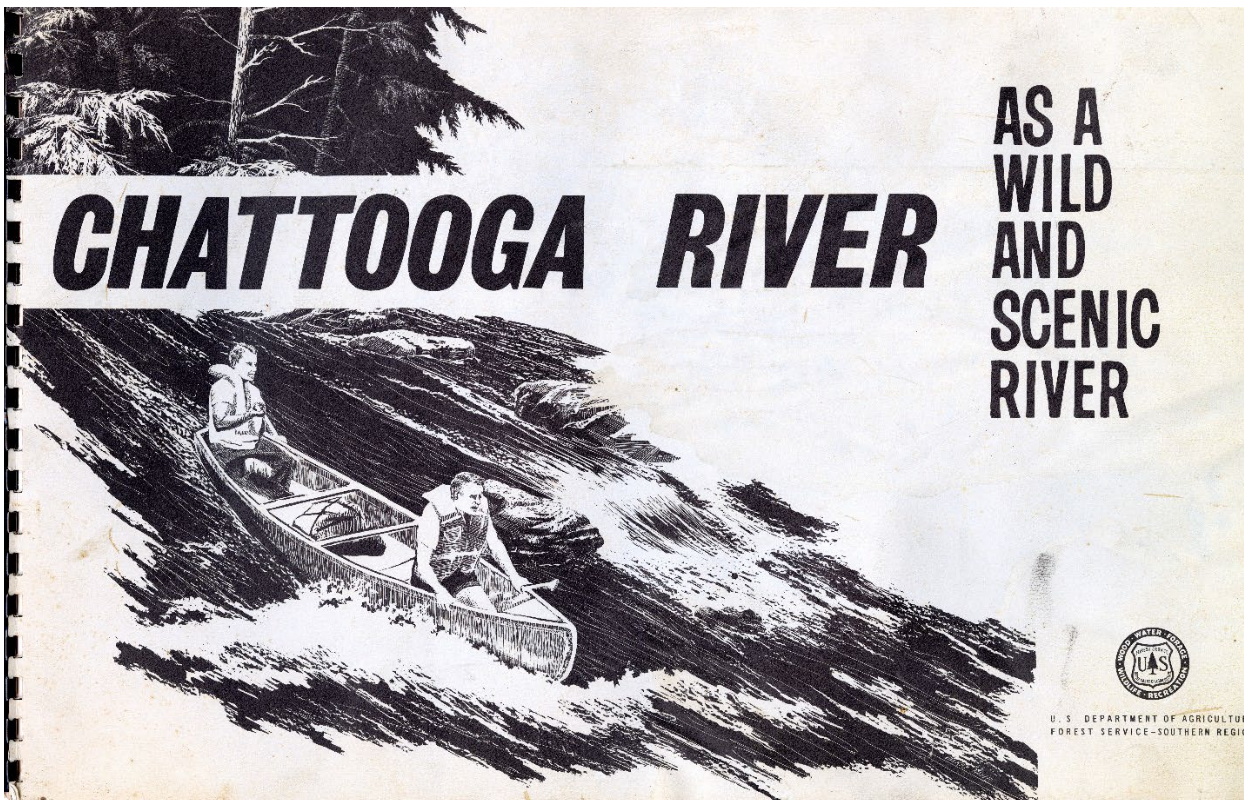
Institute for the Environment
Highlands Field Site
2021 Internship Research Reports



Highlands Biological Station

Highlands, North Carolina

The 2021 IE class floating down the West Fork of the Chattooga River after successfully navigating the Class II rapid Big Slide. The iron bridge is part of the Chattooga River Trail.

COVER IMAGES

Front cover: *The cover page of the 1971 US Forest Service summary report recommending the designation of the Chattooga River as a National Wild and Scenic River.*

Back cover: *Image of Whiteside Mountain from the Chattooga River from page 13 of the 1971 US Forest Service summary report recommending the designation of the Chattooga River as a National Wild and Scenic River. This photo was taken near the Sliding Rock microplastics sample site.*

INTRODUCTION

The term "Anthropocene" was popularized in 2000 by atmospheric scientist Paul J. Crutzen and refers to the proposed new geologic epoch signified by the enormous impact caused by humans to land, air, and water. For the 2021 Fall Semester, students enrolled in the Institute for the Environment Highlands Field Site program explored the theme of the Anthropocene through the lens of southern Appalachian ecology and culture. They scrambled down to the mouth of the most polluted tributary of the mighty Chattooga River, Stekoa Creek, and sampled microplastics in the headwaters of this same National Wild and Scenic River. They rode bicycles around Cades Cove and learned about the Park Service's struggles to balance preservation with tourism, and they slogged up Clingman's Dome (known as *Kuwahi* to the Cherokee, a sacred place where the Great White Bear holds Council) during Tropical Storm Fred, the same storm that dumped a massive deluge of rain in the headwaters of the nearby Pigeon River (named for the Passenger Pigeon, a bird that numbered in the billions but in the lifespan of one human and because of humans, is now extinct), causing record flooding that damaged or destroyed over 500 homes and killed 6 people.

Climate change, pollution, biodiversity loss, invasive exotics, habitat fragmentation – it is easy to become overwhelmed and distraught in this new world we've created. But the students also learned that there is still a great deal of beauty in the world and that positive changes are happening. Students kayaked down the West Fork of the Chattooga River, a river that became one of the first rivers designated as a National Wild and Scenic River in 1974 *despite* the pollution from Stekoa Creek. It is still one of the longest free-flowing rivers in the Southeast and still has crystal clear water that reveals rounded cobble and speckled trout. And despite the 12 million-plus visitors that visit Great Smoky Mountains National Park annually, you can still take a 10-mile hike through magnificent old-growth and not pass another human being, though you will certainly pass by slithering salamanders, glistening clubmoss, and some amazing mushrooms. And maybe even a wandering black bear. The students learned that sacred Cherokee mounds, such as Kituwah and Cowee, once simply plowed over, are now being preserved.

Perhaps most importantly, the students were reminded that learning and life are best experienced not through an iPhone or via Zoom, but through immersive experiences in the outdoors. It is one thing to read about a Northern Saw-whet Owl, but it is quite another to gently hold one in your hands, feeling the lightness of the feathered body and gazing into the large golden eyes that can see in darkness. And students may have heard about old-growth forests but pulling a DBH tape around an Eastern Hemlock that is over 150 cm (5 feet) in diameter and later learning, through coring the tree, that the tree dates to 1761, makes that experience real and relevant. Through these experiences, the students came away with a better understanding of not only how to conduct quality research, but also gained a deeper connection to this good Earth, especially the Highlands Plateau and the southern Blue Ridge Mountains.

- Dr. Rada Petric, Dr. Jim Costa, and Jason Love, IE Highlands Field Site

ACKNOWLEDGMENTS

On behalf of the IE-HFS class of 2021, we would like to thank the individuals who took time from their busy schedules to provide meaningful experiences for the students and provided support and mentorship to the students. We thank internship mentors Mark Hopey (Blue Ridge Bird Observatory), Jason Meador (Mainspring Conservation Trust), Dr. Todd Pierson (Kennesaw State University), Lori Williams (North Carolina Wildlife Resources Commission), Joel Scott (US Forest Service, Coweeta Hydrologic Laboratory), and Dr. Chris Oishi (US Forest Service, Coweeta Hydrologic Laboratory) for their guidance and counsel throughout the independent research projects. Dr. Jerry Miller (Western Carolina University) drove to Highlands nearly every Friday to share his knowledge on measuring stream discharge, plotting hysteresis curves, and thinking critically about the results for the students' Capstone Project.

We thank Lilly Knoepp (Blue Ridge Public Radio), Brent Martin (Alarka Expeditions/Blue Ridge Bartram Trail Conservancy), and John Lane (retired English professor, Wofford College) for bringing out the creative side of students in the *Communicating Science in the Anthropocene* course. Dr. Susan Cohen, Troy Walton, and Brian Naess (UNC Institute for the Environment) helped lead the *Remote Sensing and Landscape Analysis* course; thanks to Dr. Gary Wein (Highlands-Cashiers Land Trust) for sharing his GIS expertise with the students as part of this course. The students were fortunate to hear from a range of guests this year as part of their *Southern Appalachian Environmental & Cultural History* and *Human Impacts in the Southern Appalachians* courses: Jeremy Lloyd (Great Smoky Mountains Institute at Tremont), Dr. Tom Belt (Cherokee Nation citizen and retired Cherokee language professor, Western Carolina University), Roy McClure (Macon County resident and avid bear hunter), Paul Super (NPS Appalachian Highlands Science Learning Center), Lamar Marshall (Southeast Heritage Associates), Mary Polanski (Women's History Trail of Franklin), Dr. Ben Laseter (Mainspring Conservation Trust), Kyle Pursel (Highlands-Cashiers Land Trust), Chris Widga (Gray Fossil Site), Sadie Jones (Digital Marketing Strategist), and George and Karen Powell (Highlands Aerial Park). The Powells also generously gave the IE program a major discount on the students' zip-lining tour. Bob Hathcock and Angela Faye Martin served as two of our guides down the West Fork of the Chattooga River, which was an *amazing* trip; thanks to HBF President Jennie Stowers for providing her personal kayaks to make this trip possible for the students.

