trees have had the opportunity to recolonize open canopy areas in plots where rhododendron has been burned (RR7 and RR10). Additionally, this study could be extended with an examination of the location of trees that experienced increased growth using aerial photography at Coweeta to test if growth response is related to proximity to hemlock.

Conclusion

The growth of some hardwood trees in hemlock stands increased significantly from 2000 to 2007. This suggests a response to hemlock decline and mortality as HWA infestation was detected in early 2000s. Increased growth was detected in samples representing most hardwood species, except for oak. The comparative lack of a visible reaction to hemlock mortality in the oaks may be attributable to the age of the oaks.

Acknowledgements

I would like to thank Dr. Sandra Hawthorne for her guidance as my mentor throughout the entirety of this project. I would also like to thank Dr. Chelcy Ford and staff members at Coweeta Hydrologic Lab for assisting me with my project and providing the resources for me to carry it out. Lastly, thank you to the Highlands Biological Station staff for connecting me with this research project, and for reviewing my drafts with Dr. Hawthorne and providing feedback.

Literature Cited

Altman, J., Fibich, P., Dolezal, J., & Aakala, T. (2014). TRADER: A Package for Tree Ring Analysis of Disturbance Events in R. Dendrochronologia, **32**(2), 107-112. doi:10.1016/j.dendro.2014.01.004

Brewer, P. W. (2016). Tellervo: A Guide for Users and Developers. Tucson, AZ: Laboratory of Tree-Ring Research.

Bunn, A. G. (2008). A Dendrochronology Program Library in R (dplR). Dendrochronologia, **26**(2), 115-124. doi:10.1016/j.dendro.2008.01.002

Coweeta Hydrologic Laboratory. (n.d.). Retrieved December 05, 2016, from http://www.foresthistory.org/ASPNET/Places/ResearchSites/Coweeta.aspx

Davis, D. D., Fromm, M. S., & Davis, M. D. (n.d.). Impact of the Hemlock Woolly Adelgid on Radial Growth of Eastern Hemlock in Pennsylvania. Proceedings of the 15th Central Hardwood Forest Conference.

Ford, C. R., Elliott, K. J., Clinton, B. D., Kloeppel, B. D., & Vose, J. M. (2011). Forest Dynamics Following Eastern Hemlock Mortality in the Southern Appalachians. Oikos, **121**(4), 523-536. doi:10.1111/j.1600-0706.2011.19622.x

Fraver, S., & White, A. S. (2005). Identifying Growth Releases in Dendrochronological Studies of Forest Disturbance. Canadian Journal of Forest Research, **35**(7), 1648-1656. doi:10.1139/x05-092

Grissino-Mayer, H.D. 2001. Evaluating Crossdating Accuracy: A Manual and Tutorial for the Computer Program COFECHA. Tree-Ring Research **57**(2):205-221.

Nowacki, G. J., & Abrams, M. D. (1997). Radial-Growth Averaging Criteria for Reconstruction Disturbance Histories from Presettlement-Origin Oaks. Ecological Monographs, **67**(2), 225. doi:10.2307/2963514.

Orwig, D. A. and D. R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. Journal of the Torrey Botanical Society **125**: 60-73.

Orwig, D. A., Foster, D. R., & Mausel, D. L. (2002). Landscape Patterns of Hemlock Decline in New England due to the Introduced Hemlock Woolly Adelgid. Journal of Biogeography, ***29***(10-11), 1475-1487. doi:10.1046/j.1365-2699.2002.00765.x

Small, M. J., C. J. Small, and G. D. Dreyer. 2005. Changes in a hemlock-dominated forest following woolly adelgid infestation in southern New England. Journal of the Torrey Botanical Garden **132**: 458-470.

USFS, Northeastern Area, Forest Health Protection. (2015, April 17). Northeastern Area. Retrieved December 01, 2016, from http://na.fs.fed.us/fhp/hwa/.

INVESTIGATING THE RELATIONSHIP BETWEEN *CASTANEA DENTATA* DIMENSIONS AND THE PRESENCE OF *CRYPHONECTRIA PARASITICA*

ALEXANDRA R. TRIBO

Abstract. American chestnuts (*Castanea dentata*) were integral to early subsistence culture and wildlife dynamics in the Appalachian Mountains prior to the introduction of a devastating parasitic ascomycete (*Cryphonectria parasitica*) imported from Japan that rendered the species functionally extinct with a threatened genome, despite prolific sprouting from old root systems. Due to the major economical and sociocultural impact of this loss, there have been vigorous efforts in chestnut genetics, blight dynamics, and restoration techniques. Breeding programs are currently backcrossing American chestnut with Chinese chestnut (*Castanea sativa*) in order to express blight resistant features. If chestnuts are able to grow strong enough to reproduce before infection, it may result in the natural evolution of resistant species. In this study, three sites in the Southern Appalachians with concentrations of American chestnut were evaluated to determine if height and diameter were determining variables with respect to presence of blight. This was accomplished through taking dimensions of chestnut coppices, bark sampling, and visual confirmation of positive cultures in the laboratory. The results suggest that blight is more likely to be present in trees with greater height, diameter, and average number of stems per coppice. These results overall indicate that if large populations of American chestnut sprouts are concentrated in generally confined areas, the high levels of pathogen are always present but greatest in areas with largest sprouts. These results are valuable since breeding efforts during last 50 years had developed resistant (tolerant) backcross hybrids and are being planted in reforestation studies through the southeastern United States. Knowledge of areas with heavy pathogen inoculum pressure dictates possible areas to avoid during early establishment of seedlings with tolerance to the pathogen. A more thorough understanding of chestnut blight population dynamics will be beneficial for successful restoration efforts of blight-resistant American chestnut hybrids into its historical range in the Eastern hardwood forests.

Key words: American chestnut; chestnut blight; Castanea dentata, Cryphonectria parasitica; *height; diameter; disease; fungus; fungal disease*

INTRODUCTION

Few species have had as much of a significant sociocultural, economical, and ecological impact in the Appalachians as the American chestnut. *Castanea dentata* comprised between 20% and 30% of all standing trees in the region, with usual trunk diameters of over eight feet (Davis 2000). Settlers, Cherokee, and wildlife relied on the chestnut for a variety of purposes. Its fruits were known to blanket the forest floor, feeding nearby fauna en masse with its significant mast (Lord 2005, Burke 2013). Its decay-resistant wood was sought after and used for construction of furniture, fences, shingles, and more (Davis 2000, Burke 2007). It was also responsible for bringing the leather tanning industry into the region (Davis 2000). The American chestnut served a paramount ecological role, as its rich canopy of over one hundred feet provided cover, shade, and nutrients, for lower tree species and shrubs (Rellou 2002). The majesty of the American chestnut has since been eclipsed by the widespread epidemic of a parasitic fungus, *Cryphonectria parasitica* (formerly *Endothia parasitica*), which was introduced from a Japanese nursery in 1904 (Anagnostakis 2000). By 1913, enough trees had been decimated to warrant investigation by the USDA (Conolly 2007). The death of the American chestnut is arguably the mark of the end of subsistence culture in the Appalachian Mountains, and scientists have urgently been exploring modes of restoration through backcrossing with Chinese chestnut (Inman 1987, Burnham 1988, Davis 2000, Horton 2010).

Colloquially known as chestnut blight, *Cryphonectria parasitica* is an infective fungus that specifically targets susceptible chestnuts, penetrating the cracks and natural wounds of the tree until its capillaceous filaments fan out underneath the bark (Missouri Botanical Garden). The pathogen ultimately interrupts the cambium and disrupts the functional xylem and phloem, inhibiting nutrient and water transportation (Smith 2012). This results in browning of leaves and localized bark mortality (Anagnostakis 2000, Smith 2012). Once the fungus erupts through the dead bark as stromata, it is usually spread via two mechanisms. Small animals and insects transport sticky condia as the reproductive structures from cankers are trapped by feet, fur, and feathers (Rellou 2002). Ascospores are carried by wind, and more specifically, are ejected into the air after triggering from a rainstorm, easily infecting nearby chestnut sprouts (Anagnostakis 2000). Chestnut blight typically will kill a chestnut before it becomes sexually mature, prohibiting any resistance to develop. Although few mature chestnuts survive, sprouting from old chestnut root systems is prolific, yet ephemeral, because *Cryphonectria parasitica* does not affect the root system (Huang 1998, Smith 2012). In fact, it is conjectured that the root stocks of most surviving chestnuts are at least a half a century old or older and have withstood disease, timbering, fires, acid rain, and weather extremes (Burke 2007). All existing chestnuts are usually saplings, and due to its inability to reproduce to any significant degree, the American chestnut is considered a threatened species (Rellou 2002). Once a significant canopy species, blight has constrained American chestnut to an understory species with a distinctly different niche, altering the ecology of Appalachian forests (Griffin 1992, Burke 2012). Most chestnuts today are seen in fairly xeric, well-drained upper slopes with a southerly aspect and poorly developed understory (McCarthy 2012). Recent studies indicate that there is still considerable genetic diversity amongst chestnut sprouts, especially in the Southern United States, but its genome may deteriorate if not conserved in blight-resistant trees (Huang 1998, Kubisiak and Roberds 2003, Dane and Sisco 2014).

There has been limited research thus far relating American chestnut size and number of sprouts that impact inoculum levels. Previous research shows that there may be evidence for a positive correlation between tree size and presence of fungal diseases (Falk et al. 1989, Griffin et al. 2003, McCann and MacDonald 2012).

During the course of this study, a total of 510 American chestnut coppices were tagged, mapped, and assessed for blight at three disparate sites: Yellow Mountain trailhead, Reynold's Gap, and Watershed 7 (with the latter two sites both located in proximity to Coweeta Hydrological Station). Collected bark samples were plated and incubated in the laboratory in order to verify the presence of blight. My primary objective is to examine our results and, by utilizing mapping techniques in combination with recorded measurements, determine whether or not there are any patterns exhibited in any three sites that may provide insight about the nature of *Cryphonectria parasitica*. My prediction is that there will be a positive correlation between *Castanea dentata* dimensions and coppice size and presence of blight, given that larger trees have more surface area available for the development of multiple fungal entry areas and subsequent infection. We therefore investigated whether or not height, diameter, and number of stems per coppice played a role in vulnerability to chestnut blight, or if blight infection is random and not consistent with any known variables.

MATERIALS AND METHODS

Data Collection

I tagged, mapped, and collected field samples from 170 American chestnut coppices at three different sites. Site 1 (YM) is located just beyond the Yellow Mountain trailhead off of Cole Gap Road, within the Highlands, North Carolina boundary. Site 2 (Reynold's Gap, abbreviated RG) and site 3 (Watershed 7, abbreviated WS) are both located on the property of Coweeta Hydrologic Laboratory in Otto, North Carolina on opposite facing slopes.

At all three locations, I flagged each chestnut coppice (defined here as an isolated cluster of trees at least one meter from a separate cluster) with the site code (YM; WS; RG) and an identification number (1-170) so that they could be located and identified easily for data collection at a later date. Each coppice was subsequently marked using a Garmin Montana 650t hand-held GPS to record its location and later be entered into a GIS system for analysis. Dimensions (height and diameter) were taken from each individual tree in every flagged coppice. Stem height was scored based on a scale in five-foot increments (table 1.) and diameter was recorded in centimeters to two significant figures. For the sake of uniformity, all diameter measurements were performed near the base of the tree. Finally, I visually assessed each chestnut stem individually for the presence of blight based on morphological characters.

A sterilized scalpel or box cutter was used to cut two small bark samples from trees that indicated blight-infected tissue. I placed the samples in small, plastic containers that were labeled by site and number. Samples were stored at room temperature, then inundated with a 20% sodium hypochlorite solution for 30 seconds before being placed separately in potato dextrose agar plates. The plates were incubated for 14-21 days and checked in 3-5 day increments. Each plate was then visually assessed for confirmation of *Cryphonectria parasitica* presence based on cultural characters.

TABLE 1. AMERICAN CHESTNUT HEIGHT SCORING SYSTEM

Score	Range (in feet)
1	1-5
2	6-10
3	11-15
4	16-20
5	21-25
6	25<

Statistical Analysis

Means of American chestnut sprout heights, diameters, and chestnut blight cultures were analyzed as a series of combined experiments – combined across site – using the GLM procedure of SAS (SAS Institute, Cary, NC). Means were separated using Fisher's protected least significant difference (LSD) test if significant ($p=0.05$). Chi-square analysis was run to compare 301 coppices visually identified as blighted versus identification by culture bark isolation data. The purpose of this last analysis was to compare the frequency of diseased sprouts between locations.

RESULTS

Means of height and diameter were taken at three sites (RG, WS, YM) to determine if there was a significant relationship between chestnut dimensions and presence of *Cryphonectria* *parasitica* (table 2). Average height was significantly higher at Reynold's Gap (1.70556) than either Watershed 7 (1.34236) or Yellow Mountain (1.34464). Average diameter was significantly greater at the Reynold's Gap (1.4367) site than either Watershed 7 (1.1005) or Yellow Mountain (1.0755) as well, an expected result given the mean heights. Watershed 7 produced only a slightly greater average diameter than Yellow Mountain.

TABLE 2. American chestnut stem height and diameter means at each sampling site (n=509).

Plot Type	Height[A]	Diameter (cm)
Reynold's Gap	1.70556A	1.4367A
Watershed 7	1.34236B	1.1005B
Yellow Mountain	1.34464B	1.0755B
(LSD)	0.1662	0.2246

[A] Height was measured using a scale: 1=1- 5ft, 2=6-10ft, 3=11-15ft, 4=16-20ft, 5=21-25ft, 6>25ft.

Coppices verified as infected with blight (1.65658) exhibited significantly greater mean height in range of size classes (table 3) than non-infected coppices (1.51005). Similarly, by a larger margin, coppices infected with blight (1.4378) possessed a greater diameter than non-infected coppices (1.2442).

TABLE 3. American chestnut stem height and diameter means based on presence of chestnut blight.

Presence of Blight	Height[A]	Diameter (cm)
Yes	1.65658A[B]	1.4378A
No	1.51005B	1.2442B
(LSD)	0.1895	0.2462

[A] Height was measured using a scale: 1=1- 5ft, 2=6-10ft, 3=11-15ft, 4=16-20ft, 5=21-25ft, 6>25ft.

Reynold's Gap exhibited a significantly greater mean height (1.7971) and mean diameter (1.5331) than Watershed 7 (1.4692; 1.2737) and Yellow Mountain (1.4269; 1.1490). Watershed 7 and Yellow Mountain produced values comparable with one another in height and diameter (table 4).

TABLE 4. Mean height and diameter of blight infected American chestnut stems (n=170).

Plot Type	Height[A]	Diameter (cm)
Reynold's Gap	1.7971A	1.5331A
Watershed 7	1.4692B	1.2737B
Yellow Mountain	1.4269B	1.1490B
(LSD)	0.2315	0.3007

[A] Height was measured using a scale: 1=1- 5ft, 2=6-10ft, 3=11-15ft, 4=16-20ft, 5=21-25ft, 6>25ft.

Following field collection of bark samples, samples were plated and cultured for identification of blight infection. Bark samples collected in the field were not as reliable a form of blight infection identification as bark samples cultured in the laboratory. A chi-square analysis confirmed that visual identification in the field was not as adequate for recognition of blight infection than laboratory cultures (table 5). Approximately one-third of bark samples from all three sites proved infected for blight presence. Reynold's Gap shows an accuracy percentage of 33.9%, Watershed 7 at 28.7%, and Yellow Mountain at 29.1%.

TABLE 5. Chestnut blight field identification accuracy based on cultures.

Plot Type	Accuracy	Percentage (%)	Chi-Square
Reynolds Gap	56/109	33.9	<0.001
Watershed 7	41/102	28.7	<0.001
Yellow Mountain	37/90	29.1	<0.001

Reynold's Gap exhibited the greatest number of coppices with stems over 7.62 meters (25 ft) in height. Additionally, Reynold's Gap had the greatest number of stems in the 4.88-6.10 m (16-20 ft), 6.40-7.62 m (21-25 ft), 3.35-4.57 m (11-15 ft), and 0.30-1.52 m (1-5 ft) ranges as well. Figure 1 shows that Reynold's Gap had the largest trees and greatest amount of total sprouts.

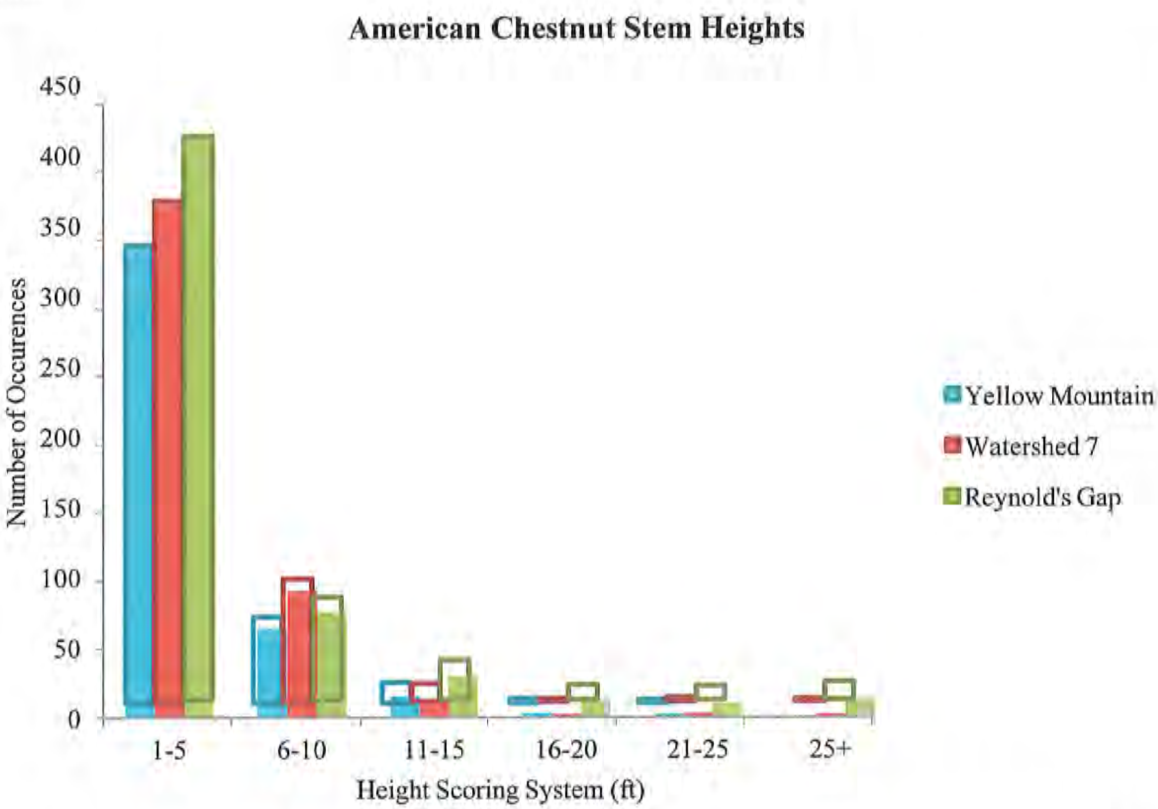


FIG 1. A comparison of stems occurring in each size class at Yellow Mountain, Watershed 7, and Reynold's Gap.

Reynold's Gap chestnut coppices had the greatest average number of stems per coppice while Yellow Mountain had the lowest average number of stems per coppice. A comparison between the mean numbers of stems in a coppice (fig. 2) with the number of coppices infected per site indicates that there may be a relationship between chestnut blight and greater numbers of stems.

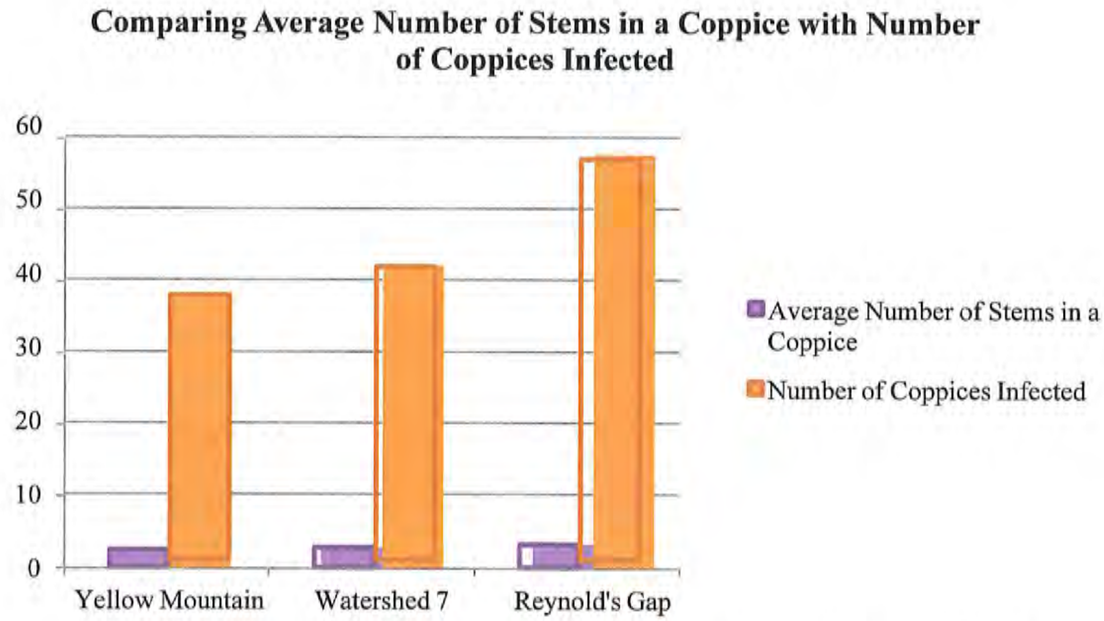


FIG 2. A comparative assessment of mean number of stems in a coppice and number of coppices infected across three sites.