We also appreciate the HBS and HBF staff who contributed their time and expertise to the program this year: David Ford, Katie Cooke, Rachel Martin, Patrick Brannon, Charlotte Muir, Paige Engelbrektsson, and Winter Gary. Special shout-out to Mike McMahan for helping students in the workshop with their various projects. A big thank you to Holly Theobald for serving as the RA in the Valentine House and help to ensure the house was kept clean and orderly. And finally, a very special thanks to the Highlands Biological Foundation for supporting this important program by providing funding for the Highlands Field Site Director position and pitching in to ensure the students felt welcome and had the best experience possible, from paying for kayaks to hosting dinner for the students.

- Dr. Rada Petric, Dr. Jim Costa, and Jason Love, IE Highlands Field Site, December 2021

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Morning splendor at Purchase Knob, Great Smoky Mountains National Park.

USING DENDROCHRONOLOGY TO STUDY GROWTH PATTERNS OF EASTERN (*Tsuga canadensis)* AND CAROLINA (*Tsuga caroliniana)* HEMLOCKS IN WESTERN NORTH CAROLINA

SCOUT ALLEN AND PETER WINIKER

Abstract. Eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) differ in preferred habitat, genetic makeup, appearance, and range; however, they share some similarities as well, the most salient of which is infestation by the hemlock woolly adelgid (*Adelges tsugae*). This invasive aphid has decimated populations of eastern and Carolina hemlock across the United States in the past few decades, leaving them both at high risk of becoming functionally extinct. In this study, we focused on two stands of hemlocks: one known old-growth stand of eastern hemlocks in Highlands, North Carolina; and a suspected old-growth stand of Carolina hemlock at Whiteside Mountain in adjacent Jackson County. For this study, we cored hemlocks from both stands and utilized dendrochronology procedures, standards, and software to analyze patterns in the tree rings. We created a chronology from the tree-ring growth patterns and compared it to regional rainfall records to establish a correlation between rainfall and growth for both species. Only the Carolina hemlocks experienced a positive correlation between increased rainfall and growth productivity. We also compared the stands' relative responses to discrepancies in mean annual temperature to examine how weather patterns affect the two species differently. Overall, the stand of Carolina hemlocks is more vulnerable to variations in rainfall and temperature than eastern hemlocks.

Key words: Carolina hemlock, dendrochronology, eastern hemlock, southern Appalachian Mountains, Tsuga canadensis*,* Tsuga caroliniana

INTRODUCTION

The eastern hemlock (*Tsuga canadensis* (L.) Carr.) is a coniferous evergreen that thrives on moist, high-elevation slopes, generally between 600-1000 m. Eastern hemlocks are found from southern Quebec to Alabama and are found in abundance in the southern Appalachian Mountains. Eastern hemlocks are a mesophytic species that prefer north-facing slopes of sheltered ridges, as well as rich coves and valley flats (Rentch et al. 2000). Among North American trees, the eastern hemlock is one of the longest-lived, with some individuals living for over 800 years, reaching a diameter of two meters, and growing to 40 meters in height (Hart and Shankman 2005). Eastern hemlock is a foundation species, meaning that its presence within an ecosystem is reflected in the species residing within the ecosystem, as well as in the amount of available light and soil moisture available for nearby organisms (Ellison et al. 2005). Eastern hemlocks often reside within old-growth forests, which foster unique ecosystems of their own. Old-growth stands or stands that are relatively undisturbed by humans and which have numerous old trees (Hunter 1989), experience competition for resources such as light, nutrients, and water, at a higher intensity than young- or second-growth stands (Bigelow et al. 2019). This increased competition for resources can create a larger stressor for trees in old-growth stands than even that of climate change (Bigelow et al. 2019). However, eastern hemlock is a shade-tolerant species, and usually fares well in competitive environments.

Carolina hemlock (*Tsuga caroliniana* Engelm.) is endemic to the southern Appalachian Mountains and ranges from northeastern Georgia to southwestern Virginia (Rentch et al. 2000). Unlike the eastern hemlock, the Carolina hemlock is typically found on dry, exposed, and nutrient poor slopes, meaning they are often found on balds and rocky mountain tops (Rentch et al. 2000). Although eastern hemlocks are often considered northern species, their range extends further south than that of the Carolina hemlock (Rentch et al. 2000). Though the two species share a morphological resemblance, the Carolina hemlock is more genetically similar to the hemlocks of the Pacific northwest and Asia than the eastern hemlock (Rentch et al. 2000, Havill et al. 2008). Carolina hemlocks are often found in proximity to red maple (*Acer rubrum* L.), chestnut oak (*Quercus montana* Willd.), serviceberry (*Amelanchier* spp.), Rhododendron (*Rhododendron* spp.), as well as sometimes eastern hemlock, although they generally prefer different habitats (Rentch et al. 2000).

Populations of both Carolina and eastern hemlocks have been decimated in the last few decades by an invasive aphid called the hemlock woolly adelgid (HWA: *Adelges tsugae* Annad). The HWA poses a threat to the survival of both species; both the eastern and the Carolina hemlock are expected to be functionally extinct throughout much of their ranges by 2050 (Austin et al. 2016). Native to Japan, the predatory HWA has found a suitable ecological niche in populations of eastern hemlock ranging from the forests of New England and the Mid-Atlantic, and more recently, the Southern Appalachians (Krapfl et al. 2011). Models of the HWA migration in the southern United States, which is the southernmost point of the hemlock's native range, predict a mean HWA migration rate of 12.6 km/year since 1990 and show HWA migration speed decreasing in colder temperatures (Evans and Gregoire 2007).

The increase in hemlock mortality due to the HWA causes lasting changes in local ecosystems and species biodiversity. Hemlock is a foundation species and is one of the only trees able to propagate under a thick rhododendron canopy. Rhododendron, particularly rosebay rhododendron (*Rhododendron maximum* L.), can inhibit seed germination and seedling growth as it moves in to dominate the understory and spaces left behind by deceased hemlocks (Roberts et al. 2009). This decrease in biodiversity and near-monoculture dominance of rhododendron can have lasting effects: making areas more prone to erosion and landslides due to a shallower and weaker root system; increased flooding as hemlock trees are not present to absorb excess rainfall; exacerbated effects of drought in the summer without as much canopy cover; and an overall increase in stream temperature, which could have negative impacts on aquatic species (Webster et al. 2012).

According to Speer (2010), dendrochronology "examines events through time that are recorded in the tree-ring structure or can be dated by trees." Trees can be used as long-term bioindicators, as they can keep a record of past temperature, rainfall, fire, and natural disasters (Speer 2010). The oldest eastern hemlock ever recorded was cut down in 1893 in Luzerne County, Pennsylvania; the rings were "carefully counted," and revealed that the tree was 969 years old (USDA 1943). Multiple studies have been conducted using dendrochronology to study eastern and Carolina hemlocks to answer various questions. DeMaio (2008) used dendrochronological analysis on eastern hemlock stands in Maine and determined that decreased growth in their master chronology occurred during or directly following drought events. Another experiment used a dendrochronological study of eastern hemlocks to examine the relationship between crown condition and changes in radial growth associated with infestation by the HWA and found a predictable pattern of hemlock vulnerability at light and moderate HWA infestations (Rentch et al. 2009). Austin et al. (2016) used dendrochronology to determine dates, growth rates, and radial growth patterns of a Carolina hemlock stand, and used these data to conclude that Carolina hemlock populations in the southern Appalachians are projected to increase in stress and vulnerability as climate change progresses, and populations infested with the HWA are especially vulnerable.

For this study, we utilized dendrochronology practices to extract information about two old-growth stands of hemlock trees; one comprised entirely of eastern hemlocks, and the other comprised mainly of Carolina hemlocks, with only a few eastern hemlocks present. We cored trees from known and suspected old-growth eastern and Carolina hemlock stands, with the goal of gathering data about remaining Southern Appalachian hemlock stands. As the HWA poses an increasingly impending threat to both eastern and Carolina hemlock, it is imperative to conduct studies on these communities before they succumb to these pests. Large and old-growth trees have a large impact on the carbon economy and health of the ecosystems around them (Stephenson et al. 2014); we can use knowledge about hemlocks to further our understanding of the forest as it is now, as well as how it could be changed if hemlocks become functionally extinct. In the southern Appalachian region, mean annual precipitation and intra-annual distribution has changed, leading to more severe droughts (Ford et al. 2011). One of our objectives is to use our data to help determine the age of our two stands, and whether they are truly old-growth; if they are, their conservation status could be heightened, possibly resulting in a greater effort expended on their preservation. We expect to find a correlation between increased rainfall and growth productivity for both eastern and Carolina hemlocks. We will also compare data from our respective eastern and Carolina hemlock cores to examine differences in the species' ages, as well as their stands' sensitivity to changes in rainfall. We expect to see higher sensitivity to changes in precipitation in Carolina hemlocks, as they grow on shallow soils that dry out quickly. We also hope to establish a reference point for future hemlock research in the Southern Appalachians.

MATERIALS & METHODS

Site Description

We selected sites of known old-growth eastern hemlocks at the Highlands Biological Station (HBS) Coker Trail, Macon County, and the suspected old-growth stand of isolated Carolina hemlocks at Devil's Courthouse on Whiteside Mountain, Jackson County. The Coker site on the HBS property is an Appalachian Acidic Cove Forest with hardwood and conifer stands dominated by eastern hemlock. The undergrowth of this Acidic Cove Forest is primarily composed of rosebay rhododendron and dog hobble (*Leucothoe fontanesiana* (Steud.) Sleumer), with very few herbaceous plants (Costa 2012). Located at an elevation of approximately 1,190 m, this forest grows in a steep ravine, has characteristically low soil pH, and has a small second-order stream running through it. The Coker site has been in the possession of HBS since 1962, and the forest has been left undisturbed as a research area. The hemlock woolly adelgid was first noted in the area in 2003, and most large hemlocks in the Coker stand were treated in 2004 and approximately every 4-6 years thereafter (Love, pers. comm.).

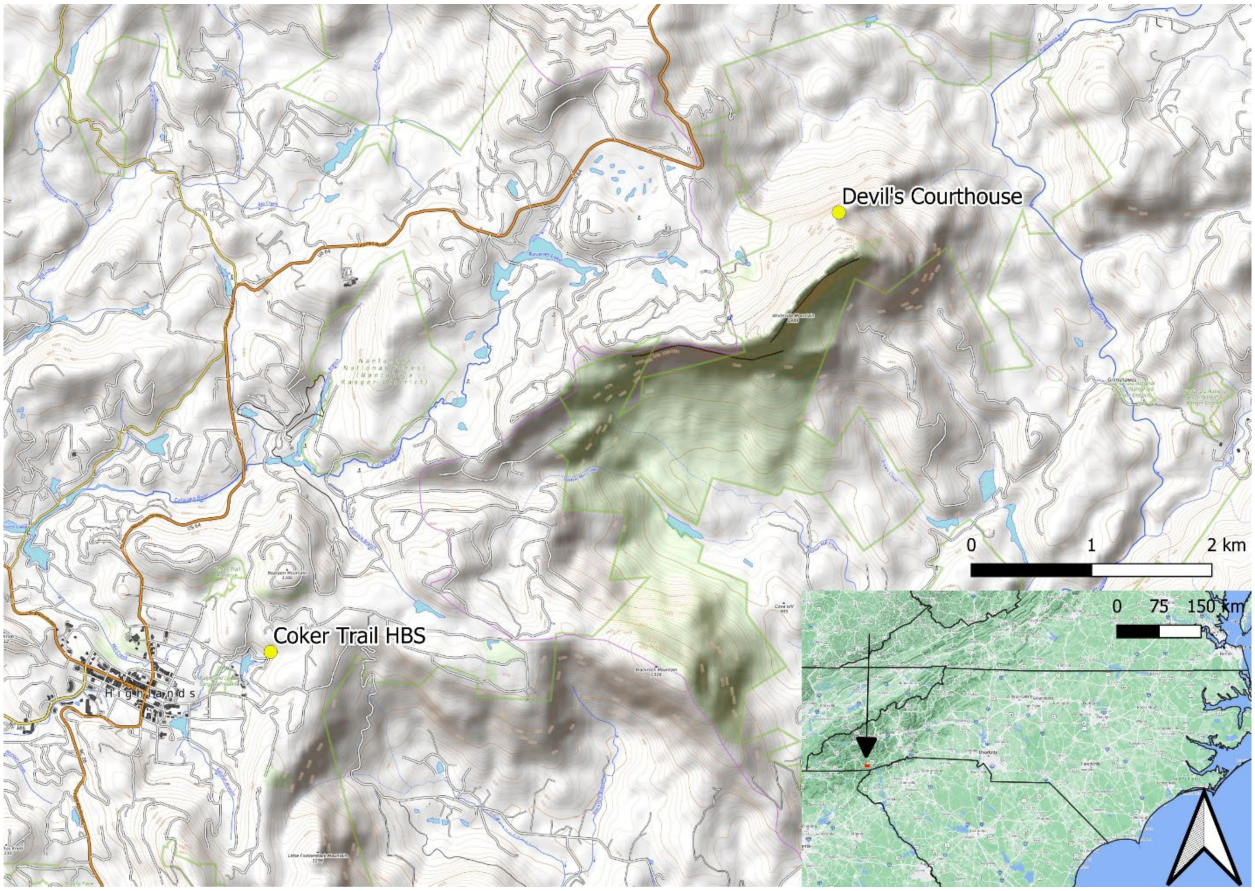


FIG. 1. Main map displays Coker and Devil's Courthouse sites in Macon County NC. Inset map displays site location in the broader context of the state of North Carolina.

Devil's Courthouse on Whiteside Mountain is a mixed stand of eastern and Carolina hemlocks, with pitch pine (*Pinus rigida* P. Mill.) also present on the same sparse rocky outcropping at an elevation of approximately 1,430 m. Heaths such as mountain laurel (*Kalmia latiofolia* L.) and rosebay rhododendron dominate the understory. The soil layer is thin: a spongy mass of decomposed organic matter on bedrock. The hemlocks at Devil's Courthouse are all untreated for HWA, and surrounding hemlocks along the side of the mountain showed signs of HWA infestation. Whiteside Mountain was pre-colonially a part of the Cherokee Nation but was divvied up to settlers in the mid-1800s. The mountain subsequently came under the ownership of various private families and enterprises and was logged in 1943 before the U.S. Forest Service acquired it in 1970 (Spencer et al. 2017). It is possible that the sparse outcropping of hemlocks on Devil's Courthouse was left untouched by this logging and remains old-growth.

Due to the close temporal proximity of both hemlock sites, we used the same dataset for precipitation and temperature to find annual averages and run regression analyses against our average annual tree growth data. Based on our NOAA climate dataset, our study region experiences an average overall temperature of 17.06° C annually, with an average daily precipitation of 0.58 cm (NOAA 2021).

Dendrochronological Sampling

We began our coring process by recording the coordinates of each tree using a Garmin handheld GPS. We also measured diameter at breast-height (DBH) of each cored tree. We tagged unmarked trees using aluminum nails and labels and recorded their numbers before sampling. If the tree had any scarring or portions of distress, we chose a core placement that would avoid those sections, as they could exclude some outer rings of the tree. We utilized 5 mm increment bores of various lengths; before we began removing samples, we greased the shaft of the borer with beeswax to ensure the easy insertion and removal of the borer from the tree, and then began drilling a hole into the tree. We extracted two core samples from each tree at 90 degrees from each other; at Devil's Courthouse, the Carolina hemlocks had not yet been treated for the HWA, and due to this and the general rarity of Carolina hemlock trees, the US Forest Service only allowed us to take one core each from ten trees. We cored each tree as close to ground height as possible while allowing enough room to turn the borer handle. We extracted our cores with the borer perpendicular to the ground. Each time we bored at least halfway through the trunk, with the goal being to pierce the "pith," which is the soft, innermost ring of the tree. The pith of a tree can be skewed to one side for a variety of reasons, so it is common to miss the pith, even when the borer's path goes through the center of the tree (Speer 2010).

Once we were satisfied with the depth of the borer, we inserted the "spoon," a slender strip of metal used to pull the core through the center of the hollow borer and began to remove the core. As we pulled the core out of the borer, we slid it into a straw, which we used for transportation back to the lab. Paper straws are ideal, as they allow the core to begin to dry as they are transported, but plastic straws are much more accessible, so the majority of our tree cores were stored in those with the ends taped off. Sometimes the cores came out in broken pieces, so we had to carefully place the fragments into the straw in the same order in which they were extracted. Once in the straw, we labeled the cores' "pith" and "bark" ends, as well as including the initials of the individual that cored the tree, the date, the tree's DBH, the location of the extraction (such as "Devil's Courthouse"), and the tree species. Once the borer was removed from the tree, we recorded the "aspect," or direction in which the borer was inserted, of the hole with a compass, and measured the height of the hole from the ground. At Devil's Courthouse, we also used a chainsaw to remove cross-sectional discs from three dead trees: two Carolina hemlocks, and one eastern white pine.