At the Watershed 7 and Yellow Mountain sites, blight was present in coppices with greater mean height per stem (fig. 3). At Reynold's Gap, blight was present in coppices with lower mean range of height.

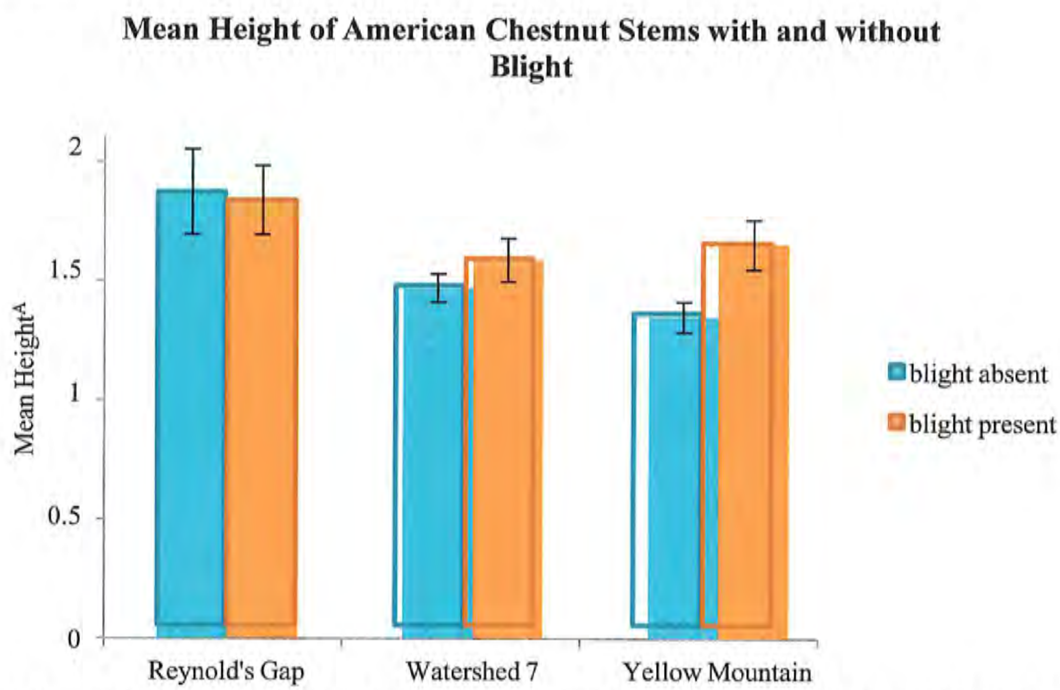


FIG 3. Comparative measurements of mean height with and without verified blight infection. [A] Height measured using a scale: 1=1- 5 ft, 2=6-10 ft, 3=11-15 ft, 4=16-20 ft, 5=21-25 ft, 6>25 ft.

At all three sites, average diameter in centimeters was greater for American chestnut stems infected with blight than they were for stems that were not infected with blight.

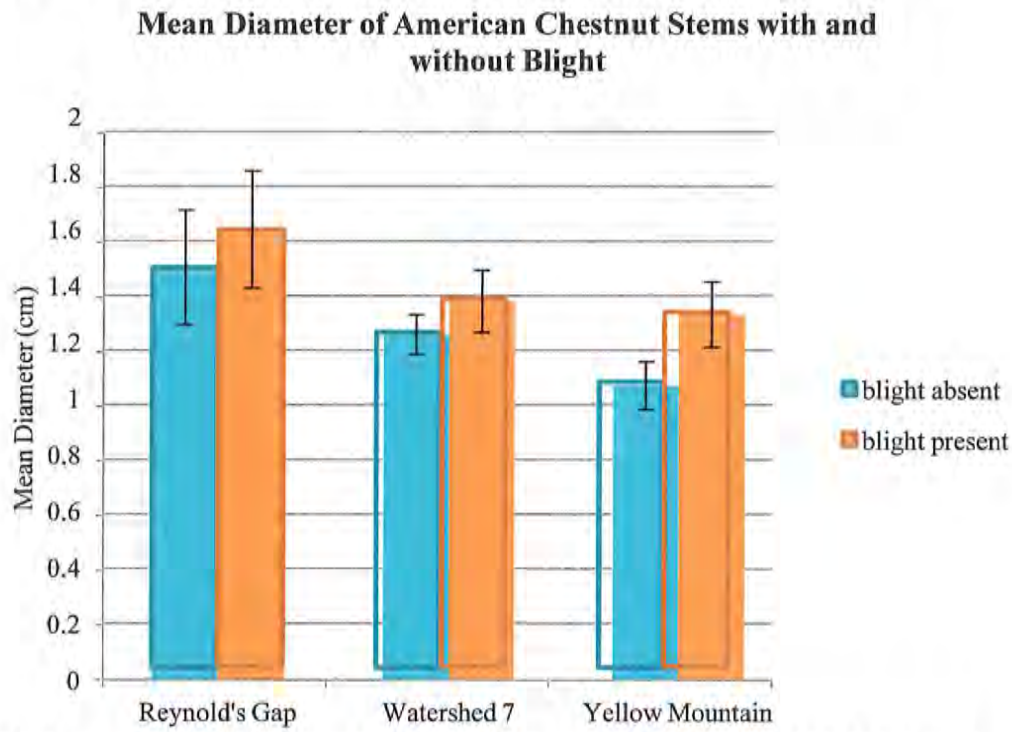


FIG 4. Comparative measurements of mean diameter with and without verified blight infection.

Reynold's Gap had significantly greater mean height mean diameter than either Yellow Mountain or Watershed 7 that did not significantly differ from one another in height or diameter. In general, coppices infected with blight are greater in height and diameter. Average number of stems per coppice at Reynold's Gap was greater than Watershed 7 and Yellow Mountain. Greater average number of stems per coppice is correlated with blight populations.

DISCUSSION AND CONCLUSIONS

In this study, we examined whether American chestnut dimensions and presence of *Cryphonectria parasitica* were meaningfully related in a measurable pattern. My results demonstrate that there may be a positive correlation between chestnut size and vulnerability to blight. For two out of three sites, blight infection was associated with a greater size class, with only a slight disparity in the Reynold's Gap site, possibly influenced by the greater number of sprouts therein, which far surpassed the other two sites (fig. 3). All three sites demonstrated that blight infection occurred in trees with a larger stem diameter (fig. 4). Finally, a comparative assessment of average number of stems per coppice at each site with number of coppices infected suggests that blight is more likely to proliferate in chestnut coppices that have greater numbers of stems (fig. 2). Chi square analysis indicates that field identification and culture confirmation did not agree well (table 5); this result is likely attributed to our conservation in choosing samples, opting to take samples from trees that showed even the slightest deviation in morphological characteristics.

Reynold's Gap chestnuts are greater in average height and diameter than Watershed 7 and Yellow Mountain by a notable margin (table 1). Greater averages in these dimensions, and therefore larger trees, may be attributed to local slope, soil type, and moisture conditions that support heavy chestnut sprouting. Additionally, Reynold's Gap boasts a greater average number of stems per coppice than the other two sites, although percentage of infection is aligned fairly congruently amongst the three sites (fig. 2, table 5). Because Reynold's Gap has the greatest average number of stems per coppice, it dominates the other two sites in most size classes (fig. 1). Collectively, all sites demonstrate size class distribution that is consistent with previous assessments (Scrivani 2011).

Larger trees will have more surface area and therefore a greater opportunity for cracking and wounding, which are easy entry points for *Cryphonectria parasitica*. Larger trees will likely have greater branching nodes as well, a common location of fungal entry as the constant sway and new growth splits the bark (Smith 2012). This may explain why my results show that trees with greater dimensions have greater incidences of infection. It is unclear why blight correlates with a greater average number of stems per coppice; perhaps this is due to the fact that blight can travel more readily and without substantial obstacles between stems in a coppice rather than from tree to tree. There's a chance that dead central chestnut stumps are able to easily and quickly infect circumferential sprouts. Close field observation suggests also that chestnut sprouts with smooth, darkened bark appeared conspicuously healthier than lighter, striated bark, which usually had more crevices through which the opportunistic blight may enter. One study suggests that there is heartier sprouting in coppices with a dead central stump, which may be related to the chestnut's resource allocation properties (Stevens et al. 2014). It follows suit, then, that the heavy coppicing in Reynold's Gap is associated with greater concentrations of blight.

These results are consistent with previous studies pertaining to tree dimensions and likelihood of fungal pathogen infection (Falk et al. 1989, Griffin et al. 2003, McCann and MacDonald 2012). These results may also pave the path for establishing the age at which blight is most likely to girdle and destroy. This offers insight to researchers attempting to develop resistant strains by providing a dimensional frame within which to check experimental sprouts for resistance. A major impediment to American chestnut recovery is its inability to survive long enough to sexually reproduce and give rise to genetically evolved trees with nature-induced resistance. These results provide understanding into chestnut blight dynamics so we can better deduce how to manipulate chestnut growth, distribution, and breeding so that it may have a greater chance of evading or surviving infection.

Successful reintroduction of the American chestnut will require far more than the development of blight-resistant hybrids. Since there is evidence that forest canopy is affected by habitat types, slope, shade tolerance, moisture, soil, and associated species, it is important that further research investigates the preferences of the American chestnut (Bass 2002). It will also be beneficial to the ongoing effort to restore this cherished tree to decipher where there are concentrated areas of greatest genetic diversity. It is also worthwhile to study how blight infection responds to changes in external stimuli like light, temperature and moisture. These research ventures combinatorially would allow scientists to determine optimal conditions that both facilitate maximum chestnut growth and occlude or retard chestnut blight development when considering areas for reforestation with pathogen-tolerant seedlings.

This study in its entirety proposes that incidences of *Cryphonectria parasitica* are more significant in trees that are larger and with greater levels of coppicing ability. This information should be considered when developing strategies for the full resurgence of *Castanea dentata* into eastern deciduous forests. Further diagnostic studies on blight dynamics and chestnut tolerance in communities will be valuable in this effort.

ACKNOWLEDGEMENTS

I would like to thank Coweeta Hydrologic Laboratory for allowing me to use their vehicles to access hard-to-reach areas via precarious mountainside dirt roads and to study and sample chestnuts on their land. I would also like to thank my partner, Alex Kellogg, for assisting me with sampling, measuring, and culturing, and Dr. Sarah Workman for tirelessly making suggestions and edits for this paper and the data therein. I'd like to thank Dr. Jim Costa for supervising our semester and being an enthusiastic resource. I'd like to thank Dr. Richard Baird for mentoring me through this research process and always being immediately available for troubleshooting and guidance. Finally, I'd like to acknowledge the value of the following plants to the completion of this project: *Coffea arabica*, *Ilex paraguariensis*, and *Humulus lupulus*.

LITERATURE CITED

Anagnostakis, S. L. 2000. Revitalization of the Majestic Chestnut: Chestnut Blight Disease. The American Phytopathological Society. Retrieved September 2016. Web.

Bass, Q. 2002. Talking trees: the Appalachian forest ecosystem and the American chestnut. Journal of the American Chestnut Foundation, **16**(1), 42-56.

Burke, K. L. 2007. History of chestnut survival in the Appalachians (prehistory to present). Journal of the American Chestnut Foundation, **21**(2), 18-26.

Burke, K. L. 2012. Niche contraction of American chestnut in response to chestnut blight [Abstract]. Canadian Journal of Forest Research, **42**(3), 614-620.

Burke, K. L. 2013. Chestnuts and wildlife. Journal of the American Chestnut Foundation, **27**(2), 8-13.

Burnham, C. R. 1988. The restoration of the American chestnut. American Scientist **76**: 478-487.

Conolly, N. Barbara. 2007. Chestnut blight: Cryphonectria parasitica. Plant Disease Diagnostic Clinic. Cornell University College of Agricultural and Life Sciences. Retrieved 28 Oct. 2016. Web.

Dane, F., and P. Sisco. 2014. Genetic diversity of American chestnut is highest in the Southern US. Journal of the American Chestnut Foundation, **28**(1), 9-15.

Davis, D. E. 2000. Where there are mountains: an environmental history of the Southern Appalachians. University of Georgia Press, Athens, Georgia, U.S.

Falk, S. P., D. H. Griffin, and P. P. Manion. 1989. Hypoxylon canker incidence and mortality in naturally occurring aspen clones. Plant Disease **73**: 394-397.

Griffin, G. J. 1992. American chestnut survival in understory mesic sites following the chestnut blight pandemic. Canadian Journal of Botany, **70**(10), 1950-1956.

Griffin, J. M., G. M. Lovett, K. C. Weathers, and M. A. Arthur. 2003. The distribution and severity of beech bark disease in the Catskill Mountains, N.Y. NRC Research Press **33**:1754-1760.

Horton, T. 2010. Continuing saga of the American chestnut. American Forests Magazine **115** (4).

Huang, H. 1998. Restoring the American chestnut to its native range: genetic variation in the American chestnut and selection strategies for recurrent parents. Journal of the American Chestnut Foundation, **12**(2), 27-40.

Inman, L. L. 1987. Proposed strategies to preserve and restore the American chestnut. The Journal of the American Chestnut Foundation **2**:3–6.

Kubisiak, T. L., and J. H. Roberds. 2003. Genetic variation in natural populations of American chestnut. Journal of the American Chestnut Foundation, **16**(2), 43-49.

Lord, W. 2005. Wildlife food. Journal of the American Chestnut Foundation, **19**(1), 29-33.

McCann, D. P. and W. L. MacDonald. 2012. Preliminary report of ecological factors influencing incidence and severity of beech bark disease in the Appalachian region. Proceedings, 18th Central Hardwood Forest Conference; 2012 March 26-28; Morgantown, WV; Gen. Tech. Rep. NRS-P-117. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 169-181.

McCarthy, B. 2012. The ecology of chestnuts: how and where they grow. Journal of the American Chestnut Foundation, **26**(4), 12-15.

Missouri Botanical Garden. Chestnut Blight. http://www.missouribotanicalgarden.org/gardens-gardening/your-garden/help-for-the-home-gardener/advice-tips-resources/pests-and-problems/diseases/cankers/chestnut-blight.aspx

Rellou, J. 2002. Chestnut Blight Fungus (Cryphonectria parasitica). Retrieved October 09, 2016. Web.
Scrivani, J. 2011. Forest inventory and analysis: where does the chestnut stand today? Journal of the American Chestnut Foundation, **25**, 3rd ser., 17-19.
Smith, A. H. 2012. Introduction to chestnut blight. Journal of the American Chestnut Foundation, **26**(2), 9-11.
Stevens, D. L., K. Soltau, A. D. Baines, and A. M. Jars. 2014. American Chestnut Sprout Dynamics [Abstract]. Acta Horticulturae, **1019**, 223-228.

PREDICTING GOLDEN-WINGED WARBLER (*VERMIVORA CHRYSOPTERA*) NESTING HABITAT USING GEOGRAPHIC INFORMATION SYSTEMS MODELING

LAUREN E. WHITENACK

Abstract: Golden-winged warbler (*Vermivora chrysoptera*) populations are rapidly declining in the southern Appalachian Mountains, the southernmost extent of their breeding range. Little is known of their presence in the southwestern counties of North Carolina. To predict potential Golden-winged warbler breeding sites in Graham, Jackson and Macon Counties, I created a habitat model with ArcGIS using elevation, slope, vegetation type, and soil type data from known breeding sites in the area, along with a convolution filter to detect edges. Using the model, I was able to locate predicted sites on Google Earth and verify habitat characteristics such as presence of herbaceous and shrub layers, and adjacency to mature forest. I mapped 215 potential sites to survey during the summer 2017 breeding season.

Key words: GIS; Golden-winged Warbler; Graham County; habitat model; Jackson County; Macon County

INTRODUCTION

Golden-winged warblers (*Vermivora chrysoptera,* Parulidae) are migrant songbirds that breed from southeastern Canada into the northern-midwestern and northeastern extents of the United States, as well as in select moderate to high elevation sites in the Appalachian Mountains as far south as northern Georgia (Confer et al. 2011). After migrating south through the eastern half of the U.S., these warblers winter in Central and South America (Confer et al. 2011).

Within their breeding range, Golden-winged warblers are found in early successional habitats located near mature hardwood forests (Patton et al. 2010). Bakermans et al. (2015) found that in the mid-Appalachian region of their breeding habitat, Golden-winged warblers prefer early successional habitats with greater herbaceous cover (mean 13.1% versus 8.1% for sites of no detection). Aldinger and Wood (2014) found nest sites had an average distance to forest edge of 28.9 m (approx. 95 ft). The necessity for herbaceous, shrub and mature forest layers can be explained by the breeding behavior of these warblers. The songbirds build their nests on the ground in the herbaceous layer or just above the ground in shrubs (Klaus and Buehler 2001). Shrub cover provides protection from predators (Greenberg et al. 2002) while surrounding mature hardwood forest is needed for male perches, nesting material and foraging ground (Confer et al. 2011, Patton et al. 2010, Rossell 2001). In the southern Appalachian Mountains, Golden-winged warblers are most commonly found above an elevation of 610 meters (approx. 2,000 ft.) in patches with mature deciduous forest comprising 50-75% of the landscape within 2.5 kilometers (Roth et al. 2012).

For over 40 years, Golden-winged warbler populations have been declining at an average of 2.5% per year, and their breeding range has been shifting northward and shrinking (Buehler 2007). This species is especially vulnerable in the southern Appalachian Mountains, the southernmost extent of their breeding range, where they are disappearing at rates of around 8% per year (Sauer et al. 2014). Habitat loss due to development or maturation of early successional habitat, hybridization with Blue-winged warblers (*Vermivora cyanoptera*), whose range is expanding into the Golden-winged warbler range, and brood parasitism by Brown-headed cowbirds (*Molothrus ater*) have all contributed to this decline (Confer et al. 2011). The U.S. Fish and Wildlife Service is currently reviewing a petition to list Golden-winged warblers under the

Endangered Species Act (USFWS 2013). The decline of Golden-winged warbler populations augments the need for research and understanding of the breeding habitat and ecology of this species. The Golden-winged Warbler Working Group was founded in 2003 to facilitate communication and collaboration among scientists who work to produce region-specific guidelines for the conservation of these warblers throughout their breeding and wintering ranges (Roth et al. 2012).

Knowledge of the numbers and locations of Golden-winged warblers during their breeding season in the southern Appalachian Mountains is limited. In the past decade, the North Carolina chapter of the Audubon Society has collected data on the presence of breeding Golden-winged warblers in western North Carolina. In this study, I developed a predictive habitat model using NC Audubon Society breeding site data in order to better understand habitat availability for Golden-winged warbler populations in this region. Development of this model constitutes the first stage of a two-part study, the second of which will involve refinement and ground-truthing of the model in the summer of 2017.

METHODS

In this study, I first analyzed data collected for the GOWAP/GWWA Atlas Protocol, a survey procedure initiated by the Cornell Laboratory of Ornithology and carried out by the NC Audubon Society in western North Carolina to locate occupied and potential Golden-winged warbler breeding sites (Swarthout et al. 2009). The data I analyzed were collected from 1999 through 2013. Using ArcGIS 10.4™ (ESRI, Inc.), I clipped the occupied points to only include those located within Jackson, Macon and Graham counties, NC, which gave me a sample size of 31 breeding sites—18 in Graham County, seven in Jackson County and six in Macon County. Using a Digital Elevation Model (LIDAR collected by NC Floodplain Mapping Program and processed by NC DOT), I generated slope, aspect and curvature layers for the selected counties. County soil data were obtained from the United States Department of Agriculture Web Soil Survey and vegetation height and type data from the United States Forest Service LandFire Data Distribution Site. Next, elevation, slope, aspect, curvature, soil type, and vegetation type values were extracted for all 31 sites.

Using the data collected in ArcGIS, I developed a predictive model to locate potential Golden-winged warbler habitat by creating new elevation, slope and vegetation type raster layers that included only the ranges of values or categories corresponding to where known breeding sites were located. The elevation layer included elevations of 2,000 to 5,000 feet (610-1,524 m) and the slope layer included slopes of 0 to 37%. The vegetation type raster included Developed-Low Intensity, Developed-Roads, Southern Appalachian Oak Forest, Southern and Central Appalachian Cove Forest, Central and Southern Appalachian Montane Oak Forest, Eastern Cool Temperate Urban Herbaceous, Eastern Cool Temperate Developed Ruderal Deciduous Forest and Eastern Cool Temperate Developed Ruderal Grassland. A new soils vector layer was created from only those soils in which the warblers were previously found. I reclassified the original vegetation type raster data into five categories: open developed land and herbaceous, shrub, forest height 5-10 meters, forest height 10-15 meters, and forest height 15-20 meters in order to run a Gradient North convolution filter in "Image Analysis" for edge detection.