Before mounting our cores for sanding, scanning, and analysis, we dried the cores to ensure that no water remained to distort the core when mounted; we achieved this with a low-temperature oven for some of our samples, and the rest were air-dried by cutting slits in the straws and leaving the samples in a dark, dry, place for about a week. We used a metal rod to carefully push the cores from their straws, going slow and, if fragmented, stopping to mount the core piece by piece onto a mounting board with a small semicircular channel for the core to sit in. Examining the cores for correct orientation is imperative for getting good tree ring analysis of each core after it is sanded flat (Speer 2010). A "shiny" stripe can be seen running down opposite sides of each core, due to the nature of the wood grain within the core. These shiny stripes can be glimpsed when held up to light, and they indicate the lateral sides of the core, with the wood grain running vertically in between them when viewing the end of the core head on. Each core has two "shiny" sides that are the lateral sides of the core; we used this hint along with the direction of the wood grain to determine on which side to mount the core. We used wood glue to bind the "top" or "bottom" of the core pieces to their mounting boards, allowing the glue to dry a few days before handling. We also sanded one side of each cookie flat using 100 grit sandpaper on a belt sander and mounted them onto small squares of plywood using wood glue and clamps. Once the cores and cookies were dry, we sanded them using a handheld oscillating sander, starting with 100 grit sandpaper and gradually increasing the grit, finishing with 320. A few of the cores had very narrow rings that were difficult to read so we used 600 grit paper on these samples to define the rings more clearly. Our goal was to leave the cores with a flat surface with the tree's rings visible.

Dendrochronology Analysis

We used an Epson Expression 12000XL scanner to scan our sample cores at a resolution of 0.00847 mm per pixel. Using a naming system including tag number, core order (A or B), and date collected, we saved the .jpg of each sample core to a laptop drive. To age our samples and determine width between annual rings, we used Coorecorder 9.6. The primary focus during our data collection was to ensure that neighboring ring coordinates were perpendicular to one another's respective growth ring. If neighboring coordinates were not perpendicular across the gap between rings, the resulting width collected would be too large of a value, implying greater growth for the tree that year and skewing the results of our data. We utilized tools in Coorecorder such as "Auto-place" and "Help-line" to visually aid in our selection of the location most perpendicular to the previous growth ring coordinate. We used the tool "CrackMarker" and the option for multi-point placement on the same growth ring to avoid or account for cracks and damage in our core samples. Unmarked cracks and measurements of damaged wood would also skew the results of our sample growth from year-to-year. We counted growth rings for each sample to the pith or nearest to the pith as we could get. If a sample did not reach the pith or missed it slightly, we used the tool "Set distance to pith" to estimate the location of the pith relative to the growth rings closest to the center. After estimating the location of the pith, Coorecorder calculated the average width between the last five growth rings and allowed us to use this value to estimate the number of rings remaining between the last measured ring and the pith. Once pith was identified, if able to be located, we exported the coordinate .pos file for each sample to their respective "Coker" or "Devil's Courthouse" folders, grouping the samples by location for ease of analysis of each tree stand in CDendro 9.6.

We used CDendro 9.6 to create master chronologies of growth trends and extract tabular annual growth data for hemlocks at both Devil's Courthouse and the Highlands Biological Station Coker stand. These two master chronologies, one for each site in the form of a .wid file, can be used in future dendrochronological research and on-the-fly Coorecorder dating of trees in this region. The annual growth from each sample core was extracted into Microsoft Excel with 0.001mm accuracy for correlation and regression analysis with our annual climate data.

Climate Data

We accessed National Oceanic and Atmospheric Administration (NOAA)'s Online Weather Data tool to obtain precipitation and temperature records for Highlands, NC. We utilized the "monthly summarized data" tool to extract records of average temperature and rainfall, their earliest beginning in 1893 (all of the data had an unexplained gap from 1905 to 1909). Both precipitation and temperature were provided in monthly averages of daily values. We then averaged the values from the months of April – September to obtain a value of mean daily precipitation and temperature during hemlocks' active growing season. We focused on growing season metrics because hemlocks are most active and sensitive to atmospheric changes during these months, and aren't very productive in winter, despite being evergreen trees (Speer 2010). Precipitation data was provided in inches, and temperature was recorded in degrees Fahrenheit. Using these combined growth data, we will be able to find any correlation present with regional rainfall data and potentially explore the causes of release events through analysis of local historical patterns of the HWA, species extinction in the same ecological niche, and other environmental factors.

RESULTS

Our results show a weak, negative relationship between Coker hemlocks and daily average growing season precipitation (FIG. 4) and a stronger, positive relationship between Devil's Courthouse trees and daily mean growing season precipitation (FIG. 5). Our higher elevation stand of Carolina hemlocks at Devil's Courthouse shows a slight negative trend with mean monthly temperature (FIG. 11), and the Coker stand shows an extremely weak relationship with temperature overall (FIG. 10). We broke down our ring-width chronologies into roughly 20-year periods using a "moving-window" analysis and compared the growth from both stands to growing season precipitation records for these periods. For the Coker stand, we observed both negative and positive correlations with growing-season precipitation, compared to the entire chronology's correlation, which had an almost-flat trendline with an R^2 value of 0.0054 (TABLE 1); the moving-window analysis' R^2 values range from 0.0021 to 0.585 (TABLE 2). The moving-window figures at Devil's Courthouse also show more variation than that of the entire chronology, which has an R^2 value of 0.0725 (TABLE 1). The R^2 values for the moving-window analyses range from 0.151 to 0.324 (TABLE 2), with three of the plots sporting positive trendlines, and one negative. We found a higher correlation between Devil's Courthouse moving-window trendlines and the original Devil's Courthouse chronology than we found between the Coker moving-window trendlines and the original Coker chronology. In efforts to examine lag effects of precipitation on our annual stand growth, we matched mean annual tree-ring growth for both of our stands to precipitation records from the year before for our correlation analyses. The Coker stand results showed only a slightly more negative relationship with this lag data (FIG. 8) than with the standard, unlagged precipitation data (FIG. 4) but, the Devil's Courthouse stand analysis resulted in a weaker positive relationship with the lagged data (FIG. 9) than with the unlagged data (FIG. 5).

TABLE 1. Regression outputs for various analyses between growth trends and climate data.

A) Coker and Devil's Courthouse comparative growth trends

Correlation Values	R^2 Values
0.02802736	0.00078553

B) Yearly ring-growth correlation to mean daily precipitation during growing season

Stand	Correlation Value	R^2 Value
Devil's Courthouse	0.26929087	0.072517573
Coker	-0.073791708	0.005445216

C) Yearly ring-growth correlation to mean daily temperature during growing season

Stand	Correlation Value	R^2 Value
Devil's Courthouse	-0.295127091	0.0871
Coker	-0.038729833	0.0015

D) Yearly ring-growth correlation to mean daily precipitation during growing season, lagged by 1 year

Stand	Correlation Value	R^2 Value
Devil's Courthouse	0.05477225575	0.003
Coker	-0.13820274961	0.0191

TABLE 2. Moving window analyses for yearly-ring growth correlation to mean daily precipitation during growing season.

Stand	Time Frame	Correlation Value	R^2 Value
Coker	1893-1913	-0.13114877	0.0172
Coker	1914-1934	-0.615223537	0.3785
Coker	1935-1955	0.553172667	0.306
Coker	1956-1976	0.764852927	0.585
Coker	1977-1997	0.045825757	0.0021
Coker	1998-2021	0.281957444	0.0795
Devil's Courthouse	1948-1968	0.569209979	0.324
Devil's Courthouse	1969-1989	-0.122882057	0.0151
Devil's Courthouse	1990-2010	0.39987498	0.1599

Devil’s Courthouse	2011-2021	0.554526825	0.3075

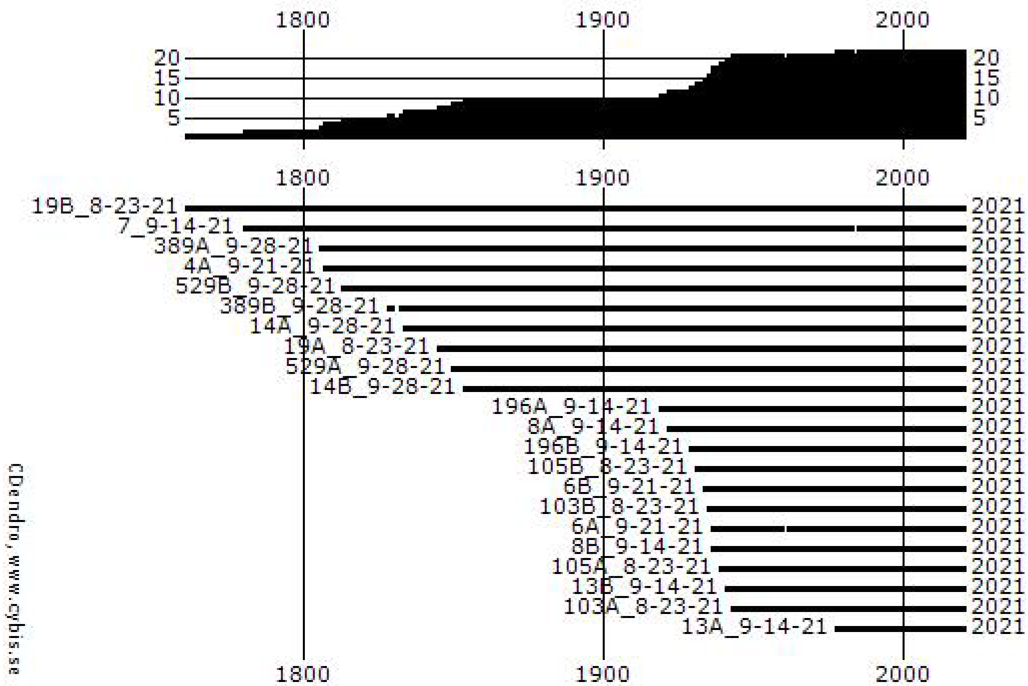


FIG. 1. Record of all eastern hemlock sample cores taken from Highlands Biological Station Coker trail.