After inputting the new elevation, slope, vegetation type, and edge analysis raster layers in "Raster Calculator", I clipped the resulting raster layer by the new soil vector layer to exclude any pixels that were not associated with a soil type of an occupied site. The resulting model highlighted small patches of land at detected edges where the soil type, elevation, slope and vegetation type matched or fell within the appropriate range based on those locations where breeding warblers were detected.

To test the edge analysis and overall model, 175-meter buffer polygons were created around each of the known breeding site points, which is the maximum distance of approach to a broadcast call for other woodland warblers (Betts et al. 2005). I ensured that the model predicted habitat within each of these polygons.

Using the predictive model, the LandFire vegetation height and type layer, and Google Earth satellite imagery, I manually plotted potential breeding sites in Macon, Jackson and Graham Counties in ArcGIS. Using roads as reference points, patches of land predicted to be suitable habitat by the model were matched with satellite imagery to verify that the patch had sufficient edge habitat. I looked for a visible shrub or herbaceous layer surrounded by mature forest then plotted 215 total points at potential sites.

RESULTS

Extracting values from the raster and soils layers to the occupied points, I found that within the study area, Golden-winged warblers have been found at elevations ranging from 2,046 to 4,570 feet (624-1393 m) in patches with slope values of 2.5% to 36.3% (table 1). Thus, the model includes elevations from 2,000 to 5,000 feet and slopes of 0-37%. Breeding sites were associated with 19 different soil types, which were used in the model as well (table 1). Aspect and curvature had too large of ranges to be useable in the model.

TABLE 1. Plot characteristics of known Golden-winged warbler breeding sites in Graham, Jackson and Macon Counties, NC.

Point	County	Elevation (ft)	Slope (%)	Aspect	Curvature	Soil Type*
1	Graham	2562	25.6	231.3	-0.25	ScF
2	Graham	2171	23.7	324.3	1.5	JtD
3	Graham	2789	24.7	277.0	1.5	SbF
4	Graham	3259	23.3	295.8	0.5	ScF
5	Graham	3065	28.3	26.9	0.5	SpE
6	Graham	2907	33.5	15.4	-0.5	SbF
7	Graham	2576	4.5	341.6	-0.5	LnC
8	Graham	3732	13.1	20.4	2.5	SpE
9	Graham	3522	9.6	129.0	-1.25	CsF
10	Graham	2623	19.0	273.1	1.5	SpE
11	Graham	3122	31.2	71.9	1.75	SbF
12	Graham	4362	26.2	257.6	-0.25	CrE
13	Graham	2597	4.5	71.6	-0.5	UdE
14	Graham	2842	23.4	241.6	-0.5	SvD
15	Graham	3686	7.6	81.9	-0.5	CsF
16	Graham	3357	11.9	67.2	1.5	ScE
17	Graham	2602	6.6	337.6	0.0	FvA
18	Graham	2046	3.2	83.7	0.25	JbE
19	Jackson	3552	11.7	95.2	1.25	TwC
20	Jackson	3759	15.1	173.4	1.0	EdE
21	Jackson	3944	36.3	227.8	-0.25	EdE

22	Jackson	3590	18.3	259.1	-0.75	TwC
23	Jackson	3125	20.8	152.6	0.5	TwC
24	Jackson	4570	2.5	171.9	-0.5	BuD
25	Jackson	3517	4.5	198.4	-2.5	TwC
26	Macon	3714	23.7	100.7	0.25	CuE
27	Macon	3537	22.8	143.5	-0.25	EdF
28	Macon	3352	13.0	141.6	1.25	Ud
29	Macon	3473	6.6	53.7	0.5	Ud
30	Macon	3667	5.1	81.9	0.0	Ud
31	Macon	3432	27.1	66.3	-2.0	EvF

*see tab. 2

Extracting values from the raster and soils layers to the occupied points, I found that within the study area, Golden-winged warblers have been found at elevations ranging from 2,046 to 4,570 feet (624-1393 m) in patches with slope values of 2.5% to 36.3% (table 1). Thus, the model includes elevations from 2,000 to 5,000 feet and slopes of 0-37%. Breeding sites were associated with 19 different soil types, which were used in the model as well (table 2). Aspect and curvature had too large of ranges to be useable in the model.

TABLE 2. Soil type descriptions corresponding to where Golden-winged warblers were found in Macon, Jackson and Graham Counties.

Soil Type Abbreviation	Soil Type Description
BuD	Burton-Craggey-Rock outcrop complex, windswept, 8 to 30 percent slopes, stony
CrE	Cheoah-Jeffrey complex, 30 to 50 percent slopes, rocky
CsF	Cheoah-Jeffrey complex, 50 to 95 percent slopes, very rocky
CuE	Cullasaja-Tuckaseegee complex, 30 to 50 percent slopes, stony
EdE	Edneyville-Chestnut complex, high precipitation, 30 to 50 percent slopes, stony
EdF	Edneyville-Chestnut complex, high precipitation, 50 to 95 percent slopes, stony
EvF	Evard-Cowee complex, 50 to 95 percent slopes, stony
FvA	Fluvaquents, ponded, 0 to 3 percent slopes, frequently flooded
JbE	Junaluska-Brasstown complex, 30 to 50 percent slopes
JtD	Junaluska-Tsali complex, 15 to 30 percent slopes
LnC	Lonon-Northcove complex, 8 to 15 percent slopes, bouldery
SbF	Snowbird loam, 50 to 95 percent slopes, stony
ScE	Soco-Stecoah complex, 30 to 50 percent slopes, stony
ScF	Soco-Stecoah complex, 50 to 95 percent slopes, stony
SpE	Spivey-Santeetlah complex, 30 to 50 percent slopes, very bouldery
SvD	Spivey-Whiteoak complex, 15 to 30 percent slopes, bouldery
TwC	Tuckaseegee-Whiteside complex, 8 to 15 percent slopes
Ud	Udorthents, loamy
UdE	Udorthents-Urban land complex, 15 to 95 percent slopes

Each of the vegetation type, elevation, slope, edge and soil layers eliminated portions of my study site that were considered unsuitable breeding habitat (fig. 1 and 2).

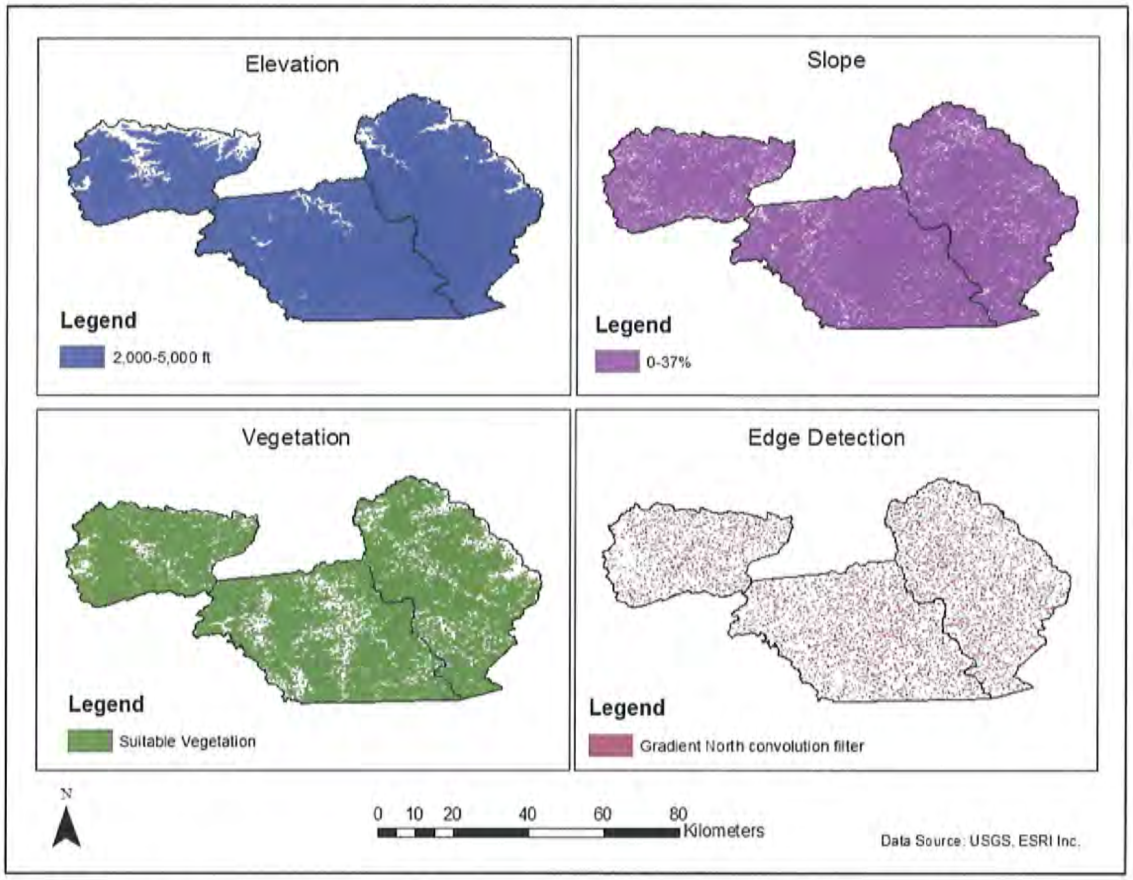


FIG. 1. Vegetation, elevation, slope and edge detection raster layer components of the predictive model. The colored portion is suitable and the white is unsuitable breeding habitat for Golden-winged warblers.

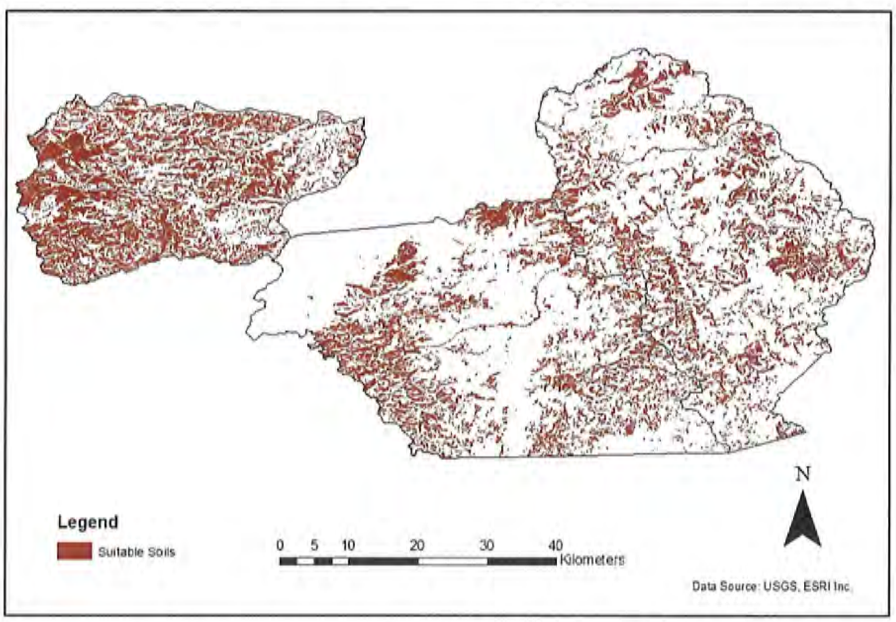


FIG. 2. Suitable soil types according to occupied Golden-winged warbler breeding sites.

Adding the five model components resulted in one raster layer that represented predicted suitable habitat (fig. 3).

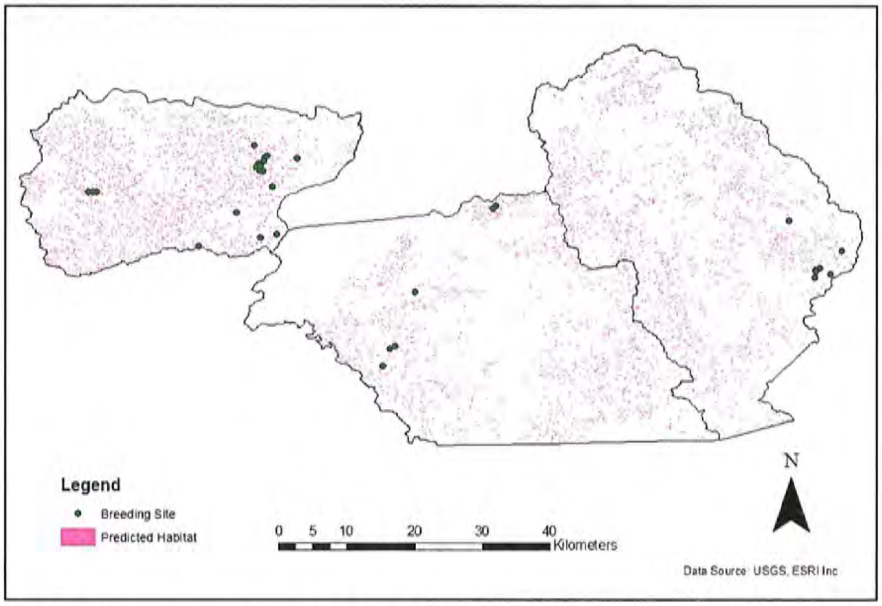


FIG. 3. Map of known breeding sites and potential Golden-winged warbler breeding habitat predicted by the model developed in ArcGIS.

Only one known site was not associated with predicted habitat within a 175-meter buffer (fig. 4).

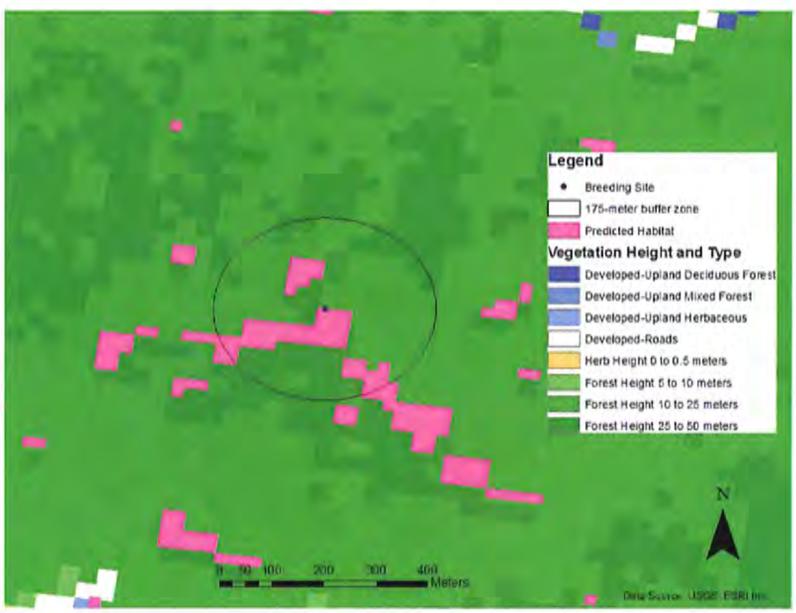


FIG. 4. Example of a potential Golden-winged warbler breeding site, a 175-meter buffer and location of habitat predicted to be suitable by the model.

I plotted 215 new potential sites to survey for breeding Golden-winged warblers (fig. 5).

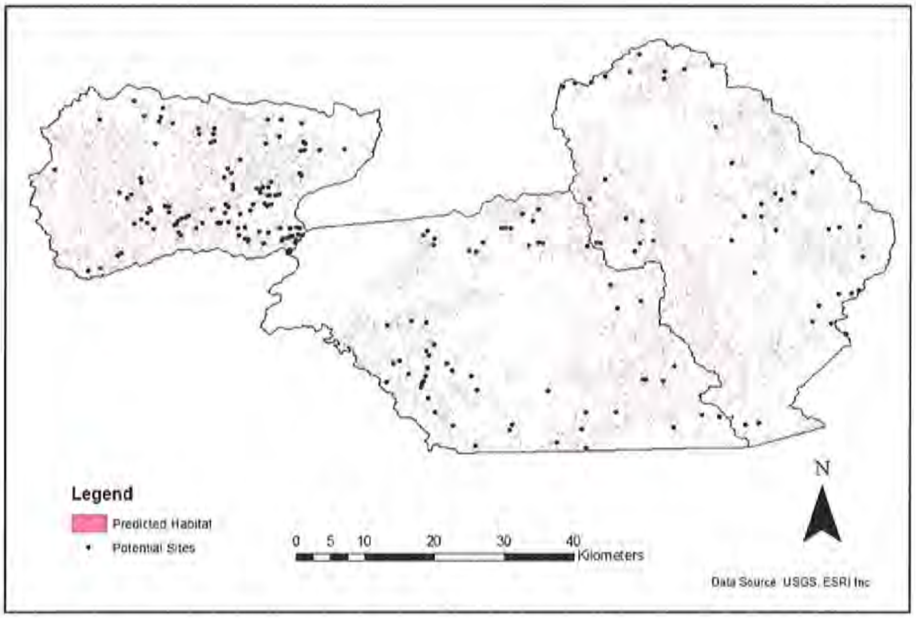


FIG. 5. Predictive model for Golden-winged warbler breeding habitat with overlaid potential broadcast survey sites.

DISCUSSION

The predictive model was successful in identifying patches of suitable Golden-winged warbler habitat but did not sufficiently narrow down the options, resulting in heavy reliance on Google Earth satellite imagery for verifying potential sites. The discrepancy between the edges detected in the LandFire data and those visible on Google Earth may be due to my inability to clearly distinguish edges on Google Earth, and the fact that the LandFire data is from 2012 and Google Earth imagery is from 2015. Additionally, the known breeding sites were found from 1999 to 2013, a range of time that would allow for noteworthy landscape changes. Patches that were early successional habitat in 1999 may now be in much later successional stages and no longer suitable breeding habitat. Reliance on known breeding sites for predicting suitable habitat is thus inherently flawed.

In the spring of 2017, broadcast surveys will be performed at some of the potential sites I plotted, after which I will perform additional analyses to determine how best to perfect the model for use in the following year. In the upcoming year, I will be adding more components to the model, including spectral fingerprint and other edge analyses. The new data collected in the spring of 2017 will increase the sample size and provide more recent data to build a more accurate model for use in the 2018 breeding season. Edge analysis can be improved by using multiple convolution filters, since using just a Gradient North filter may cause bias and not catch all edges. Additionally, I can try a different reclassification scheme for the vegetation height data, creating either more or fewer classes, and changing the groupings of vegetation heights. Since I used the reclassified raster layer when applying the convolution filter, a different reclassification system may improve the accuracy of the filter. If possible, more recent data with smaller pixel sizes may also improve the predictive ability of the model, since Golden-winged warblers have specific microhabitat preferences including presence of herbaceous, shrub and forest layers.

ACKNOWLEDGEMENTS

I would like to thank Dr. Gary Wein, who contributed many hours helping me with ArcGIS, Gail Lemeic, who served as my mentor for the semester, Tasmia Zaman, who worked alongside me on her related project, Dr. James Costa, who advised me on the development of my senior honors thesis and Dr. Sarah Workman for editorial comments and advice on the progression of the project.

LITERATURE CITED

Aldinger, K. R., and P. B. Wood. 2014. Reproductive success and habitat characteristics of Golden- winged warblers in high-elevation pasturelands. The Wilson Journal of Ornithology **126**(2): 279- 287.

Bakermans M. H., B. W. Smith, B. C. Jones, and J. L. Larkin. 2015. Stand and within-stand factors influencing Golden-winged warbler use of regenerating stands in the central Appalachian Mountains. Avian Conservation and Ecology **10**(1): Article 10.

Betts, M. G., A. S. Hadley, and P. J. Doran. 2005. Avian mobbing response is restricted to territory boundaries: experimental evidence from two species of forest warblers. Ethology**111**: 821-835.

Buehler, D. A. 2007. Restoration and enhancement of reclaimed surface mines as habitat for disturbance-dependent species in the Cumberland Mountain. Final General Challenge Grant Report to the National Fish and Wildlife Foundation.

Confer, J. L., P. Hartman, and A. Roth. 2011. Golden-winged warbler (*Vermivora chrysoptera*). The Birds of North America Online, Cornell Laboratory of Ornithology. 7 November 2016. Web.

Environmental Systems Research Institute (ESRI). 2016. ArcGIS Desktop: 10.4. Redlands, California, USA.

Greenberg, C. H., C. R. Rossell, Jr., and D. B. Johnson. 2002. A comparison of artificial nest predation in hurricane-created gaps and closed canopy forest of the southern Appalachians. Journal of the North Carolina Academy of Science **118**: 181-188.

Klaus, N. A., and D. A. Buehler. 2001. Golden-winged warbler breeding habitat characteristics and nest success in clearcuts in the southern Appalachian Mountains. Wilson Bulletin **113** (2): 297-301.

Patton, L. L., D. S. Maehr, J. E. Duchamp, S. Fei, J. W. Gassett, and J. L. Larkin. 2010. Do the Golden-winged warbler and Blue-winged warbler exhibit species-specific differences in their breeding habitat use? Avian Conservation and Ecology **5**(2): Article 2. 29 September 2016. Web.

Rossell, Jr. C. R. 2001. Song perch characteristics of Golden-winged warblers in a mountain wetland. Wilson Bulletin **113**(2): 246-248.