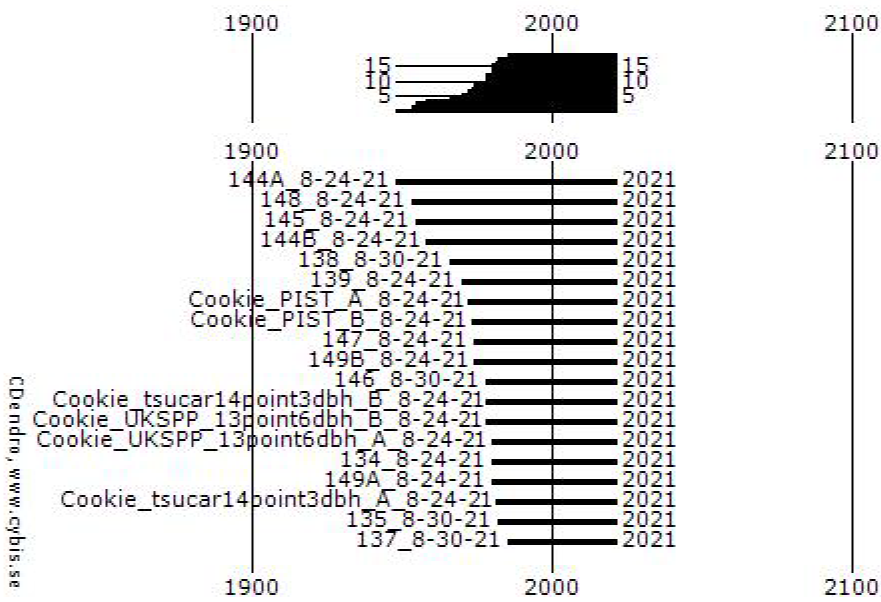


FIG. 2. Record of all sample cores and cookies taken from Devil’s Courthouse on Whiteside Mountain. The majority are Carolina hemlocks, but also included are a few eastern hemlocks, eastern white pines, and a Table Mountain pine.

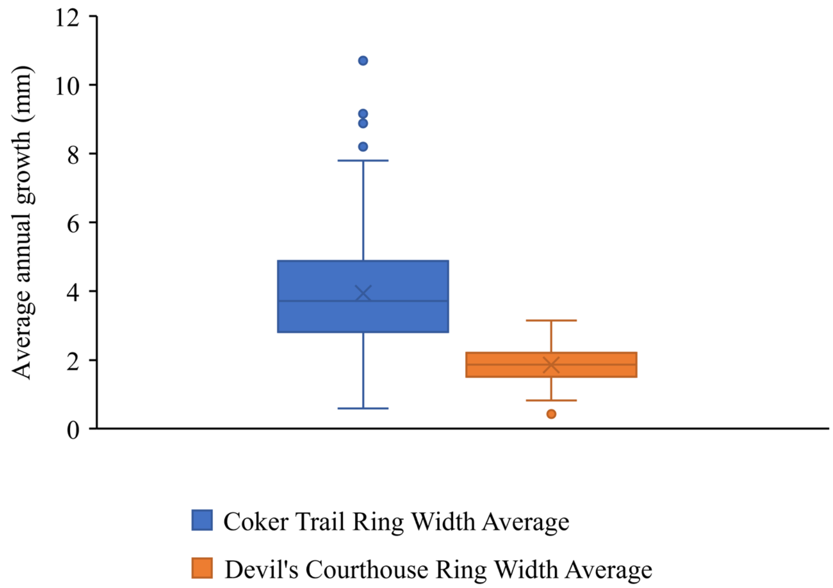


FIG. 3. Box and whisker plot of the average annual growth values between Coker and Devil's Courthouse sites. Smaller hemlocks on Devil's Courthouse showed less variability and less average annual growth than the old-growth forest on Coker trail.

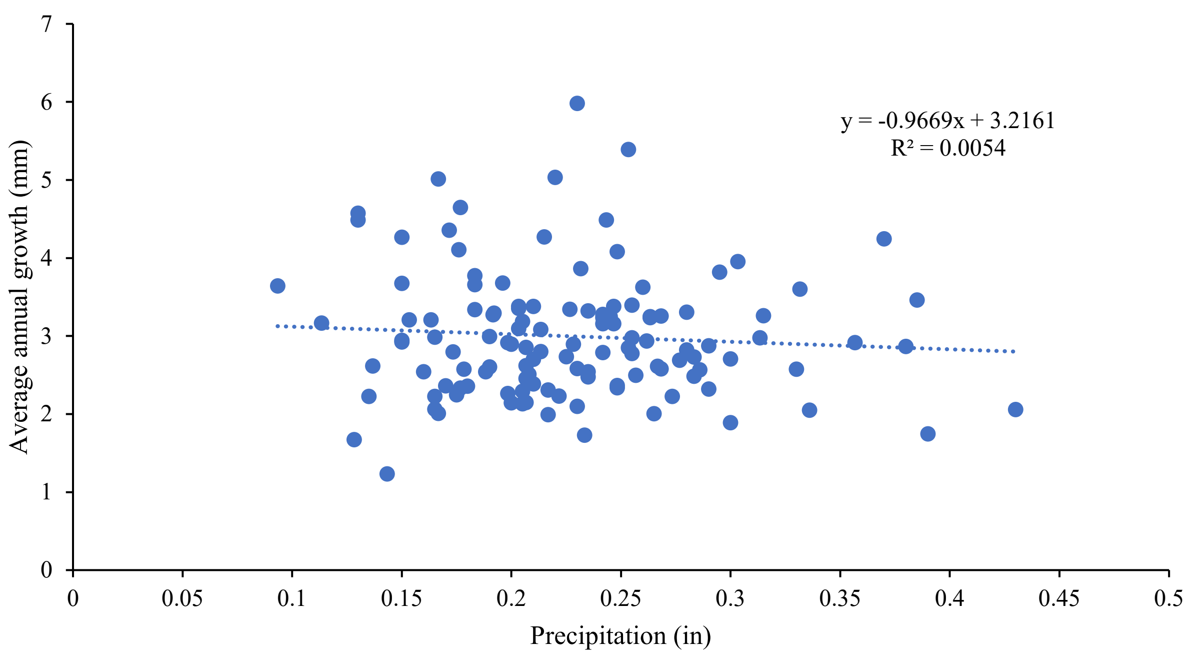


FIG. 4. Regression plot of Coker average annual growth vs. mean daily growing season precipitation by year. These two variables have a slight negative correlation with a correlation value of -0.0738 (TABLE 1).