Roth, A. M., R. W. Rohrbaugh, T. Will, and D. A. Buehler, editors. 2012. Golden-winged warbler Status Review and Conservation Plan [Internet]. Golden-winged warbler Working Group. 7 November 2016. Web.

Sauer, J. R., J. E. Hines, J. E. Fallon, K. L. Pardieck, D. J. Ziolkowski, Jr., and W. A. Link. 2014. The North American Breeding Bird Survey, Results and Analysis 1966-2012. Version 02.19.2014. USGS Patuxent Wildlife Research Center, Laurel, MD.

Swarthout, S. B., K. V. Rosenberg, R. W. Rohrbaugh, and R. S. Hames. 2009. Golden-winged warbler Atlas Project. 24 September 2016. Web.

United States Fish and Wildlife Service [USFWS]. 2013. Species of Concern: Golden-winged warbler. 20 September 2016. Web.

A PATCHY FUTURE FOR GOLDEN-WINGED WARBLERS?: USING PATCH ANALYSIS TO CONTEXTUALIZE HABITAT PREDICTOR MODELS WITHIN THE LANDSCAPE

TASMIA ZAMAN

Abstract: The Golden-winged Warbler (*Vermivora chrysoptera)* is facing serious population declines in the Southern Appalachian regions primarily due to habitat loss. Active land management is necessary to create the early-successional habitat upon which the bird depends. To locate potential habitat sites in western North Carolina, a predictive model was developed based on various physical landscape characteristics. The resulting predicted habitat patches were analyzed for size and shape. On average, the patches were too small to be considered suitable habitat. This raises the question as to whether the model needs to be adjusted or if the landscape itself is too fragmented for Golden-winged Warblers to thrive.

Key words: Golden-winged Warbler, Vermivora chrysoptera, *habitat prediction, landscape ecology, patch analysis*

INTRODUCTION

The Golden-winged Warbler (*Vermivora chrysoptera)* is a Neotropical migratory songbird. It is also one of the fastest-declining songbird species in North America. Their population is decreasing at a rate of 8.3% per year (New Jersey Department of Environmental Protection 2012). The causes for the bird's demise are habitat loss, nest parasitism by Brown-headed Cowbirds *(Molothrus ater)*, and hybridization with Blue-winged Warblers (*Vermivora cyanoptera)* (Harrelson 2015). Of these, habitat loss has had the most drastic effect. Their breeding habitat was once a contiguous range spanning northeastern North America (Larkin 2014). Within the past four decades, their range has been fragmented to the point that there are now two disjunct populations: the Great Lakes/Canada region and the Appalachians (fig. 1, Cornell Lab 2016). This disjunct has interesting biogeographical implications, as geographical barriers within a population set the stage for vicariance and potential speciation.

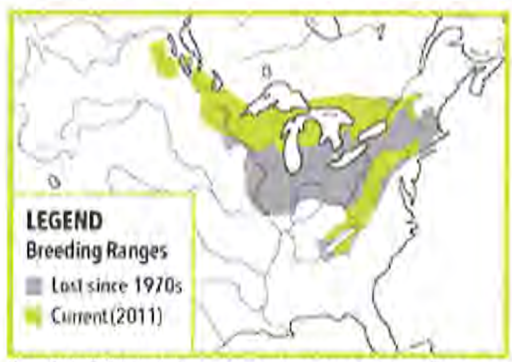


FIG. 1. This map displays the GWWA's historic and current breeding range. The disjunction between the Great Lakes and Appalachian populations is apparent. (Source: Cornell Lab of Ornithology)

Habitat fragmentation has been even more detrimental within these two now-distinct regions. In the Southern Appalachians, GWWA population has declined by 98% since the 1960s (Larkin 2014). Local habitat loss puts pressure on bird populations by limiting resource availability. This resource stress reduces breeding success.

GWWAs are habitat specialists. They require a mix of grasses for foraging, shrubs for nesting, and canopy trees for singing and defending their territory (fig. 2). These conditions are found in early successional-stage forests as regrowth generated after a disturbance event (Roth et al. 2012). Natural disturbance events include fire, flooding, and beaver dam construction. Excessive fire-extinguishing and the functional extirpation of beavers from a wide extent of GWWA range have limited the chances for early successional forest generation and maintenance. GWWAs do also thrive under a degree of human disturbance. Old orchards and pastures, roadsides, and utility right-of-way clearings have proven to contain worthy habitat (Roth et al. 2012). This is not to say, however, that these activities are sufficient habitat sources. Additionally, the traditional process of clear-cutting these areas does not always provide the appropriate mix of vegetation layers that compose suitable GWWA habitat.

FIG. 2. This utility right-of-way in the Nantahala National Forest exhibits the type of mixed habitat suitable for GWWAs. (Source: author)

The GWWA has attracted significant attention from the scientific community due to the interesting biological cases that it presents. The complex hybridization dynamics between GWWAs and BWWAs (Blue-winged Warblers) blur the lines between species to the point where some argue that they are the same species (Axelson 2016). The GWWA is also a key species for connecting conservation scientists and landowners. Managing habitat for GWWAs also benefits a host of other species of concern, such as the Appalachian Cottontail *(Sylvilagus obscurus)* and the American Woodcock *(Scolopax minor)* (GWWA Working Group). Furthermore, the bird's innate charisma lends itself as an ambassador to the public in raising awareness about endangered species.

The Golden-winged Warbler Working Group was founded in 2007 as a collaboration between scientists and landowners. The organization's goal is to reverse the bird's decline by managing for habitat and minimizing hybridization with BWWAs. The group has published a detailed conservation plan that is available to the public on their website. One facet of the plan focuses on increasing both quantity and quality of GWWA breeding and migratory habitat. This process involves identifying places where the birds have been known to breed, discovering new potential suitable locations, and then working with landowners to properly manage sites for habitat. Having more available breeding sites will be a crucial step in saving GWWA populations by addressing the main cause of their fall (Roth et al. 2012).

During the spring, volunteers from the North Carolina Audubon Society conduct call surveys at both known and potential sites occupied by GWWAs. They then record whether the birds were truly present at the site. Most of these efforts have been concentrated in the northern part of the birds' North Carolina range. More information is needed on habitat quality in the southwest regions of the state. In order to identify new sites to be investigated, we made a model to predict new locations to be investigated.

MATERIALS AND METHODS

Audubon volunteers conduct surveys in the field each spring in search of GWWAs. They record GPS coordinates of sites investigated and how many birds were observed. We plotted these points, collected in 2013, on a map of western North Carolina. We focused on the southwest corner of the state and narrowed our study area down to three counties: Jackson, Macon, and Graham.

A model was developed to highlight the areas most likely to be suitable for GWWAs. The variables used to develop the GWWA habitat model were elevation, slope, aspect, curvature, soil type, vegetation type, and boundary edge of area. This model was based on landscape characteristics of the points provided. The characteristics are listed in table 1 below. These values were consistent with the required habitat traits outlined in the GWWA Conservation Plan (gwwa.org). Each trait was made into a raster layer at 30m pixel resolution in ArcMap and assigned a score. The raster layers were added together using the raster calculator tool. Numbers were arbitrary but distinct so that it would be easy to see which habitat aspects were present or absent in the final result. The resulting layer, at this point the model, displayed clusters of pixels that yielded high raster calculator values. A high "score" indicates the presence of a majority of required habitat traits. A more detailed description of this process can be found in the paper by Whitenack (2016) in this volume.

TABLE 1. Plot characteristics of known Golden-winged warbler breeding sites in Macon County, NC.

County	Elevation (ft)	Slope (%)	Aspect	Curvature	Soil Type*
Macon	3714	23.7	100.7	0.25	CuE
Macon	3537	22.8	143.5	-0.25	EdF
Macon	3352	13.0	141.6	1.25	Ud
Macon	3473	6.6	53.7	0.5	Ud
Macon	3667	5.1	81.9	0.0	Ud
Macon	3432	27.1	66.3	-2.0	EvF

* See paper by Whitenack, this volume.

The areas highlighted by the model showed a highly-dispersed pattern, with suitable habitat being represented as isolated patches within the landscape matrix. This raised the question as to whether the model was too restrictive in its interpretation of the habitat characteristics at the previously collected points. The initial model did not include a cover analysis derived for each point where a GWWA had been sighted. A spectral fingerprint, derived from satellite imagery such as Landsat or Ikonas, can be used to improve the resolution of habitat models. However, because there is a range of error around the GWWA from its collection buffer zones of 30m and 175m were created to explore impacts on using satellite data as well as the scale of that data.

Creating buffer zones around points allows for a more open interpretation of the points and accounts for potential imprecision in point collection. Two buffer sizes were utilized: 30-meters and 175-meters. The 30-meter buffer accounts for differences in GPS coordinates. The 175-meter buffer accounts for the maximum range at which warbler calls can be heard unaided; this is pertinent as birds are more often heard than seen, and thus the GPS points recorded are not likely to represent the true location where the bird was at the time.

Enhanced Thermatic Data Plus (ETM+) derived from Landsat 7 collected on May 20, 2007 was used for this analysis. The area for this analysis was restricted to Macon County as appropriate ETM data for the other counties was not accessible. Maximum Likelihood Classification (MLC) was used to classify each pixel, by which class it has the highest probability of belong to, based on algorithm quantification of the image. This supervised classification yielded more potential sites compared to the raster calculator model, as they were not as restricted to edges. The habitat did, however, still appear highly fragmented. This was somewhat expected, as landscapes are mosaics composed of a variety of patch types dispersed throughout the area.

I sought to investigate the patches derived from the original model compared to the MLC of the ETM+ for signature classes derived from a 30m and 175m buffer around the GWWA points, and to draw patterns from the results of these their sizes and shapes using Fragstats software. The Golden-winged Warbler Conservation Plan (Roth et al. 2012) recommends that potential habitat patches less than 300 m from existing habitat be at least 5 acres (2.02 ha) in area, while patches greater than 300 m away from existing habitat should be at least 25 acres (10.1 ha) in area. To do this, I ran an analysis on patch area for each of the three models: raster model, points buffered to 30 m, and points buffered to 175 m. This was to see whether the predicted habitat is of adequate size.

The Conservation Plan (Roth et al. 2012) also recommends irregular shapes for habitat patches. They should be long and narrow to maximize the amount of edge within the patch; a square-shaped patch would remain occupied in the center due to the lack of access to the edge. To measure shape regularity, I used the Perimeter-Area Ratio function and the Shape Index function (fig. 3). While the Perimeter-Area Ratio is a useful tool for quantifying the shape of a patch, it becomes difficult to compare patches of different areas because the value is affected by area. The Shape Index corrects for differences in area by comparing the shape in question to a standard shape of the same area.

$$\mathrm{SHAPE} = \frac{.25\ p_{ij}}{\sqrt{a_{ij}}}$$

FIG. 3. The formula for Shape Index. (Source: Fragstats 2013)

RESULTS

Both of the land classifications yielded more potential habitat area than the raster model. (fig.4). The 30-meter buffer classification had the most distinct patches (30,248) as shown in table 2. The 175-meter buffer classification had the least number of distinct patches but had the highest average patch area. This is mainly due to an outlier patch that appeared to be 42600.69 ha (table 2).

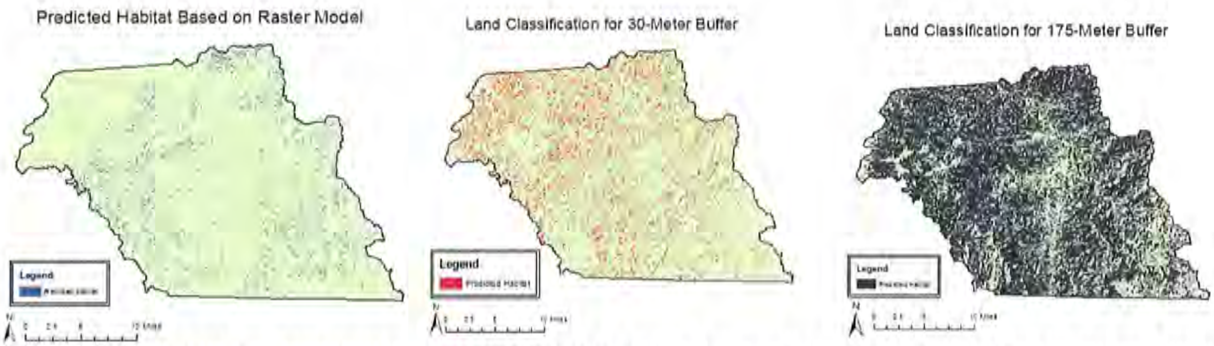


FIG. 4. The first map displays potential GWWA habitat based on the raster model. The second and third maps display the results of Maximum Likelihood Classification for the points buffered at 30 meters and 175 meters in Macon County.

TABLE 2. Patch Area statistics for all three habitat classifications. The areas are calculated in hectares.

Classification	Average	St.Dev	Max Value	Min Value	Range	Median	Total Patches
Model	0.258	0.388	15.389	0.0332	15.356	0.133	14924
30m Buffer	0.422	1.268	49.320	0.0900	49.230	0.180	30248
175m Buffer	6.882	425.956	42600.69	0.0900	426000.69	0.180	11134

Average Shape Index was fairly consistent between the three habitat classifications (table 3). The Shape Index values begin on a scale from 1, meaning a completely regular square, and increase indefinitely as shape irregularity increases. However, the 175-meter buffer had the highest maximum value (126.403) which means that it had a patch with very high irregularity.

TABLE 3. Perimeter-Area Ratio (PARA) and Shape Index Values (SHAPE) for all three habitat classifications.

		Average	St. Dev.	Max Val.	Min Val.	Range	Median
Model	PARA	1374.652	507.553	2194.065	411.3872	1782.678	1316.439
	SHAPE	1.178	0.296	4.841	1.000	3.841	1.000
30m Buffer	PARA	1136.636	239.994	1333.333	333.333	1000.000	1333.333
	SHAPE	1.238	0.532	9.696	1.000	8.696	1.000
175m Buffer	PARA	1127.271	256.101	1333.333	218.391	1114.942	1333.333
	SHAPE	1.269	1.671	126.403	1.000	125.403	1.000

DISCUSSION

Patch size is a crucial aspect of assessing whether a habitat is suitable for colonization. According to the GWWA Conservation Guide, the birds require that patches less than 300 m away from existing sites should be at least 5 acres in area, while patches over 300 m away should be at least 25 acres. Studies on GWWAs in the northern Appalachians have observed that a single pair of breeding GWWAs defended on average a territory of 10 acres. Based on both the raster model and the Fragstats analyses, very few of the patches in the study area were large enough to host GWWAs.

As edge-dwelling birds, GWWAs prefer patches with high perimeter-to-area ratios. On the landscape, this appears as long, narrow patches with feather-like extensions transitioning between grasslands and forest. Patches of more regular shape, i.e. a square, are likely to only host GWWAs on their fringes, with the inside being disregarded by the birds.

Attempts to draw patterns from the size, shape, and distribution of habitat patches are the basis of the landscape ecology concept (Forman and Godron 1981). The composition of a landscape can be described as the combined biotic and abiotic features present in a region; the differences in their responses to existing conditions, such as bedrock material, and disturbances, such as wildfires, give rise to a complex array of habitat patches. These patches are constantly changing and shifting with disturbance; as former clearings grow back into forest; new clearings are constantly being created elsewhere within the landscape (Urban et al. 1987).

Tracking spatial and temporal patterns reveals the fluid nature of landscapes. While these changes are incomprehensible on a human timescale, we can see for ourselves the landscape changes that affect the Golden-winged Warbler's chances of survival. More in-depth studies should be done to investigate the prediction and the distribution of suitable habitat for these birds if we are to ensure their continued presence in the Southern Appalachians.

LITERATURE CITED

Axelson, Gustave. 2016. Mixed-wing Warblers. Pages 16-17 *in* Living Bird (Summer 2016). Cornell University. Print. and "Golden-winged Warbler." <www.allaboutbirds.org>

Cornell Lab of Ornithology. 2016. Golden-winged Warbler. www.allaboutbirds.org

Forman, R. T. T. and M. Godron. 1981. Patches and structural components for a landscape ecology. BioScience **31**: 733–740.

Fragstats 2013. version 4 (v4.2) developed by K. McGarigal, S. Cushman, and E. Ene. original version of FRAGSTATS (v2), published in 1995. http://www.umass.edu/landeco/pubs/mcgarigal.marks.1995.pdf

Golden-winged Warbler Working Group (D. A. Buehler, chair). 2012. Golden-winged-Warbler full life cycle conservation strategy. In Roth, A. M., R. W. Rohrbaugh, T. Will, and D. A. Buehler, editors. Golden-winged Warbler Status Review and Conservation Plan. www.gwwa.org/

Harrelson, Jamie. 2015. Golden-winged Warblers *(Vermivora chrysoptera)* of the Southern Appalachians: Behavior, Ecology, and Conservation of a Declining Neotropical Songbird. Western Carolina University, Theses & Dissertations. https://libres.uncg.edu/ir/wcu/listing.aspx?id=18658

Larkin, Jeffery. 2014. Monitoring and Evaluating Golden-winged Warbler Use of Breeding Habitat Created by the Natural Resources Conservation Service Practices. A Conservation Effects Assessment Project (CEAP). USDA Natural Resources Conservation Service.

New Jersey Department of Environmental Protection. 2012. Golden-winged Warbler. New Jersey's Endangered and Threatened Wildlife. http://www.nj.gov/dep/fgw/tandespp.htm

Roth, A. M., R. W. Rohrbaugh, T. Will, and D. A. Buehler, editors. 2012. Golden-winged Warbler Status Review and Conservation Plan. www.gwwa.org/

Urban, D., R. O'Neill, and H. Shugart. 1987. Landscape Ecology. BioScience, **37**(2), 119-127. doi:10.2307/1310366

AN ASSESSMENT OF STREAM HEALTH OF THE WHITEWATER RIVER

LAURENCE A. CECIL, LAREN K. EVERAGE, WILLIAM B. HAMILTON, JORDAN E. IDDINGS, ALEXANDER W. KELLOGG, AMANDA R. MULLIKIN, BROOKE A. SMITH, ALEXANDRA R. TRIBO, LAUREN E. WHITENACK, AND TASMIA ZAMAN

Abstract. The Whitewater River is located within the Savannah River watershed in a region of the southern Appalachian Mountains that has experienced varying types of anthropomorphic disturbance. We surveyed five sites in the Whitewater River and one site in Silver Run Creek, a tributary to the Whitewater River, to assess stream health. We observed physical characteristics that affect stream ecology such as riparian zone width, erosion potential, sedimentation, channelization, and habitat availability for aquatic life. To conduct these assessments, we followed protocols from the North Carolina Habitat Assessment for Mountain and Piedmont Streams, a stream visual assessment protocol (SVAP), Rosgen's Bank Erosion Hazard Index (BEHI), Wolman's Pebble Count, and an Index of Biotic Integrity (IBI) for benthic macroinvertebrates. We found that bank stability, vegetation, and light penetration scores were relatively consistent among all sites. NC Habitat Assessment scores were lowest at the two most upstream sites in Whitewater River. SVAP scores generally decreased as distance downstream increased. IBI values suggested that the Whitewater River has been moderately impacted by anthropogenic pollutants. Further data collection in this area during a time of more standard rainfall would be useful because this survey was conducted during a period of extreme drought.

Key words: BEHI; EPT; IBI; macroinvertebrates; pebble count; Savannah River Basin; Silver Run Creek; SVAP; Southern Appalachians; Whitewater River.

INTRODUCTION

Stream ecosystems support a variety of plants, animals, and microorganisms, transport water and sediment through watersheds to croplands and towns, serve as water sources for municipal and industrial processes, and function as recreation areas. Stream conditions impact the health and diversity of aquatic and terrestrial organisms that rely on water resources. Thus, channel stability, stream health and water quality are of great importance to the surrounding area (Vannote et al. 1980, Allan and Castillo 2007). Both natural and manmade disturbances impact stream health and stability. Drought, flooding, fire, beaver activity, and landslides are natural disturbance events that impact stream ecosystems (Resh et al. 1988). Human alteration of the landscape has a documented effect on the physical, chemical, and biological characteristics of streams in watersheds (Death and Winterbourn 1995, Wolman 1967). Construction and mining activities release sediment and heavy metals into streams, dam building and stream channel alteration change flow and sediment distribution, and establishment of paved surfaces increases runoff, causing erosion and increasing pollutant transmission (Wolman 1967). Changes in both physical and chemical composition alter the distribution and abundance of organisms within streams (Resh et al. 1988). Erosion, sedimentation and pollution from runoff are all abiotic factors that can affect such change.

The headwaters of the Savannah River watershed are located in North Carolina in the Seneca sub-basin that includes the Thompson, Horsepasture, Toxaway, and Whitewater Rivers. The Whitewater River, located in Jackson County, North Carolina, received excellent bioclassification ratings when sampled from 1994 to 2009, based on benthic macroinvertebrate data collected from one site by the NC Department of Environment and Natural Resources: Division of Water Quality (NCDEQ 2012). In this study, we describe the physical and biological conditions of the Whitewater River watershed by using physical assessment methods and by collecting and analyzing macroinvertebrate samples.

METHODS AND MATERIALS

Study Area

Silver Run Creek is a tributary to the Whitewater River, which flows into South Carolina and, along with the Toxaway River, forms Lake Jocassee. The discharge from Lake Jocassee, now known as the Keowee River, ultimately joins Twelve Mile Creek near Clemson, South Carolina to become the Seneca River, which is a tributary of the Savannah River. The Seneca sub-basin covers an area of approximately 31,939 acres (fig. 1). The Whitewater River watershed is located in Jackson County, in a region that is considered to be a high elevation temperate rainforest (UCWA 2004). Average annual precipitation for nearby Cashiers, NC is 90.51 in (2.3 m), exceeding the 78.74 in (1.8 m) of precipitation needed to classify a temperate rainforest (WeatherDB 2016). On average, historical air temperatures range from a low of 25° F to a high of 84° F (NRCS 1990). To assess stream health, we surveyed six locations: one near the mouth of Silver Run Creek and five along the Whitewater River (table 1; fig. 2).

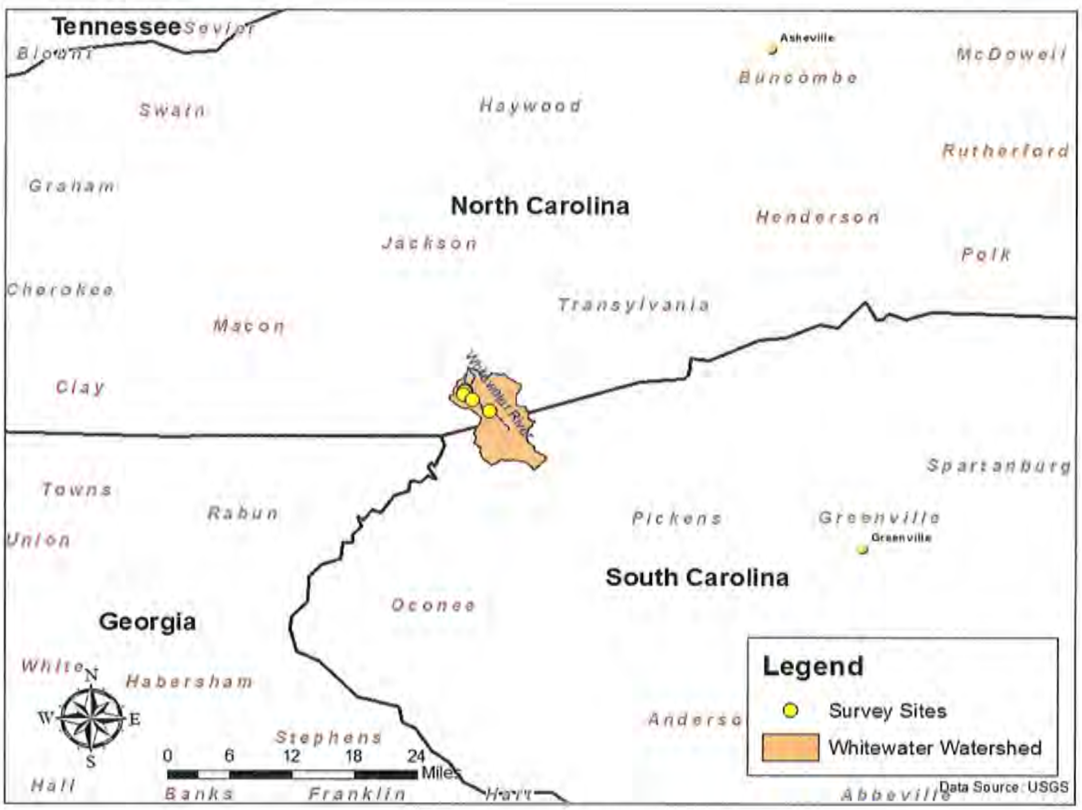


FIG. 1. All of the survey sites were located in North Carolina in the Whitewater River watershed. Map constructed using ArcGIS 10.4 (ESRI 2015).

TABLE 1. Locations of research sites referenced in the study. Sites will primarily be referred to by the listed abbreviations. Road refers to closest access point.

Site	Road	Latitude	Longitude	Survey Date
Silver Run Creek (SRC)	NC 107	35.06640 N	83.06564 W	12 Sep 2016
Whitewater River 1 (WR1)	NC 107	35.07458 N	83.06363 W	19 Sep 2016
Whitewater River 2 (WR2)	NC 107	35.06649 N	83.06577 W	19 Sep 2016
Whitewater River 3 (WR3)	NC 107	35.06621 N	83.05398 W	26 Sep 2016
Whitewater River 4 (WR4)	NC 1103	35.05343 N	83.05398 W	24 Oct 2016
Whitewater River 5 (WR5)	NC 281	35.03806 N	83.04556 W	10 Oct 2016

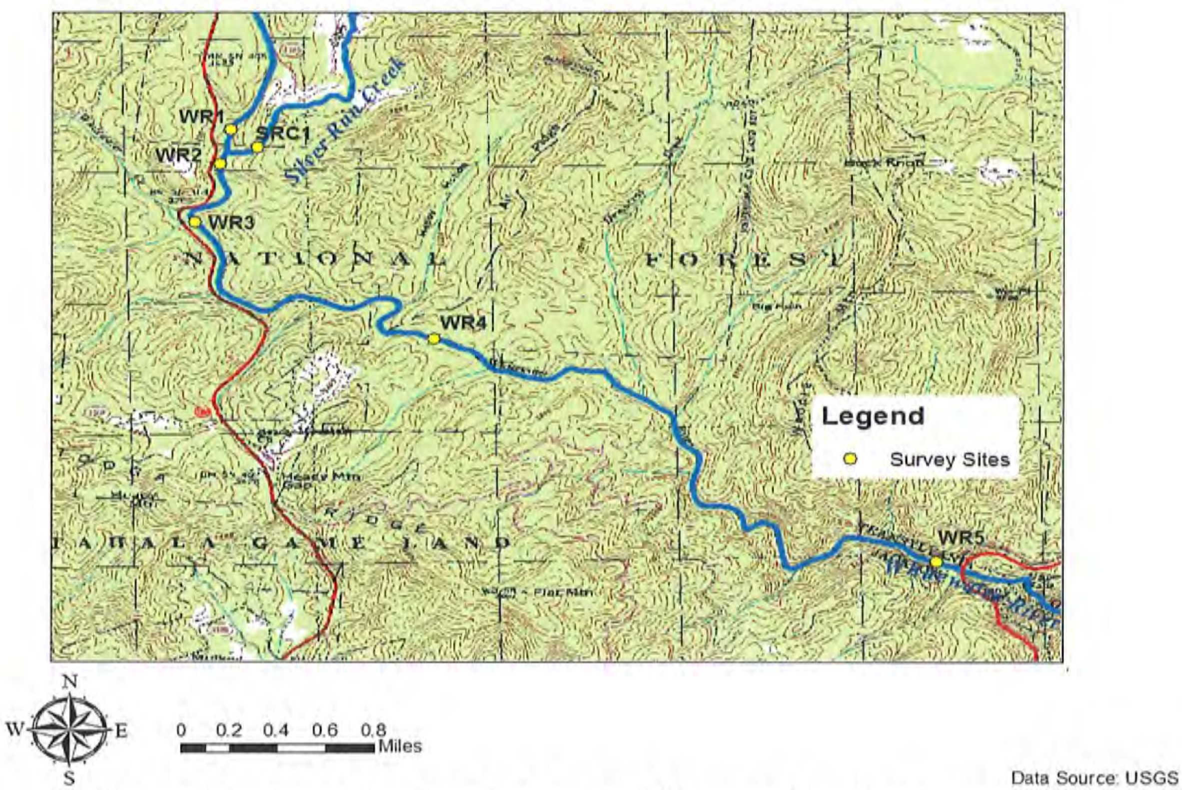


FIG. 2. Survey sites located along Silver Run Creek and Whitewater River. Map constructed using ArcGIS 10.4 (ESRI 2015).

North Carolina Habitat Assessment

In this element of the study, we examined stream reaches of 100 to 200 m in length and assigned each site a total score ranging from 1 and 100, from very poor stream conditions to very healthy stream conditions. To identify the conditions defined in the habitat assessment worksheet, we observed and recorded the stream's physical and geomorphological characteristics. We also noted the specific weather and land use conditions under which the study was conducted. We looked for evidence of channel modification, presence of instream habitat types, quality and composition of bed sediment, and bank stability and vegetation.

When looking for evidence of channel modification, we noted channelization and frequency of bends. To score instream habitat, we considered the percentage of the reach that was favorable habitat for fish and aquatic macroinvertebrates. To do this, we looked for existence of habitat types: rocks, macrophytes, sticks and leaf packs, snags and logs, and undercut banks and root mats. The score assigned to this section were based on the number of types of habitats present in the reach and the percentage of the reach available for colonization or cover. When examining bottom substrate, we looked at the entire reach to generate a score, but only looked at the riffles for embeddedness. We rated the substrate as: healthy substrate with good mix of gravel, cobble, and boulders; substrate with only gravel and cobble; substrate with mostly gravel; or substrate that is homogeneous. Within those categories, further classification was prompted based on percent embeddedness (i.e. embeddedness <20%, embeddedness 20-40%, etc.). Each classification was assigned a specific score that added up to the total instream habitat score.

We examined reaches for the presence and frequency of pools and for variety in size and dominant pool substrate. We looked at riffle habitats as well, noting whether riffles occurred frequently or infrequently. Scores were assigned based on classification of riffles as: well defined riffle and run; riffle as wide as the stream and extending two times the width of stream; riffle as wide as stream but riffle length is not two times stream width; riffle is not as wide as stream and riffle length is not two times stream width; or riffles absent. We also noted channel slope.

We also examined bank stability and erosion. We noted erosion areas on river left and river right and classified the bank vegetation (i.e. diverse trees, shrubs, and grass versus sparse mixed vegetation, etc.). We noted light penetration by classifying canopy as good, full, partial, minimal, or none. Lastly, we examined the width of the riparian vegetative zone (an area of natural vegetation adjacent to the stream) on river right and river left. We defined breaks in the riparian zone as any place on the stream which allows sediment or pollutants to directly enter the stream. We added individual section scores for the total score at the end, which can be used across sites as a quantitative comparison of individual stream habitats.

We used a spherical crown densiometer to measure canopy cover over the stream. The densiometer is divided into square sections. We counted the number of "dots" on the densiometer, which reflected open canopy while facing north, south, east, and west. Each dot represented a corner of a quadrant, with each quadrant being composed of four dots. Three points within the channel—two near the respective endpoints of the surveyed area and one in the middle—were surveyed to get an estimated average percent of open canopy. Additionally, we used a Pocket Rod to take 20 measurements at randomly selected points along the studied area of the stream in order to calculate average and maximum stream depth.

Stream Visual Assessment Protocol

The Stream Visual Assessment Protocol (SVAP) is a qualitative assessment of stream features and impacts originally developed by USDA for use by laypersons (Newton et al. 1998). It has since been modified by USDA and others (USDA 2009, LTLT 2014). The version that we used represents the unpublished precursor to the LTLT modification. We assigned a conditional assessment score of one to four, indicating poor to excellent stream health, based on ten habitat parameters. Epifaunal substrate is submerged material on the channel bottom. Embeddedness refers to how deeply sediments are deposited in the riffle and run areas. Diversity of instream habitats is measured by counting the number of riffles, runs, and pools. Sediment deposition is a survey of the presence of sediment bars within a stream. Channel flow status relates to how much water is in the stream channel. The measure of channel alteration involved surveying anthropogenic sources of disturbance or pollution, such as channelization, development, and/or agriculture. Channel sinuosity is the product of stream length divided by valley length, or a measure of the stream meander. Bank stability is a measure of stream bank resistance to change, quantified by evidence of erosion such as bank scouring and undercutting, bank collapse, and rooted vegetation. Vegetation protection refers to the percentage of a stream bank that is covered and shaded by a variety of vegetation such as trees, shrubs, flowering plants, and grasses. The riparian vegetative zone width is the measure of the amount of buffer present on each stream bank, with the best score given to a buffer zone that is greater than or equal to three times the channel width.

Bank Erosion Hazard Index

Rosgen's Bank Erosion Hazard Index (BEHI) is a protocol used to evaluate the likelihood that a section of streambank erodes, primarily during elevated stream discharge (Rosgen 2001). In this study, we calculated a BEHI value for the segment of the stream bank at each study site that appeared to be at greatest risk of erosion; values are not characteristic of the full extent of the reach. BEHI is calculated based on measuring bank height ratio (ft/ft), root depth ratio (ft/ft), root density (%), bank angle (°), and surface protection (%), each of which are then matched with an erosion risk value ranging from 'very low' to 'extreme.' We performed these measurements based on stream bank characteristics observed in the field, as defined in the Bank Erosion Hazard Index Worksheet (Rathbun 2008). As a part of this protocol, we estimated bank height by placing a Pocket Rod survey tape in the middle of the stream as a reference. From this measurement, we noted bankfull elevation as well as the visible rooting depth of bankside vegetation relative to bank height. We estimated the bank angle from the examined area of the stream relative to the surface of the water and the top of the bank. Once the initial measurements were taken, we adjusted figures either up or down in accordance with the protocol, depending upon the bank material or the presence bank stratification, to generate final BEHI values.

Pebble Count

We estimated distribution and size of bed sediment by performing Wolman's Pebble Count (Wolman 1954). We categorized sand as fine, medium, or coarse based on feel, and measured large boulders in-situ as precisely as possible. In order to obtain reach-wide pebble count result, we took ten sediment measurements at ten transects arranged to reflect the percentage of each habitat type (i.e. riffle, run, pool, and glide) within the reach, resulting in a total of 100 observations per stream reach. We measured the intermediate axes of randomly selected bed sediment with a flexible ruler and classified the sediment as silt, clay, sand, gravel, cobble, boulder, or bedrock (Wolman 1954). We used the results to develop relative and cumulative percentages of sediment size classes and to determine median grain size (D50), which is easily compared between sites.

Macroinvertebrates

We collected benthic macroinvertebrates using four different sampling methods: kick nets, D-nets, leaf packs, and timed visual sampling at each of the six stream reaches. We took four samples using each collection method, excluding visual samples. Timed visual samples were collected throughout the reach of the stream for a total of 20 minutes. We collected samples from riffles using kick nets and D-nets. When riffles were absent, we collected samples in other habitat types. We preserved insects obtained from sampling in an 80% ethanol solution and sorted specimens in the lab to order using dichotomous keys provided by Merritt et al. (2008). Orders Ephemeroptera, Plecoptera, and Trichoptera were further keyed to family to create an Index of Biotic Integrity (IBI). The EPT IBI is a biotic indicator used to gauge the health of a stream by measuring the diversity and abundance of macroinvertebrates. The IBI was determined at each individual site for the orders EPT using the following equation:

$$IBI = \frac{\sum n_i a_i}{N} \tag{1}$$

where n_i is the number of individuals in a particular family, a_i is the tolerance value associated with the family, and N is the total number of individuals in the orders EPT (Hilsenhoff 1977). Tolerance values were derived from a published list by Hilsenhoff (1988). We also determined taxonomic richness by counting the number of unique families that were present at each site. We separated families into trophic classes to provide information regarding their role within stream ecosystems. Macroinvertebrate trophic classes include shredders, scrapers, piercers, predators, and collectors (Merritt et al. 2008). Variable levels of diversity and abundance depend on how severely the organisms are affected by the presence of pollution and other environmental changes. Together, IBI and taxonomic richness can be used to qualify aquatic health and can be employed to perform comparisons between sample sites.

RESULTS

At each studied site, we took a number of measurements reflecting the physical conditions of the reach, including temperature, pH, average and maximum stream depth, channel width, and bank height (table 2). There appear to be no noticeable patterns for any condition as distance from headwaters increases, except for channel width which increases with river mile. Temperature at SRC was not taken on the day of survey and was instead recorded the following week. Temperature was not recorded for WR2.

TABLE 2. Physical conditions of research sites.

Site	Temperature (°C)	pH	Average stream depth (ft)	Maximum stream depth (ft)	Channel width (ft)	Bank height (ft)
SRC	17	5.3	1.2	2.2	40	3
WR1	16	5.13	0.39	0.77	29.5	14.1
WR2	NA	4.3	0.637	1.18	28.9	4
WR3	17	5.5	0.69	1.35	28.2	4.9
WR4	10.3	4.97	1.94	2.8	43.9	4.7
WR5	13.5	7.02	0.998	1.8	58.7	2.7

North Carolina Habitat Assessment

Component scores for each site were summed to form a cumulative habitat assessment score (table 3). Ideal scores were included as a baseline means of comparison between sites. Bank stability, vegetation, and light penetration scores were relatively consistent among all sites. In general, the quality of riffle habitats improved as distance from headwaters increased. Instream habitat and bottom substrate scores were lowest in WR1 and WR2, as were total habitat assessment scores (fig. 3).

TABLE 3. Habitat assessment scores by category. There were no riffles at WR4, so a score of 0 for riffle habitat was assigned to the reach.

Site	SRC	WR1	WR2	WR3	WR4	WR5	Ideal
Channel modification	4	5	4	5	4	4	5
Instream habitats	20	12	12	20	16	20	20
Bottom substrate	12	3	3	12	11	12	15
Pool variety	10	6	10	10	4	10	10
Riffle habitats	3	3	3	10	0	16	16
Bank stability/vegetation	12	12	11	12	12	12	14
Light penetration	7	7	10	10	7	7	10
Riparian vegetative zone width	10	10	7	7	7	10	10
Cumulative score	78	58	60	86	61	91	100

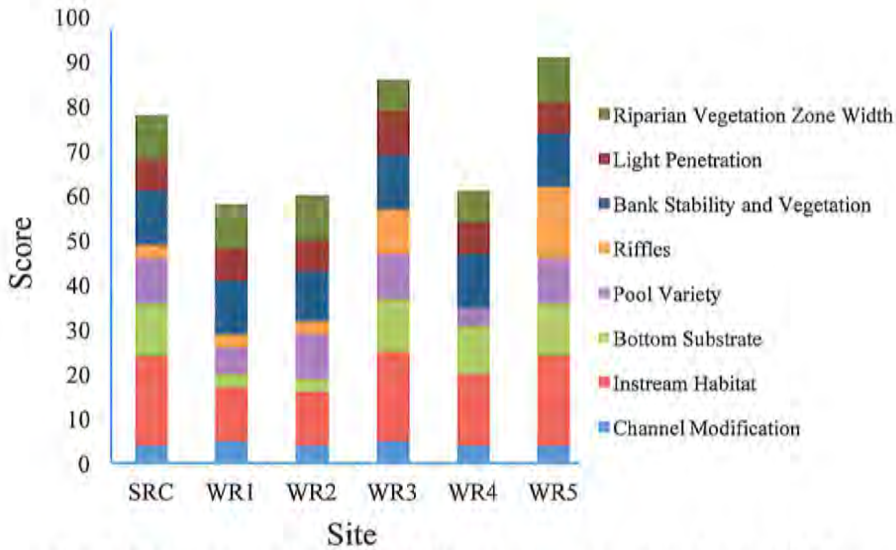


FIG. 3. Component and total NC Habitat Assessment scores for studied sites.

Stream Visual Assessment Protocol

Component scores for each site were summed to form a cumulative SVAP score (table 4). A column of ideal scores was added as a means of comparison between sites. SVAP scores generally decreased as distance downstream increased. Most categorical scores also decreased as river mile increased, except for livestock and barriers to fish movement scores which remained consistent between sites due to the absence of either issue at any of the sites (fig. 4).

TABLE 4. Stream Visual Assessment Protocol (SVAP) scores by category.

Site	SRC	WR1	WR2	WR3	WR4	WR5	Ideal
Bank condition	4	3.5	3	3.5	2.5	4	4
Streamside vegetation quantity	3.5	4	4	2.5	2.5	3.5	4
Streamside vegetation quality	4	4	4	3.5	3.5	4	4
Canopy cover	3	3	3	2	3	3	4
Riffle embeddedness	3	4	2	3	0	3	4
Trash and garbage	3	4	4	4	4	3	4
Non-trash pollution	4	4	3	4	2	4	4
Livestock	4	4	4	4	4	4	4
Pools	4	3	4	3	4	4	4
Available habitat/ Cover	4	3	3	3	2	3	4
Barriers to fish movement	4	4	4	4	4	4	4
Cumulative score	40.5	40.5	38	36.5	31.5	39.5	44
Overall (average) SVAP score	3.7	3.7	3.5	3.3	2.9	3.6	4
Class rating	Excellent	Excellent	Good	Good	Fair	Good	Excellent

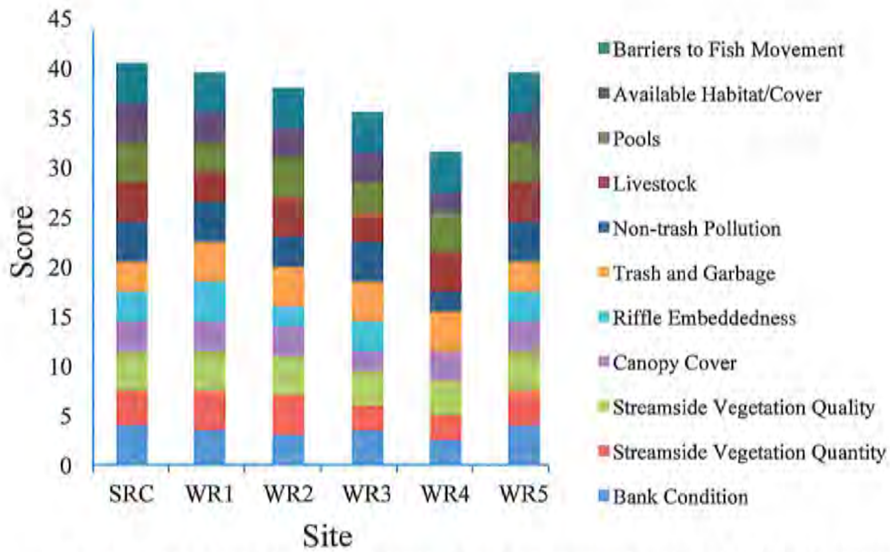


FIG. 4. Component and total stream visual assessment protocol (SVAP) scores for each studied site.