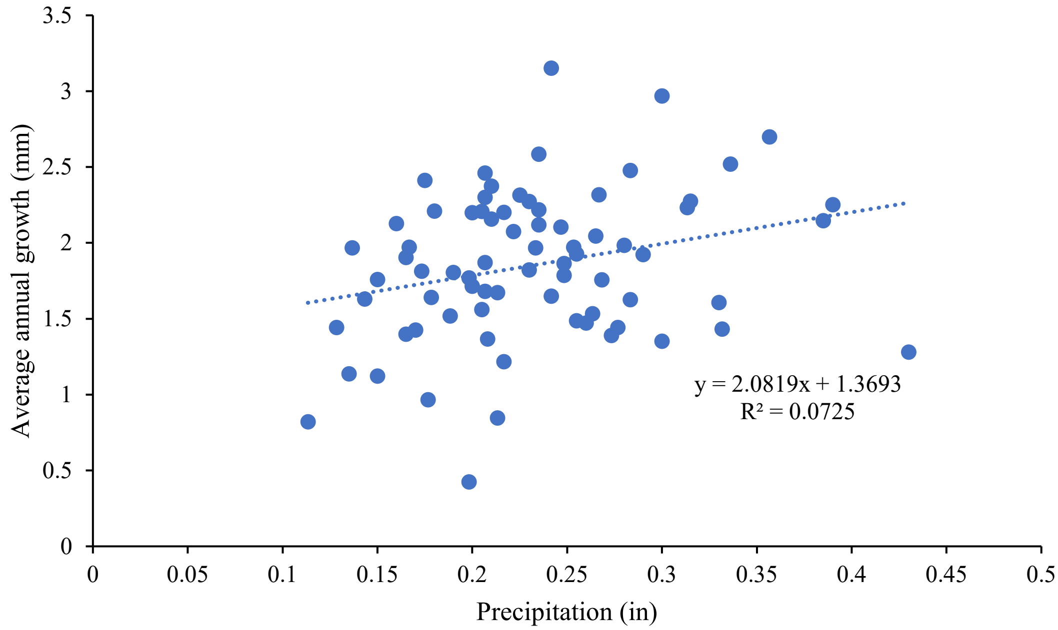
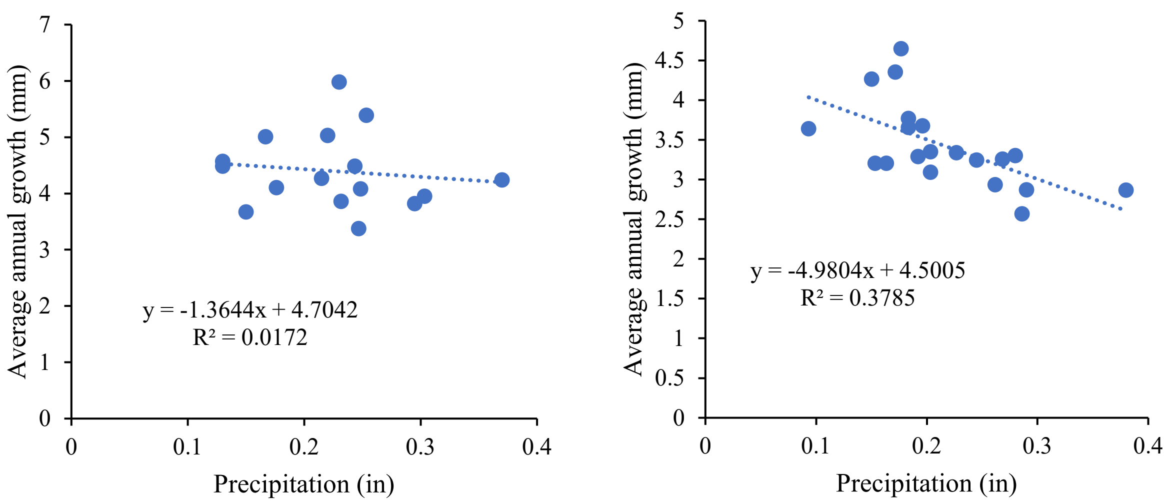


FIG. 5. Regression plot of Devil's Courthouse average annual growth vs. mean daily growing season precipitation by year. These two variables have a positive correlation with a correlation value of 0.2693 (TABLE 1).



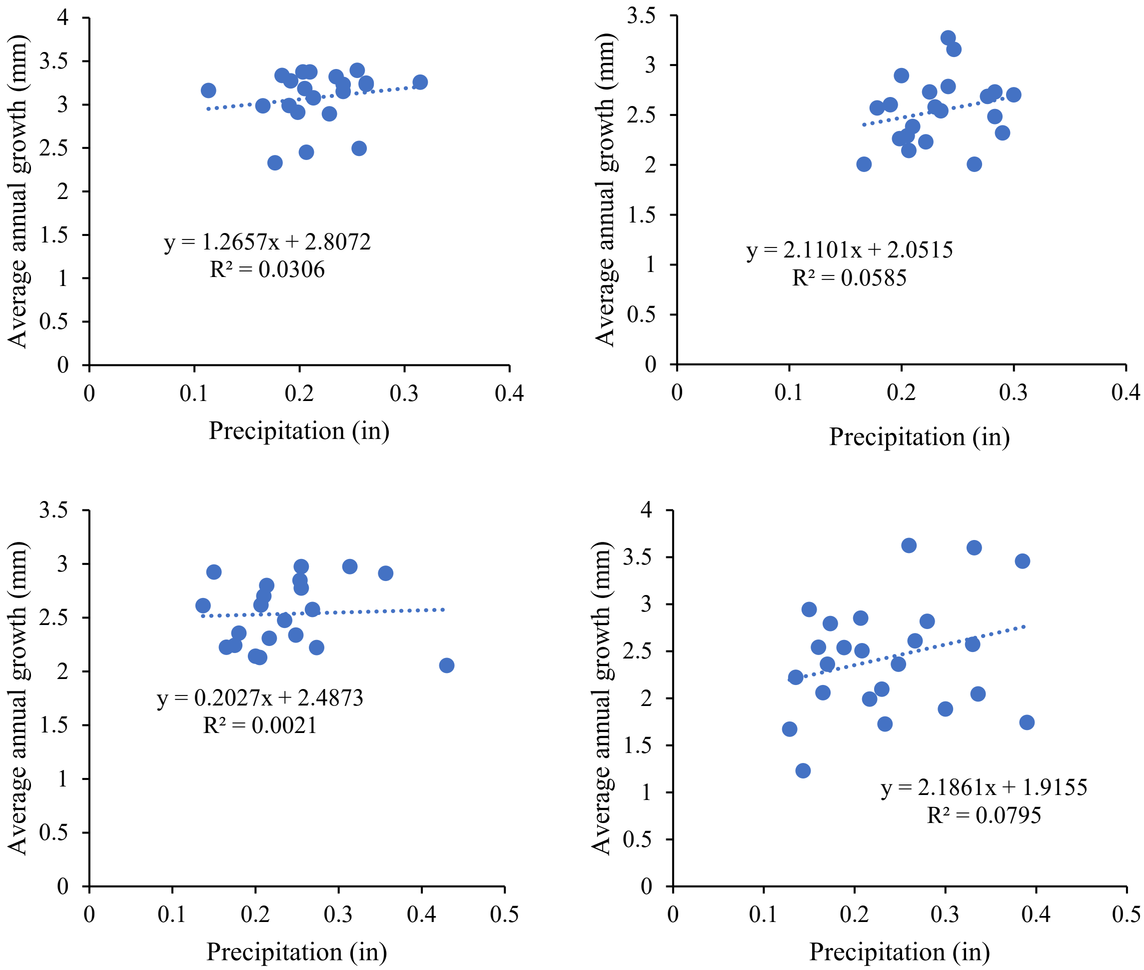
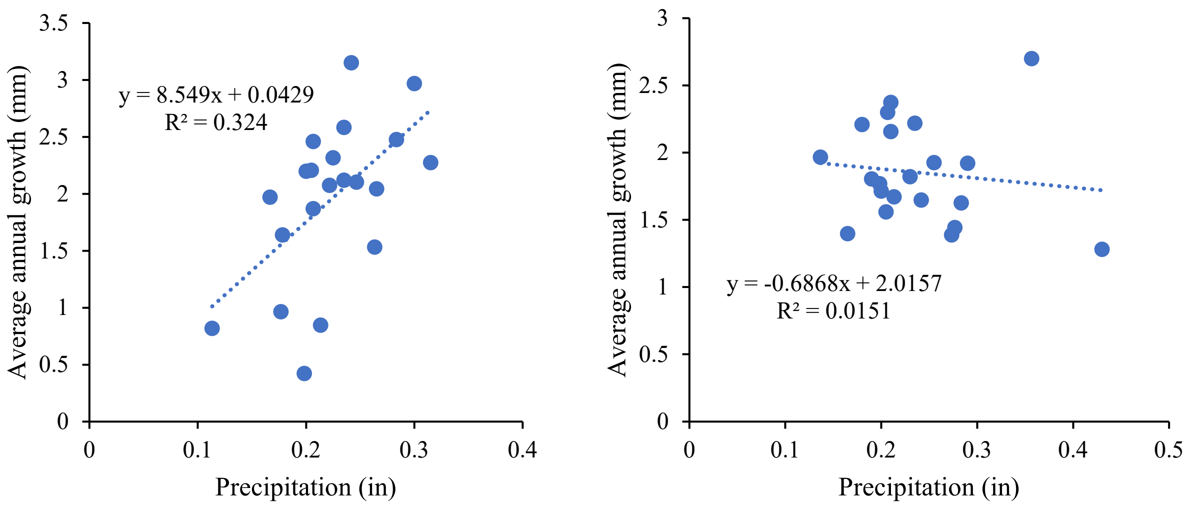


FIG. 6. Moving-window regression analysis of Coker average annual growth vs. mean daily growing season precipitation by year. From top left to lower right, read as if from a book: 1893-1913, 1914-1934, 1935-1955, 1956-1976, 1977-1997, 1998-2021.



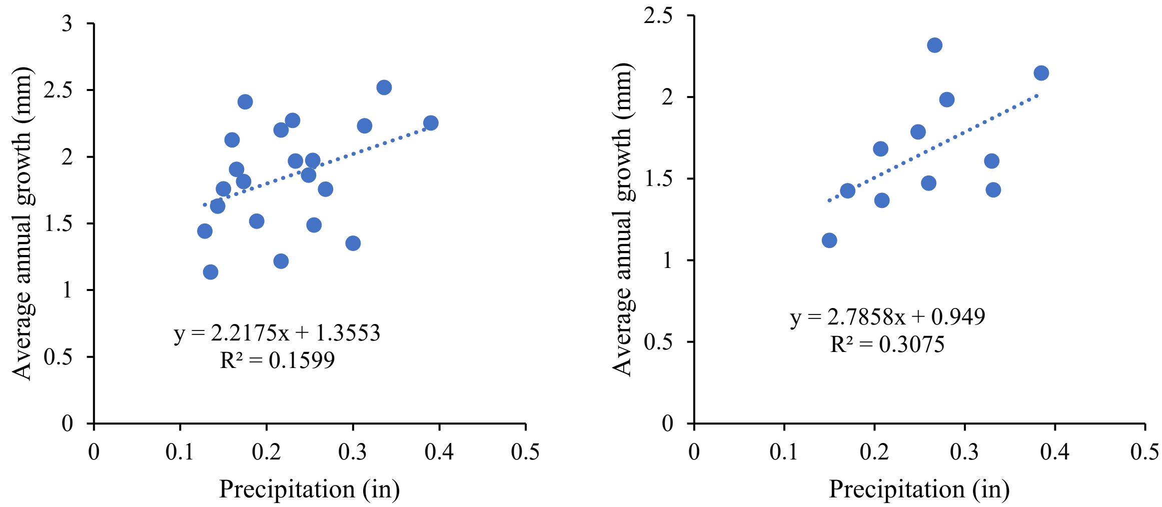


FIG. 7. Moving-window regression analysis of Devil's Courthouse average annual growth vs. mean daily growing season precipitation by year. From top left to lower right, read as if a book: 1948-1968, 1969-1989, 1990-2010, 2011-2021.

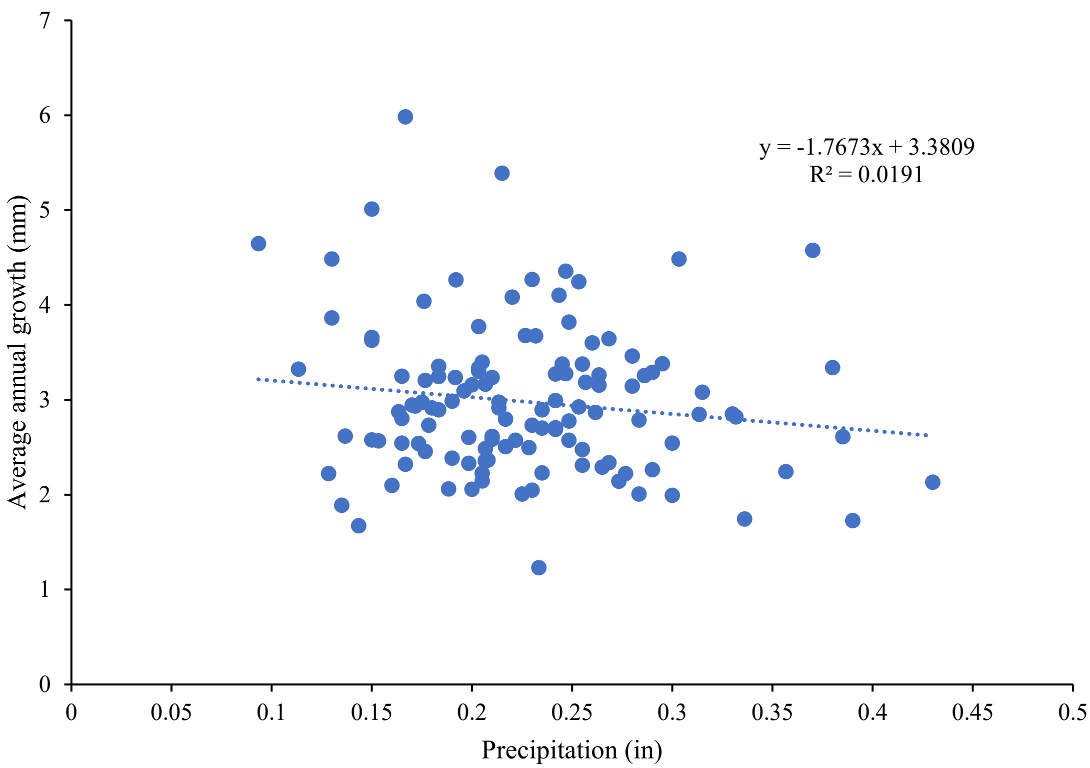


FIG. 8. Regression of Coker average annual growth vs. lagged mean daily growing season precipitation by year. Average annual growth is matched to precipitation from the year before. These variables have a negative correlation with a correlation value of -0.1382 (TABLE 1).

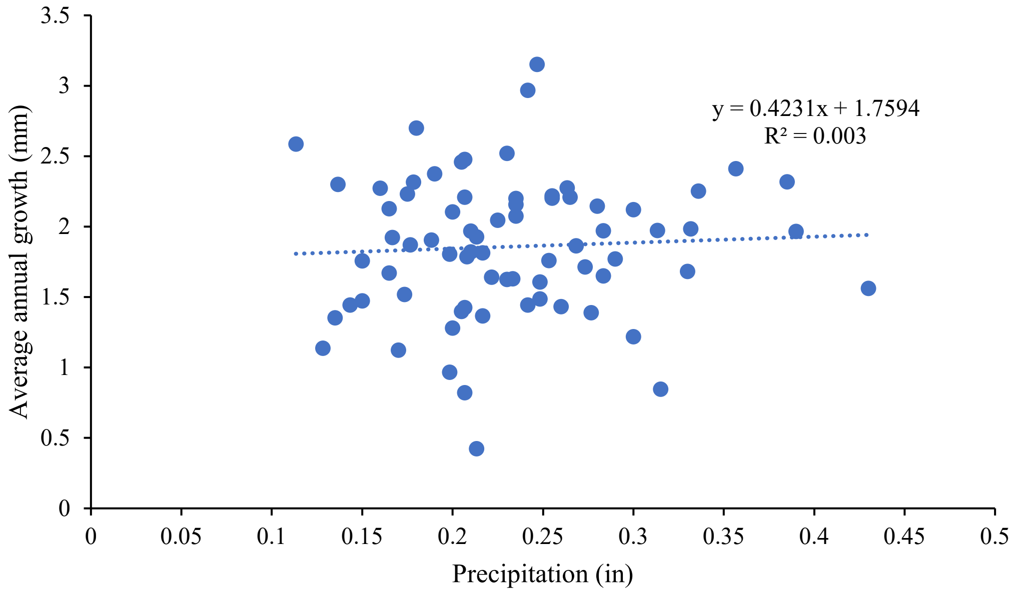


FIG. 9. Regression of Devil's Courthouse average annual growth vs. lagged mean daily growing season precipitation by year. Average annual growth is matched to precipitation from the year before. These variables have a slight positive correlation with a correlation value of 0.05477225575 (TABLE 1).

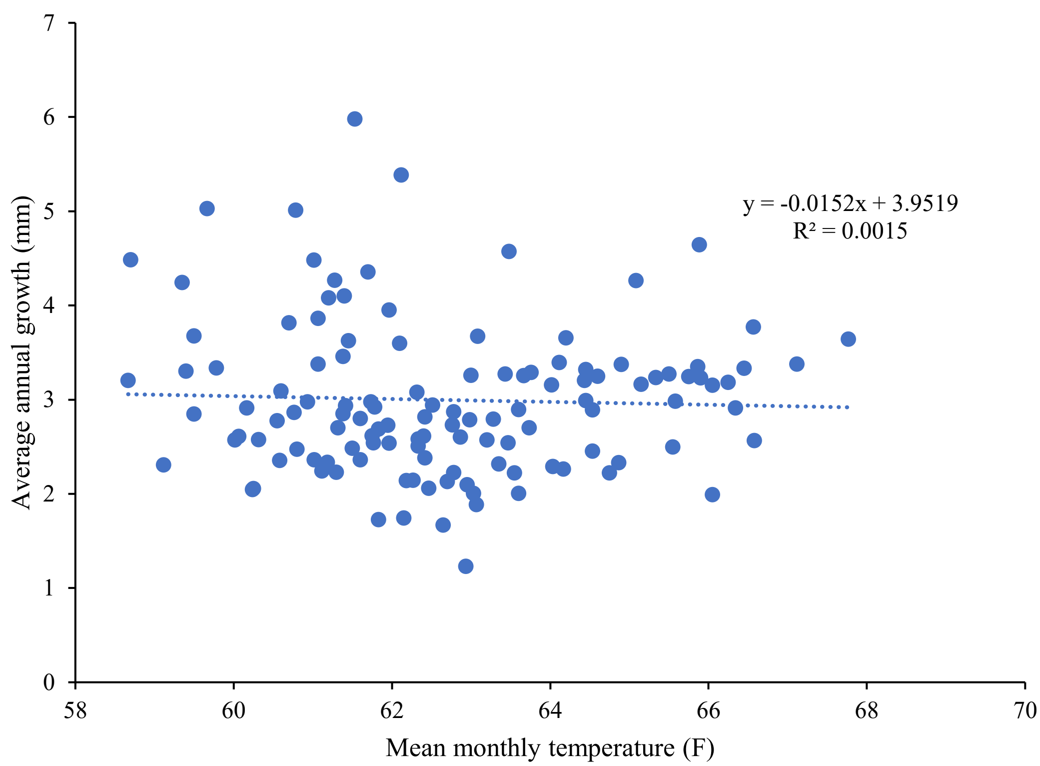


FIG. 10. Regression of Coker average annual growth vs. mean monthly temperature during growing season. These variables have a slight negative correlation, with a correlation value of -0.038729833 (TABLE 1).