The coefficient of determination (R^2) for the correlation between SVAP and NC Habitat Assessment scores was found to be 0.057, indicating that no correlation existed between total scores (fig. 5). The other linear regression was also between SVAP and NC Habitat Assessment scores, but it only includes common categories between the two assessments. These categories were instream habitats, pool variety, bank stability, and light penetration (fig. 6). We found a slightly stronger negative correlation existed between tests ($R^2 = 0.13$). This coefficient was not great enough to state that assessments were sufficiently correlated.

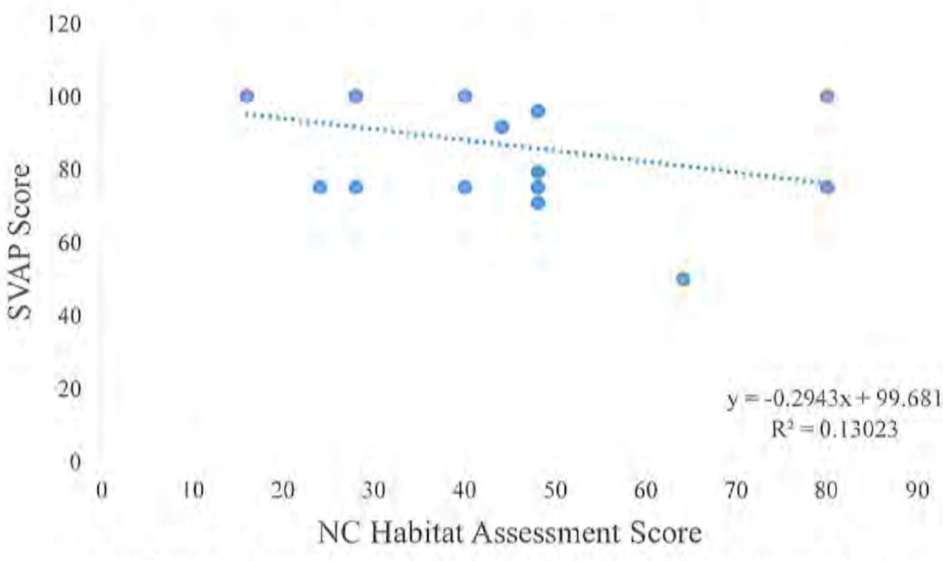


FIG. 5. Correlation between NC Habitat Assessment scores and Stream Visual Assessment Protocol (SVAP) scores.

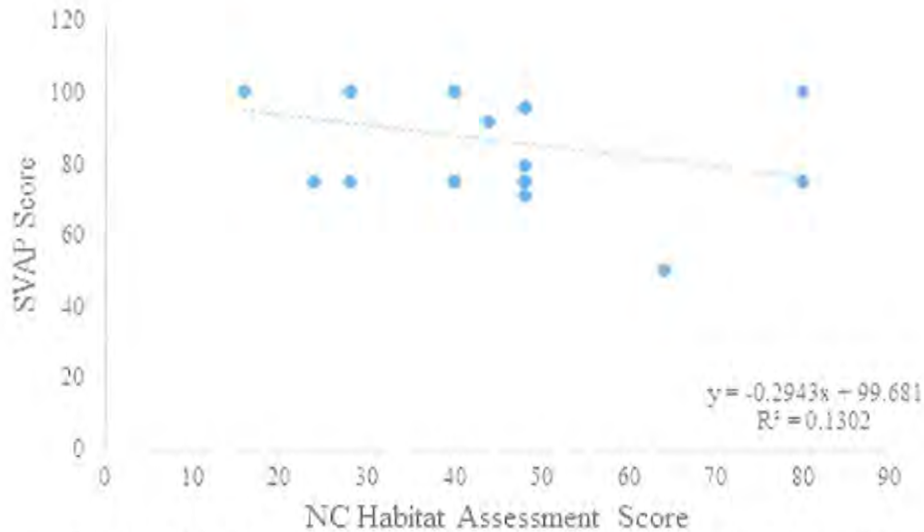


FIG. 6. Correlation between NC Habitat Assessment and SVAP scores for overlapping categories of assessment, including instream habitat, pool variety, bank stability and vegetation, and light penetration.

Percent canopy cover was generally stable across all sites (fig. 7). WR5 was the only site which did not follow the spatial trend as percent canopy cover was significantly less than the remaining sites.

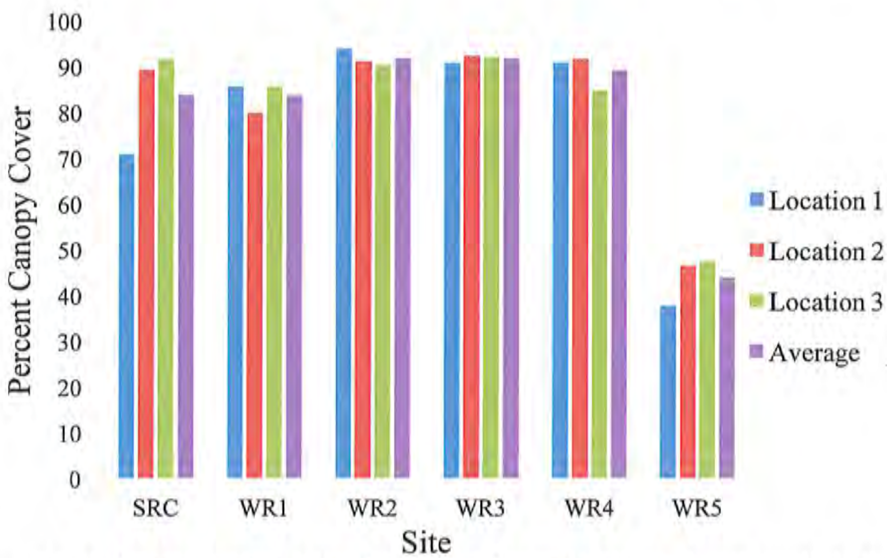


FIG. 7. Percent canopy cover for three different locations at each studied site. Locations include upstream, midstream, and downstream sections of the reach. Locations were randomly chosen, so Location 1 may not always refer to the upstream end of the reach.

Riparian vegetative zone width decreased with increasing average percent canopy cover (fig. 8). R^2 was determined to be 0.17, indicating that a weak negative correlation existed between the two variables.

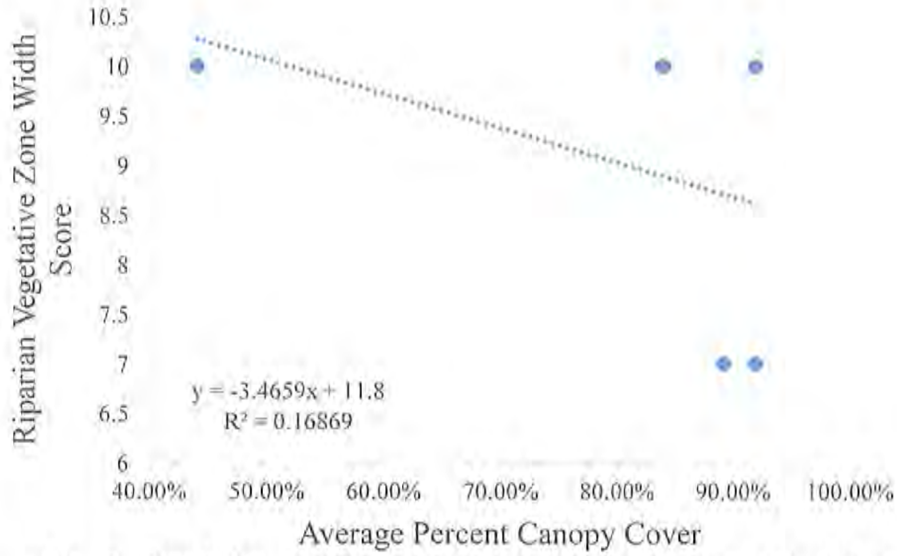


FIG. 8. Correlation between average percent canopy cover and riparian vegetative zone width scores, according to the NC Habitat Assessment.

Bank Erosion Hazard Index

BEHI scores generally decreased with increasing river mile, indicating that erosion potential for the reaches examined is highest near the headwaters (fig. 9). Most indices followed the same decreasing trend, excluding bank angle which increased with distance from headwaters (table 5). Non-adjusted BEHI scores showed the same decreasing pattern as the adjusted scores. In general, surface protection contributed the most to total scores, while bank angle contributed minimally.

TABLE 5. Indices of BEHI across sampled sites; adjusted BEHI score includes additive penalty for the presence of fine sediments.

Site	Bank height ratio	Root depth ratio	Root density	Bank angle	Surface protection	Adjustment	Adjusted BEHI	Erosion potential
SRC	8	7	10	3	10	10	48	Extreme
WR1	10	7	3	2	7	10	39	High
WR2	6	4	10	2	6	10	38	High
WR3	8	7	4	4	5	0	28	Moderate
WR4	5	2	4	3	10	0	24	Moderate
WR5	8	1	2	3	10	10	34	High

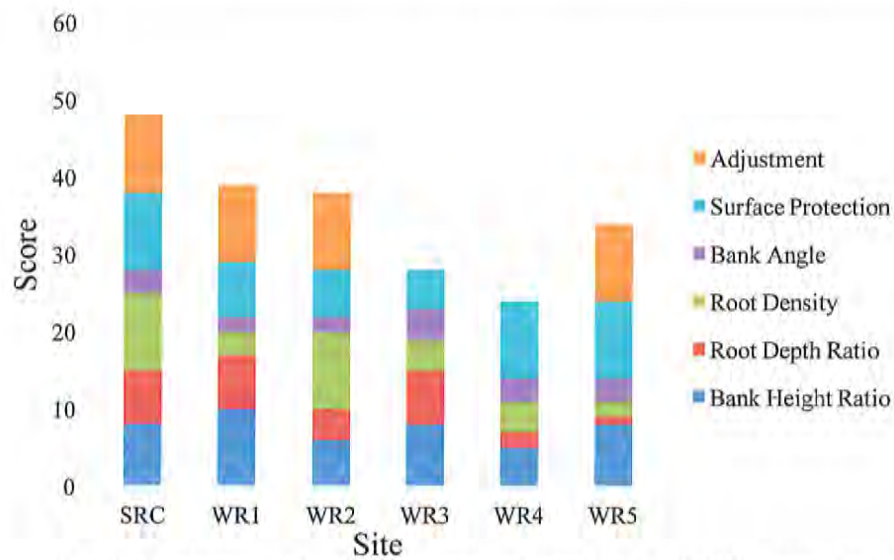


FIG. 9. Component and total bank erosion hazard index (BEHI) scores for each studied site, including adjustments for sediment types along bank where BEHI was performed.

Pebble Count

We determined relative percentages of runs, pools, and riffles/glides when setting up transects for pebble count. Morphological characteristics were most evenly distributed in WR1 and WR3, and they were least evenly distributed in WR4 (fig. 10). In most sites runs and glides composed nearly, or more than, half of all habitat types, yet WR5 was mostly composed of riffles. WR4 was absent of any riffles, and was dominated by pools.

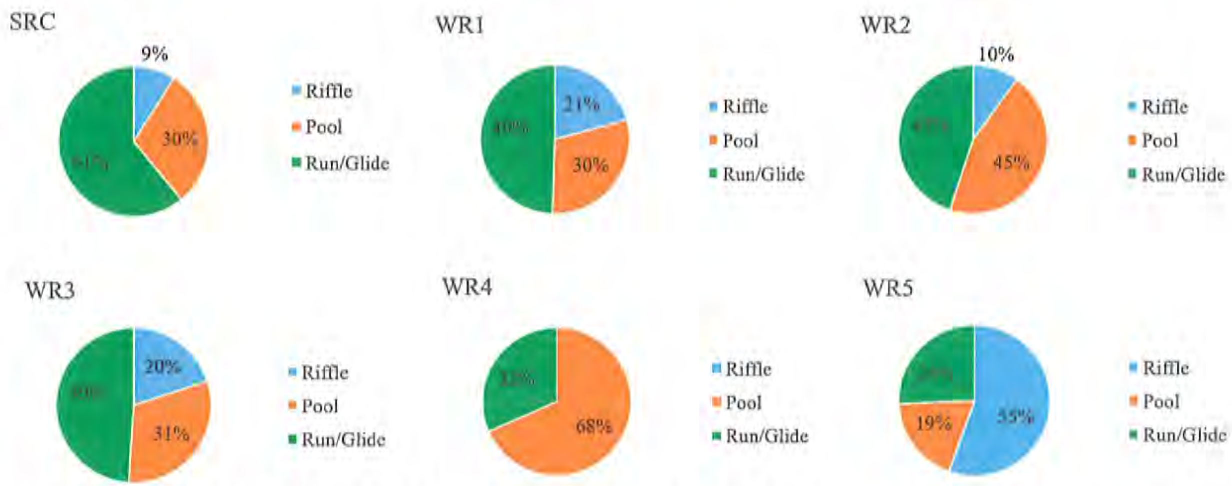


Fig. 10. At most sites roughly half of the morphology types are runs and glides. WR5 is dominated by riffles, and WR4 is absent of riffles and mostly made of pools.

The distributions of sediment particle sizes reveal a distribution with a higher number of large sediments in the Silver Run Creek site and WR5 (fig. 11-16). WR1, WR2, and WR4 show distributions with higher numbers of smaller sediments, and the distribution at WR3 is approximately normal.

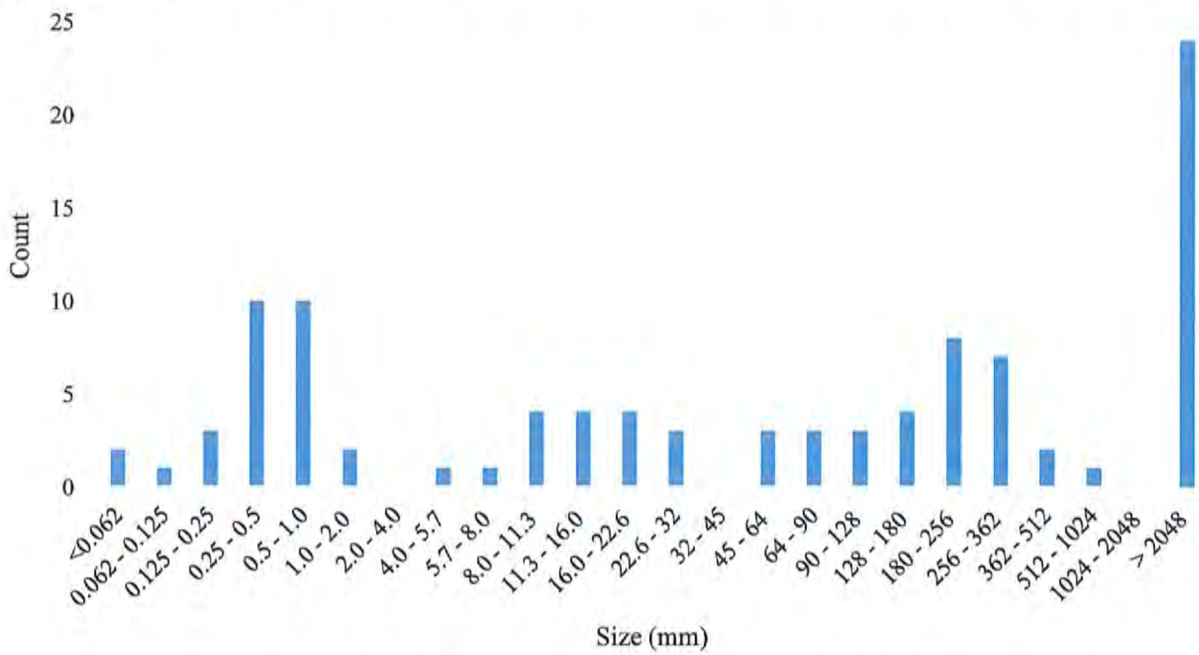


Fig. 11. Wolman Pebble Count in SRC site displaying number of sediments per size.

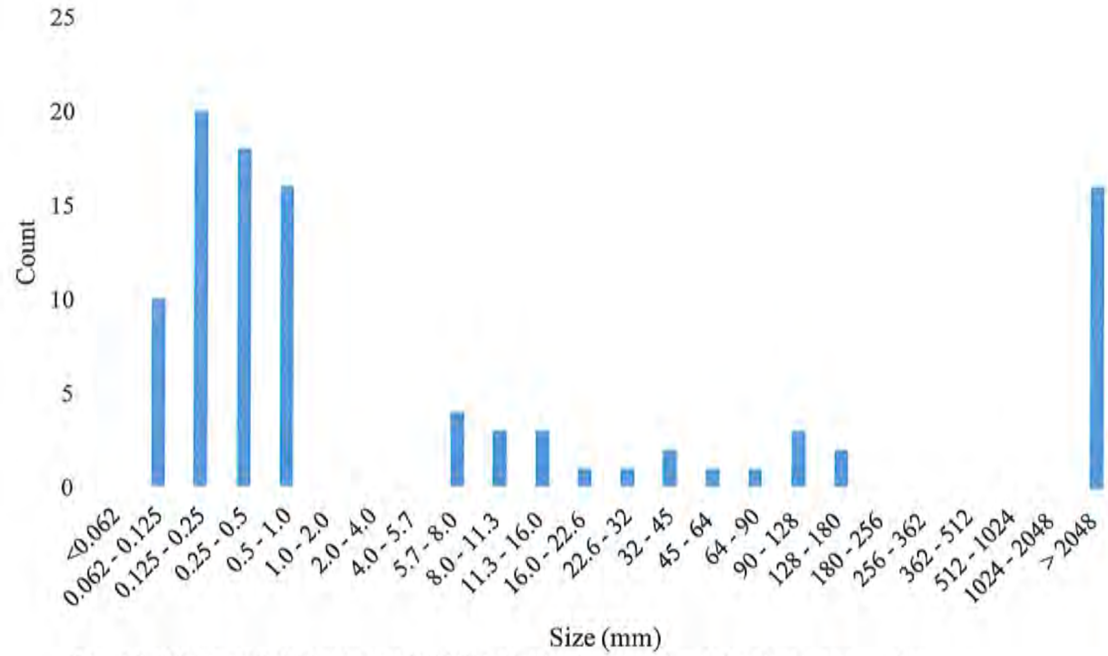


FIG. 12. Wolman Pebble Count in WR1 displaying number of sediments per size.

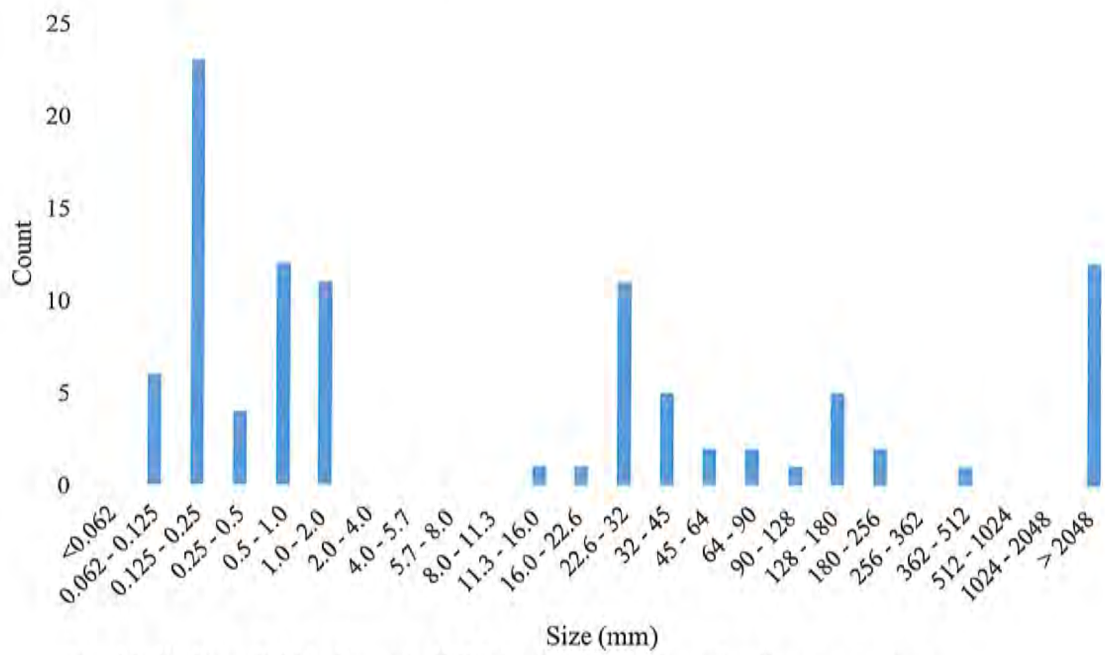


FIG. 13. Wolman Pebble Count in WR2 displaying number of sediments per size.

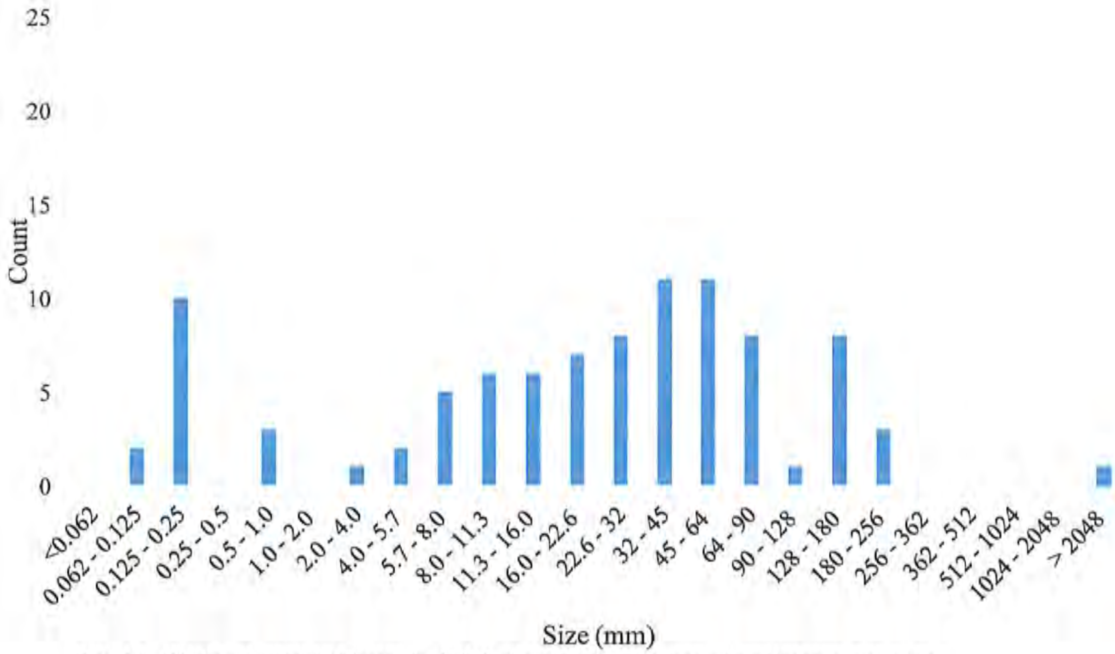


FIG. 14. Wolman Pebble Count in WR3 displaying number of sediments per size.

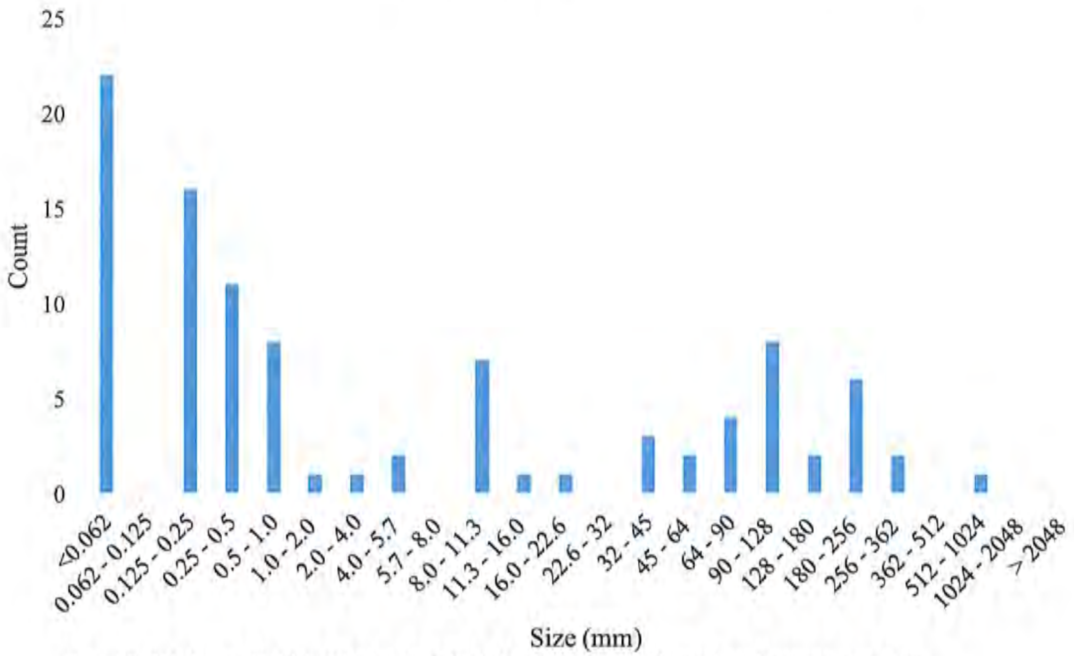


FIG. 15. Wolman Pebble Count in WR4 displaying number of sediments per size.

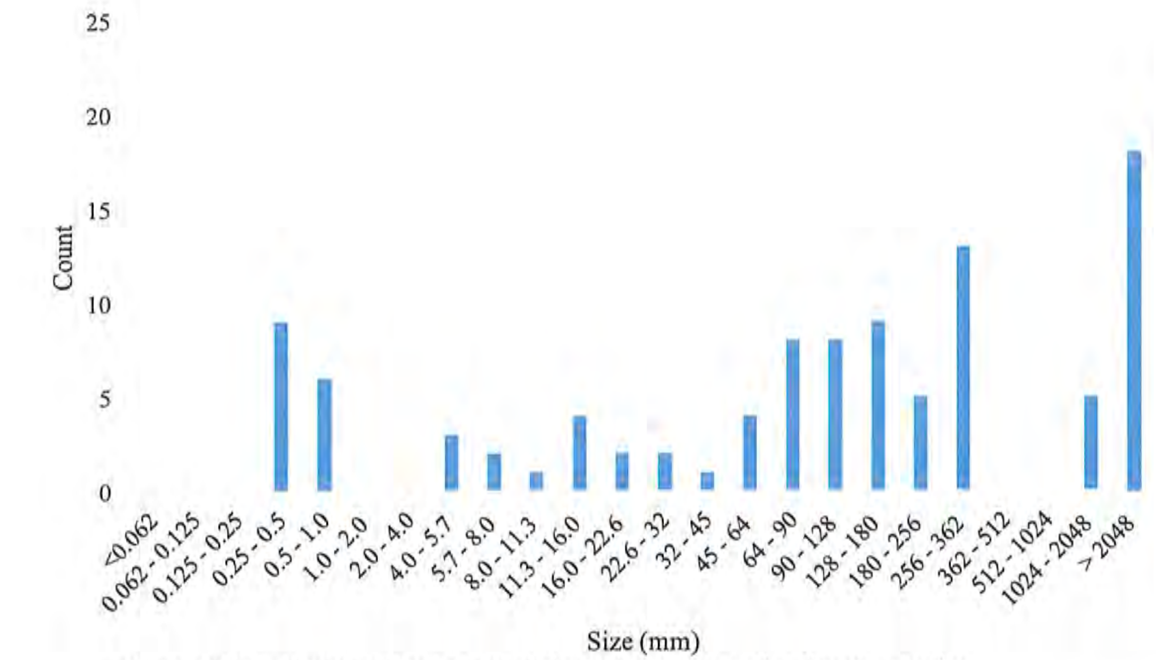


FIG. 16. Wolman Pebble Count in WR5 displaying number of sediments per size.

The D50 values (median particle size) for each stream (fig. 17) relate to the sediment size distributions and cumulative percentage rates per size class. D50 values tended to be higher in Silver Run Creek and WR5; they tended to be smaller in WR1, WR2, and WR4 (fig. 17). WR5 had the largest D50 of 128 - 180 mm, and the smallest D50 of 0.25 - 0.5 mm was at WR4.

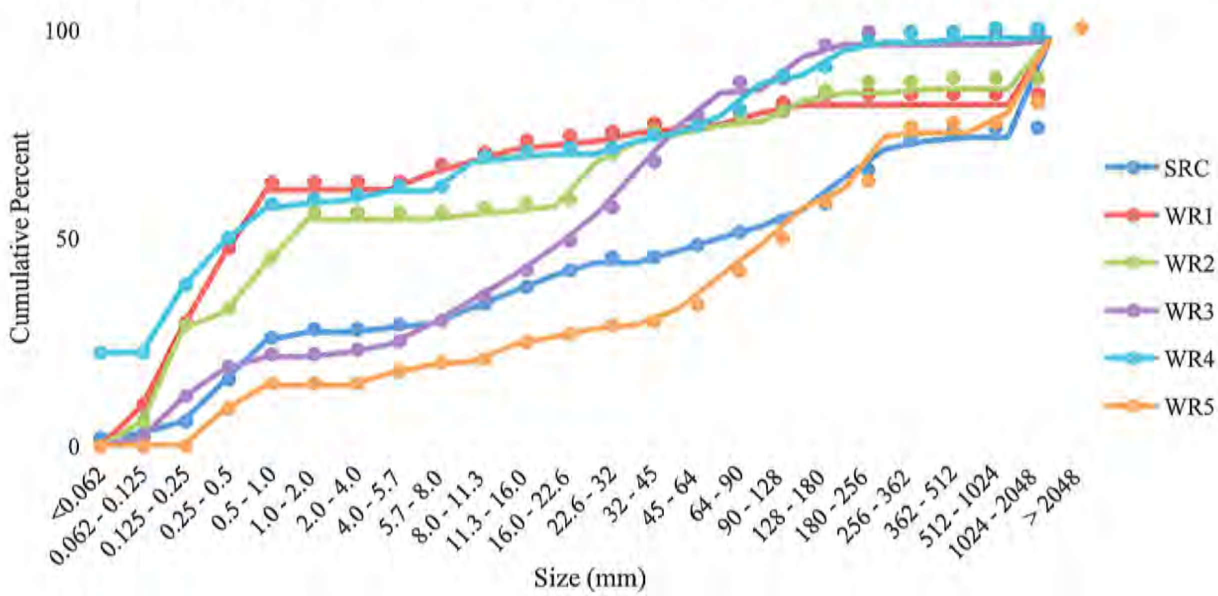


FIG. 17. Distribution of particle size in six sites in the Whitewater River watershed. D50 for each site where the line intersects 50 percent.

Median particle size (D50) relates to distance from the first site with a linear regression slope of 30.043 and an R^2 value of 0.784 (fig. 18). This means that median particle size generally increases by 30.043 mm with each river mile downstream. Median particle size is displayed as the median value of the D50 value because particle sizes are listed as a range of dimensions.

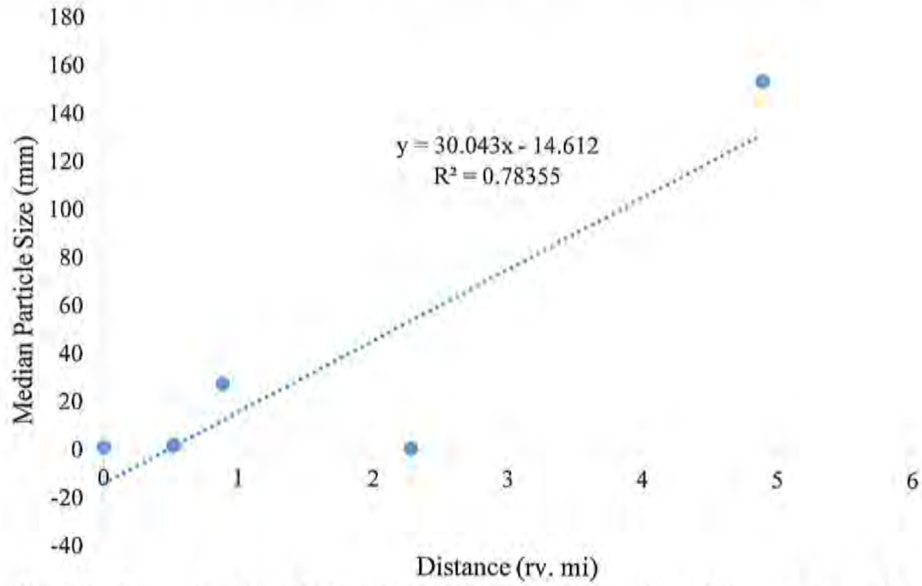


FIG. 18. The median particle size for WR1-WR5 as a function of distance from the first site (SRC is excluded because it occupies a different section of the stream). The R-squared value suggests moderate correlation, though the results did not significantly adhere to a trend line.

Macroinvertebrates

For each site, we calculated the number of individuals in the orders Ephemeroptera, Plecoptera, and Trichoptera (fig. 19). The most Trichoptera were found at WR3, while the fewest Plecoptera were found in WR1, WR2, and WR3 (fig. 19).

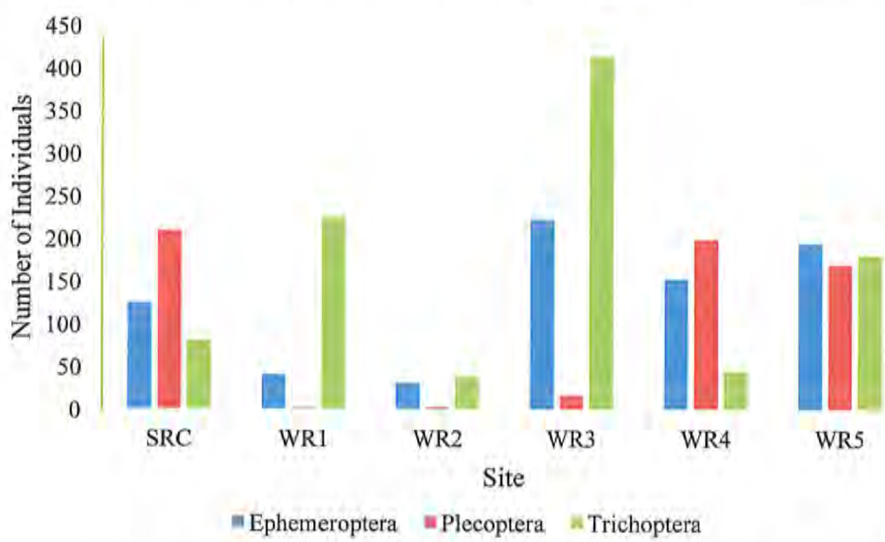


FIG. 19. Number of individuals in each order (Ephemeroptera, Plecoptera, and Trichoptera) by site.

Water quality increases with decreasing IBI values. WR4 has the lowest overall IBI value, while WR1, WR2, and WR3 have higher values (table 7). Taxonomic richness, the number of families within each order, was plotted against IBI values to determine if a correlation exists (fig. 20). A weak positive correlation was found between the two variables, yielding an R^2-value of 0.35.

TABLE 7. Comparisons of Biotic Index scores and taxonomic richness across each site for the three indicator orders, EPT.

Order	Ephemeroptera		Plecoptera		Trichoptera		Overall
Site	Index of Biological Integrity	Taxonomic Richness	Index of Biological Integrity	Taxonomic Richness	Index of Biological Integrity	Taxonomic Richness	Index of Biotic Integrity
SRC	1.144	5	1.005	3	0.661	5	2.877
WR1	0.614	3	0.007	2	3.03	7	3.760
WR2	1.722	3	0.042	3	1.958	5	3.681
WR3	1.404	6	0.037	4	2.339	8	3.800
WR4	1.431	6	0.7	6	0.436	5	1.632
WR5	1.271	7	0.531	5	1.165	8	2.991

FIG. 20. Correlation between taxonomic richness and Index of Biological Integrity for Ephemeroptera, Plecoptera, and Trichoptera at all sites.

A high proportion of scraper Ephemeroptera were found at WR1, WR2, WR3, and WR4 (fig. 21). Plecoptera tended to consist mostly of shredders (fig. 22), and Trichoptera tended to mostly include collectors (fig. 23).

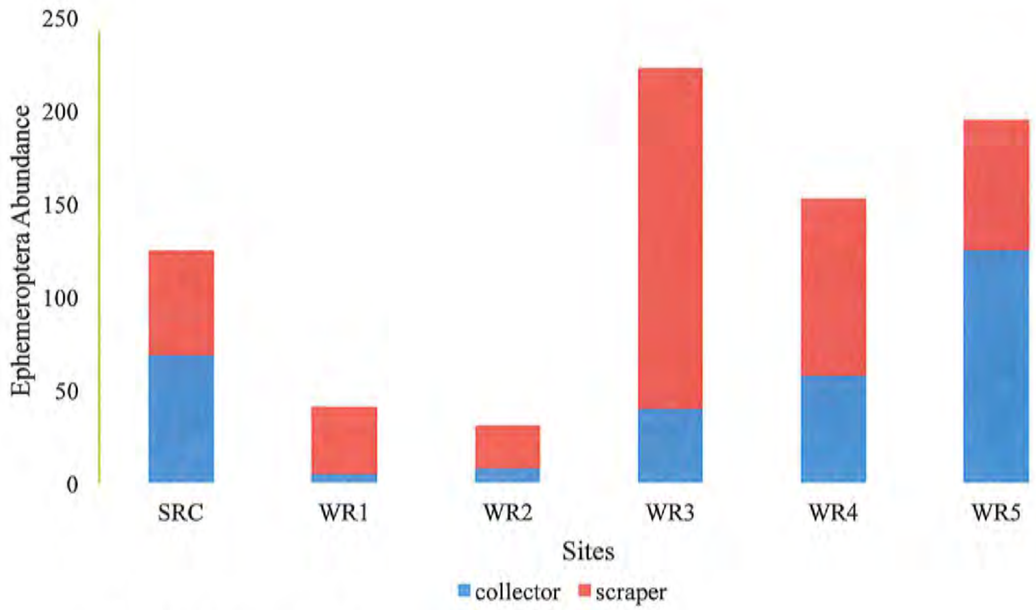


FIG. 21. Trophic levels of Ephemeroptera found at each site.

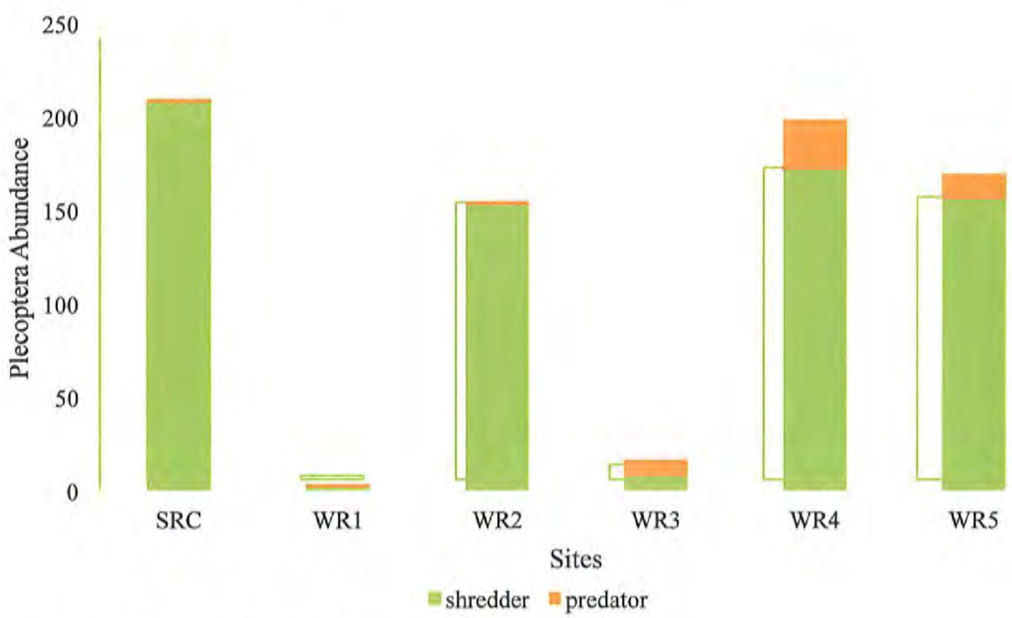


FIG. 22. Trophic levels of Plecoptera found at each site.

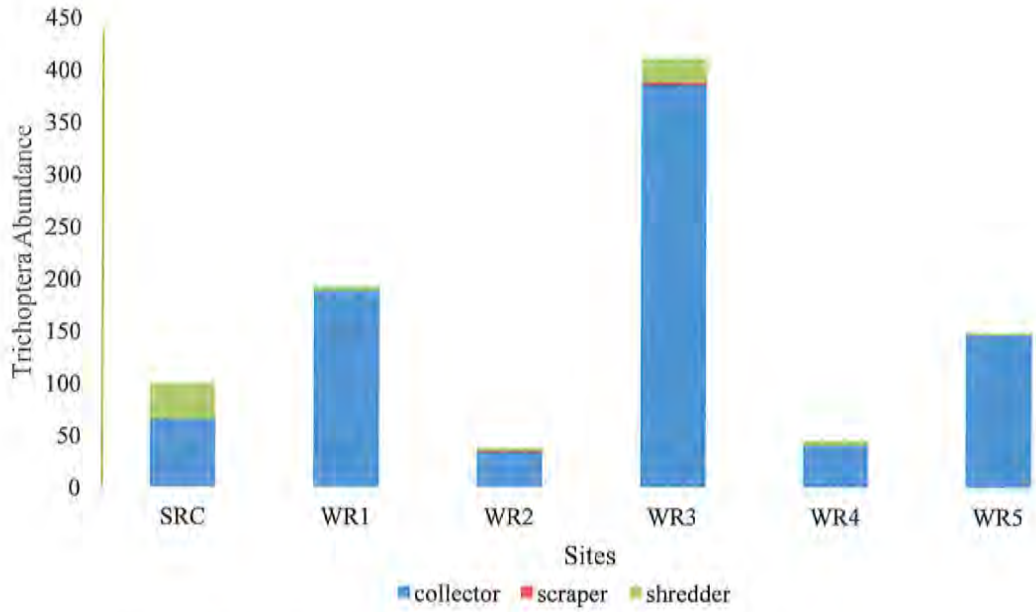


FIG. 23. Trophic levels of Trichoptera found at each site.

DISCUSSION

Habitat Assessment

The habitat assessment enabled us to analyze how natural or man-made disturbances influence stream characteristics along with documenting natural conditions. We documented the highest habitat assessment score at site WR5, which received a score of 91/100, and the lowest score at site WR1, which received a score of 58/100 (table 3). The components of the assessment that varied the most between the six sites were riffle characteristics, bottom substrate, and instream habitats (fig. 3). WR5 had wider, longer, and more frequent riffle habitats throughout the examined stretch compared to WR1.

Habitat assessment scores are greatly influenced by the characteristics of stream sediments (NCDENR 2012). Homogenous bottom substrates, regardless of sediment classification, lower total habitat assessment scores. A good mix of substrate provides more favorable habitats for colonization or cover for a variety of aquatic life. Bottom substrate scores and instream habitat scores fluctuated in unison with one another; when bottom substrate scores were lowest, instream habitat scores were also low (fig. 3). WR1 had bottom substrate that was homogeneously sand (D50 of 0.5-1.0 mm). Increased volumes of sand caused higher levels of embeddedness, which decreased the presence of instream habitats suitable for fish or macroinvertebrates. Embeddedness in WR5 was relatively low (20-40% embeddedness), so availability of instream habitats was greatest (available in >70% of the stream reach).

We found a slight negative correlation between riparian vegetative zone score and canopy cover (fig. 8). Breaks in the riparian zone resulted in a lower percentage of closed canopy because trees were absent. Analysis of temperature and pH yielded no significant conclusions that may indicate poor stream health, while channel width (table 1) increased as river mile increased.

Stream Visual Assessment Protocol (SVAP)

Cumulative SVAP score and distance from headwaters were inversely correlated for all six sites, with greater distance yielding lower scores. WR5, despite being the farthest site from the headwaters, did not follow this trend and received a relatively high score (fig. 4). This suggests that habitat suitability tended to decrease as distance from headwaters increased, with the exception of WR5. SRC and WR1 received the highest scores, 3.7/4, while WR4 received the lowest, 2.9/4. Between the six sites, bank condition, riffle embeddedness and streamside vegetation quantity appeared to be the most variable (fig. 4). The sites with the highest scores for bank condition tended to be largely free from man-made structures, while those with the lowest scores showed some signs of modification nearby. The lower scoring sites appeared to experience bank erosion as a result of runoff from these roads.

WR4, which scored the lowest both in regard to bank conditions and overall SVAP score, suffered from both residential development on one bank and proximity to a road on the other, thereby compromising bank stability. WR3 had the second lowest score due to the presence of a highway on the right bank. In contrast, the left bank was fully forested. The road reduced the right bank's vegetation quantity to less than ⅓ of the stream's width (score 1), compared to the left bank having a riparian zone that extends least 2 stream widths (score 4). There were also signs of erosion and bank failure on the right bank due to runoff from the road. Since the SVAP score is an average of left bank and right bank scores, the high scores of the left bank seemed to reduce the impacts of deterioration on the right bank. Habitat availability/cover score appeared to be the best predictor of overall SVAP score, seeing as how scores for this characteristic tended to decrease as distance from headwaters increased, with the exception of WR5. This suggests that habitat availability/cover condition is strongly influenced by the aggregate conditions of the other factors assessed in the SVAP.

The North Carolina Habitat Assessment and the Stream Visualization Assessment Protocol are both used to qualitatively describe stream characteristics. There are many categories in which they overlap, such as instream habitats, pool variety, bank stability, and light penetration. However, they differ in the ways in which they score characteristics. The scores themselves are subjective in nature, left to personal discretion in the field. Because of differences in observer interpretation, the scores for the NC Habitat Assessment and SVAP were not always consistent. A comparison between the two assessments (Figs. 5 and 6) yielded that there was no correlation between the scores of corresponding components of both assessments. The information collected on both forms is still valuable, however, when considering a site's suitability as macroinvertebrate habitat and stream health.

BEHI

As we sampled sites farther from the headwaters, we generally observed incrementally lower BEHI scores (fig. 9). This pattern indicated that erosion potential is highest near the headwaters at Silver Run Creek for this localized stretch of river. Most indices followed the same decreasing trend, excluding bank angle, which increased with distance from headwaters (table 5). Non-adjusted scores display a similar decreasing trend. Adjustment and surface protection scores contributed the most to the final scores, while bank angle contributed the least. Subsequent adjustments based on bank material are an important contribution to the final BEHI scores; we see that all sites are given an adjustment score of 10, for the presence of sandy substrate in the bank, except WR3 and WR4, which exhibited substrate types that did not significantly affect erosion potential. Stream banks at SRC, WR1, WR2, and WR5 all featured sandy substrate, which is unstable and highly erodible and alters BEHI classification negatively.

Silver Run Creek produced the highest BEHI score, indicating the most significant potential for erosion. BEHI score for this reach was calculated near the base of Silver Run Falls, a 30 foot waterfall that discharges into a large eddy pool. We believe that during high discharge events, dissipation of energy below the falls erodes the soft bank stratum at the outlet of the splash pool, driving up the BEHI score. The SRC site has maximum scores for surface protection and root density, displaying very poor, if any, vegetative protection and reinforcement on the stream bank. Additionally, SRC is a recreational site with high levels of foot traffic nearby, possibly amplifying erosion potential locally.

WR4 yielded the lowest BEHI score of all sites, despite the proximity of artificial structures (wide bridge, residence, patio adjacent to stream bank). This contradictory result is likely credited to a combination of factors, primarily the nature of the specific measurement site, which was taken on river right, opposite of the residence, where vegetation was abundant in a wide riparian zone. Additional elements include a markedly healthy root depth ratio, a small bank angle, and a bank substrate firm enough not to merit an upward adjustment in the score.

As we moved downstream from the headwaters, the overall trend of BEHI scores decreased, with the exception of WR5. While the total BEHI score for WR5 was elevated, it produced fairly low individual scores for root depth ratio, root density, and bank angle. The high score for WR5 is largely attributed to high bank height ratio, the upward adjustment for the presence of sand in the bank, and poor surface protection. This channel is wide with no substantial floodplain, facilitating a considerable amount of flood flow within the channel. The bank exhibited varying degrees of stability, with sand a predominant component at the measurement site. These conditions likely lead to accelerated levels of erosion at the location observed.

The integrity of a stream bank is highly variable because stream characteristics are transformed by weather elements, channel evolution and other external factors over time. There is minimal antecedent data available for Whitewater River to use as a reference for stream bank behavior in previous years, so it is difficult to ascertain the behavior of the bank. Additionally, these BEHI scores represent the erosion potential of narrow segments of stream and are not necessarily indicative or predictive of the overall integrity of the stream bank.

Pebble Count

Competence is defined as the ability of a stream to mobilize larger sediment class sizes. According to the River Continuum Concept proposed by Vannote et al. (1980), competency tends to decrease from headwater to mouth, while capacity, or the ability of a stream to carry a certain sediment load, increases from headwater to mouth. The pebble count distributions demonstrate that WR5 (fig. 16) and SRC (fig. 11) had larger sediments, and WR1 (fig. 12), WR2 (fig. 13) and WR4 (fig. 15) had smaller sediments. Since stream slope and velocity are interrelated, these results likely indicate that WR1, WR2, and WR4 exhibited less competence due to a lower slope although we did not conduct measurements of slope or velocity to support this. SRC and WR5 likely showed greater competency because of a steeper slope. Our study sites do not clearly display the trend in competency described by the River Continuum Concept because competency is higher in WR5 which is downstream and lower in WR1 which is further upstream. Due to the mountainous nature of the region of study, the slope of study sites was highly variable and impacted stream velocity, resulting in more variation in competency and sediment size.

Pebble count distributions and D50 values appear highly variable across the extent of the study with the lowest D50 value occurring at WR4 at 0.25-0.5 mm (fig. 17) and the highest at WR5 at 90-128 mm (fig. 17). The R^2-value of 0.784 for the median particle size versus distance plot (fig. 18) demonstrates that there is not a strong trend in particle size over distance. Again, this is likely due to a high variability in slope between our study sites.

Variety of sediment sizes within a reach increases microhabitat for fish and benthic macroinvertebrates, supporting greater local biodiversity (Vannote et al. 1980, Graça et al. 2004). Based on these criteria, sites such as WR3 with a more even distribution of sediment sizes appear to have greater habitat diversity within the stream. WR3 also had a more even distribution of habitat types consisting of 49% runs and glide, 20% riffles, and 31% pools (fig. 10). Nonetheless, it is important to consider other factors such as embeddedness and riparian zone width when assessing habitat diversity; these factors are included in the habitat assessment. Pebble counts and morphological characteristics can still be used to give an idea of habitat availability within the stream. WR4, for examples, had the smallest D50 values (fig. 17) and the most uneven distribution of habitat types with 32% runs and glides, 68% pools, and no riffles (fig. 10). These factors would contribute to the relatively low habitat score of 61 in WR4 (table 3).

Macroinvertebrates

We used EPT indices as a measure of overall stream health and, indirectly, of potential pollutants in the water, as a greater number of taxa in each order typically indicates cleaner water (NRCS 2012). Aquatic insects respond to various organic pollutants and display tolerance levels to short and long-term contaminations of water quality (NRCS 2012). The highest number of taxa was found at WR5, with 19 different families between the three orders. Plecoptera are the most sensitive to pollutants and dissolved oxygen levels (Buchanan et al. 2011). Sites with the lowest numbers of individuals in Plecoptera included WR1, WR2, and WR3. Existing literature suggests that these low numbers could indicate the presence of pollutants or low levels of dissolved oxygen (Wallace et al. 1996).

Though we did not sample water for dissolved oxygen levels or the presence of chemical pollutants, other survey methods sought to qualify these factors. The Stream Visual Assessment Protocol accounts for physical and chemical pollutants, the latter of which was only evident at WR2 and WR4. Riffles are expected to have higher concentrations of dissolved oxygen than other stream habitats (Connolly et al. 2004), so it is appropriate to use riffle presence and frequency as a qualitative indicator of dissolved oxygen.

According to the Habitat Assessment form, riffles occurred infrequently at sites WR1 and WR2, both yielding scores of 3. There were no natural riffles recorded on the Habitat Assessment form at WR4, yet high levels of macroinvertebrates within the order Plecoptera were found. In WR4, we observed that the stream bed had a large amount of leaf litter. Order Plecoptera has a high number of shredder taxa, which feed on leaf litter, which could explain the high number of Plecoptera despite the lack of riffles (NCSU 2008). Because our study sites were located in low-order streams, we would expect shredders to be relatively high in number due to the high input of leaf litter and organic matter from the forested catchment (Wallace and Webster 1996).

We also examined trophic diversity for families within the EPT orders. Ratios of trophic levels indicated the dominant ecosystem function of macroinvertebrate communities within a reach (Merritt et al. 2008). The majority of those individuals belonging to the order Trichoptera were collectors. Collectors feed in part on the broken down organic matter produced by shredders. Therefore, the high numbers of shredders in the order Plecoptera may provide the resources needed for collectors to proliferate.

An apparent lack of correlation between EPT presence and anticipated stream quality suggests that there were either discrepancies in sampling and observing riffles, that other characteristics of riffles apart from mere presence within a reach are more important for macroinvertebrates, or that chemical pollutants are having a stronger impact on EPT numbers than is reflected in other elements of the study. Since no strong relationship was evident between EPT Indices and taxa richness, it is difficult to draw conclusions. Further research testing for dissolved oxygen levels and chemical pollutants will reveal if outside inputs unrepresented in this study are having an impact on macroinvertebrate diversity within the Whitewater River system.

Sources of Error

Although we attempted to keep specimens from each site separate in the lab, we inadvertently mixed macroinvertebrate samples from WR1 and WR2. As a result, we are not able to accurately draw conclusions from these samples. Macroinvertebrate characteristics are difficult to discern by the untrained eye, so there is a possibility that we misidentified some of the specimens. Additionally, some delicate specimens were destroyed or damaged during the collection process, and some sampled from the earliest sites decomposed due to lengthy time in ethanol solution diluted by stream water. The specimens that were identified may not have yielded truly accurate results. Since we identified the insects to family level IBI, our data does not describe the exact tolerance level for each organism we found; their tolerance levels may have been different if they were further divided into genus or species.

In order to ensure that all members could practice each method of data collection, different people were assigned to collect different sets of data at each site. This introduced inconsistencies in the evaluation of qualitative data, as each researcher has slightly different standards. We did not initially collect pH and temperature for SRC when we gathered the rest of the data and subsequently took those measurements the following week. As we surveyed stream locations on different dates over the course of two months, the sites were not subject to the same environmental and seasonal conditions such as temperature, rainfall, and canopy cover at the times of data collections.

CONCLUSIONS

Our survey of six sites in the Whitewater River watershed revealed evidence that there are structural threats that may disturb ecosystem functioning in this mountain stream. While few overall trends in stream health were evident, we recorded notable instances of low habitat diversity and high risk of erosion.

NC Habitat Assessment scores were lowest at sites WR1 and WR2, where fine sediment deposition and low riffle presence resulted in sub-optimal conditions for stream organisms. BEHI values also identified that the risk of erosion may be increased during high-rainfall events at the headwaters. In contrast to the pattern expressed in Habitat Assessment scores, SVAP scores tended to decrease as distance from the headwaters increased, indicating that these assessment methods reveal separate qualities of riverine health and are not comparable. SVAP scores identified WR4 as an area of particularly low habitat quality, as it lacked riffles all together. Riparian development and modification in the form of rip-rap and a low amount of riparian vegetation as revealed by this protocol pose significant stream quality risks at WR4. However, low abundance of macroinvertebrates within the order Plecoptera may suggest that some sites had poor dissolved oxygen levels and moderate levels of pollution. High IBI values at most sites supports that anthropogenic disturbances may be impacting stream health. A lack of correlation between the results of different stream health assessments failed to identify the most heavily impacted sites, possibly suggesting that all stream sites were negatively impacted by the encompassing watershed in different ways.

Assessment scores may have been depressed by low water levels due to an extended drought across Western North Carolina at the time of the study. Further data collection in this area during a time of more typical rainfall would be useful, for the results of this study do not reflect typical fluvial conditions for the watershed.

Acknowledgements

We would like to thank Steve Foster for his mentorship and guidance throughout this project, his readiness to share his wealth of knowledge in fluvial ecology and his contagious enthusiasm for the subject, his editorial assistance, and his genuine encouragement. We would also like to thank Alyssa Fuller for her help in the field and laboratory, for teaching us to identify macroinvertebrates, and for laughing at our terrible jokes. We are grateful to Drs. James Costa and Sarah Workman and the Highlands Biological Station at large for hosting, facilitating and funding our research project and semester.

Literature Cited

Allan, J. D., and M. M. Castillo. 2007. An introduction to fluvial ecosystems. Stream Ecology: Structure and Function of Running Waters. Dordrecht, The Netherlands: Springer.

Brigham, A. R., W. R. Brigham, and A. Gnilka. 1982. Aquatic insects and oligochaetes of North and South Carolina. Mahomet, Illinois (USA): Midwest Aquatic Enterprises.

Buchanan, C., K. Foreman, J. Johnson, and A. Griggs. 2011. Development of a basin-wide benthic index of biotic integrity for non-tidal streams and wadeable rivers in the Chesapeake Bay watershed: final report to the Chesapeake Bay Program Non-Tidal Water Quality Workgroup. University of Maryland Center for Environmental Science.

Connolly, N. M., M. R. Crossland, and R. G. Pearson. 2004. Effect of low dissolved oxygen on survival, emergence, and drift of tropical stream macroinvertebrates. Journal of the North American Benthological Society, 23:251-270.

Death, R. G., and M. J. Winterbourn. 1995. Diversity patterns in stream invertebrate communities: the influence of habitat stability. Ecology 76:1446-1460.

Environmental Systems Research Institute (ESRI). 2015. ArcGIS Desktop: Build 5524 (10.4). Redlands, California, USA.

Graça, M. A. S., P. Pinto, R. Cortes, N. Coimbra, S. Oliveira, M. Morais, M. J. Carvalho, and J. Malo. 2004. Factors affecting macroinvertebrate richness and diversity in Portuguese streams: a two-scale analysis. International Review of Hydrobiology. 89:151-164.

Hilsenhoff, W. L. 1977. Use of arthropods to evaluate water quality of streams. Madison, WI: Department of Natural Resources. Technical Bulletin No. 100.

Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. Journal of the North American Benthological Society 7:65-68.

Land Trust for the Little Tennessee (LTLT). 2014. Grade your stream program planning guide. Athens, Georgia: Crawford, T. G., C. E. Mertzlufft, J. Bloodgood, S. Ortiz, E. Guinessey, C. Brownson, H. Burnett, J. Chappell, J. Howard, L. Kosen, N. Heynen, and C. Pringle.

Merritt, R.W., K. W. Cummins, and M.B. Berg. 2008. An introduction to the aquatic insects of North America. 4th ed. Dubuque, Iowa (USA): Kendall/Hunt Publishing Company.

Natural Resources Conservation Service (NRCS). 1990. Climate Narrative for Jackson County, North Carolina. www.wcc.nrcs.usda.gov/legacy/ftp/support/climate/soil-nar/nc/jackson.doc

Natural Resources Conservation Service (NRCS): Watershed Science Institute. 2012. The EPT Index. www.wcc.nrcs.usda.gov/ftpref/wntsc/strmRest/wshedCondition/EPTIndex.pdf

North Carolina Department of Environmental Quality (NCDEQ). 2008. Savannah River Basin Restoration Priorities 2008. North Carolina Ecosystem Enhancement Program. https://ncdenr.s3.amazonaws.com/s3fs-public/PublicFolder/Work%20With/Watershed%20Planners/Savannah_RBRP_hcb_22dec08.pdf

North Carolina State University (NCSU). 2008. Biotic index and feeding type calculations. NCSU Stream Restoration Program. https://www.bae.ncsu.edu/programs/extension/wqg/srp/taxonomy/biotic_index.pdf

Newton, B., C. Pringle, and R. Bjorkland. 1998. Stream Visual Assessment Protocol. National Water and Climate Center Technical Note 99–1. Washington, DC (USA): Natural Resources Conservation Service, USDA.

North Carolina Department of Environment and Natural Resources (NCDENR): Division of Water Quality. 2012. Standard Operating Procedures for Benthic Macroinvertebrates. NC Department of Environment and Natural Resources. Environmental Sciences Section, Biological Assessment Unit. Raleigh, North Carolina (USA).

Rathbun, J. 2008. Standard Operating Procedure, assessing bank erosion potential using Rosgen's Bank Erosion Hazard Index (BEHI). Michigan Department of Environmental Quality, Water Bureau, Nonpoint Source Unit.

Resh, V. H., A. V. Brown, A. P. Covich, M. E. Gurtz, H. W. Li, G. W. Minshall, S. R. Reice, A. L. Sheldon, J. B. Wallace, and R. C. Wissmar. 1988. The role of disturbance in stream ecology. Journal of the North American Benthological Society 7:433-455.

Rosgen, D. L. 2001. A practical method of computing streambank erosion rate. Proceedings of the 7th Federal Interagency Sedimentation Conference, Reno, Nevada (USA). 2:9-15.

Upper Cullasaja Watershed Association (UCWA). 2004. Upper Cullasaja Watershed Strategy and Action Plan. 1-44.

United States Department of Agriculture (USDA), Natural Resources Conservation Service. 2009. Stream Visual Assessment Protocol Version 2. Prepared by Kathryn Boyer et al. 190–VI–NBH.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. Canadian journal of fisheries and aquatic sciences 37: 130-137.

Wallace, J. B., J. W. Grubaugh, and M. R. Whiles. 1996. Biotic indices and stream ecosystem processes: results from an experimental study. Ecological Applications 6: 140–151. Web.

Wallace, J. B. and J. R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. Annual Review of Entomology 41:115 - 139.

WeatherDB. 2016. Cashiers, North Carolina Average Rainfall. 7 Dec 2016. Web.

Wolman, M. G. 1954. A method of sampling coarse river-bed material. EOS, Transactions American Geophysical Union 35:951-956.

Wolman, M. G. 1967. A cycle of sedimentation and erosion in urban river channels. Geografiska Annaler 49:385-395.