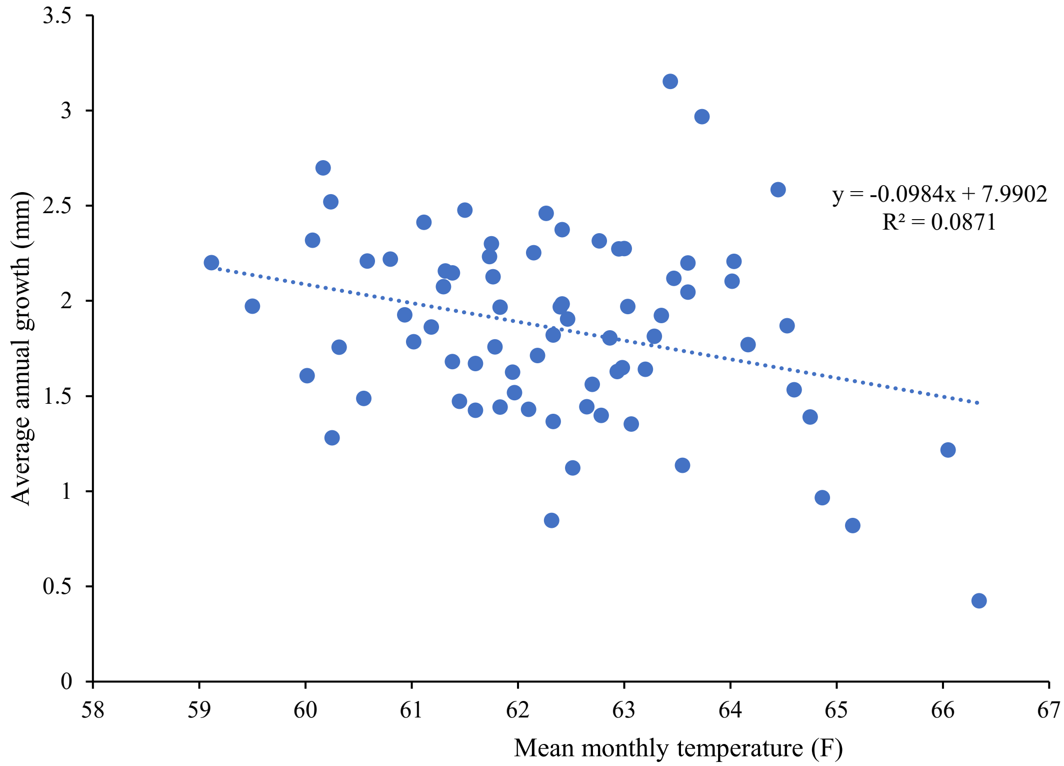


FIG. 11. Regression of Devil's Courthouse average annual growth vs. mean monthly temperature during growing season. These two variables have a negative correlation, with a correlation value of -0.2951 (TABLE 1).

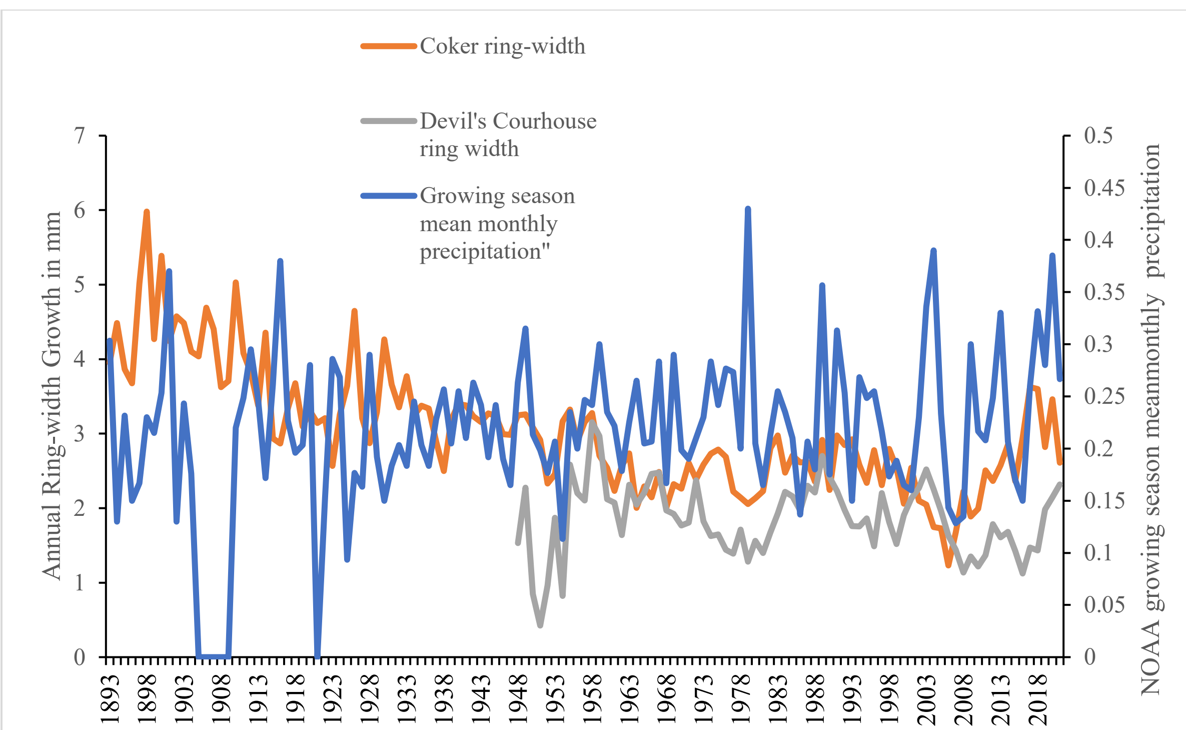


FIG. 12. Line plot comparing average annual growth of Devil's Courthouse and Coker Trail sites with NOAA mean growing season precipitation.

DISCUSSION

We were able to extract multiple insights from our tree-ring chronologies, including the ages of the trees, the stands' growth productivity in relation to precipitation averages, and possible site stability. Our oldest sample, taken from an eastern hemlock in the HBS Coker stand, suggests that the tree dates back to 1761, and many of our other cores from the Coker stand also exceeded 200 years of age. This stand of hemlocks is known old-growth, and we hoped to determine whether our hemlocks at Devil's Courthouse were old-growth as well. Our oldest core from the stand of Carolina hemlocks at Devil's Courthouse dates to 1948, only 73 years old. Although this did not fulfill our hopes of heightening the Devil's Courthouse hemlocks' conservation status, it does fit with local historical records; before Whiteside Mountain was purchased by the U.S. Forest Service in 1970, the mountain was owned and parceled by multiple families, and much of the mountain was logged in 1943 (Spencer et al. 2017). Our data suggest that the Carolina hemlocks on Whiteside Mountain are part of a second-growth stand that emerged after the logging disturbances 80 years ago.

Site conditions can influence differences in growth from year-to-year within unique tree stands. Limiting nutrients, light availability, competition for canopy cover, and groundwater access are all factors that can determine the level of growth for a stand each year (Sands et al. 1990). The Coker stand is a dense, old-growth, acidic cove forest with a stream running through the stand, while Devil's Courthouse is a rocky outcrop with its oldest trees around 70 years old, growing atop a shallow soil layer and bedrock. Depth of groundwater sources and vulnerability due to habitat have shown to greatly affect the growth and mortality of tree stands, and may be the cause of the contrasting relationships with precipitation that both of these stands experience (Braun et al. 2004). Based off of our precipitation analysis during growing seasons, and because our Devil's Courthouse samples have less groundwater resources to fall back on during dry years, they may be much more dependent on precipitation for maintaining their yearly growth. On the other hand, our Coker samples grow in a sheltered and relatively stable habitat with deep soils, and therefore may be less influenced by precipitation in their growth.

The trends found in the results of our moving-window analysis could indicate that factors other than precipitation may be affecting the Coker stand more than the Devil's Courthouse stand, and that some factors have greater influence over tree-ring development than precipitation at the Coker stand. This could be attributed to the differences in habitat at Coker and Devil's Courthouse; as the Coker stand is both more sheltered and more well-equipped to handle drought than the trees at Devil's Courthouse, precipitation levels seem to have greater influence on the growth productivity of hemlocks at Devil's Courthouse than at Coker. These data also suggest that factors other than precipitation, like nutrient and light availability, could have more of an effect on the shady old-growth stand of eastern hemlocks than precipitation.

The results from our lag analysis of precipitation are consistent with the implications of our moving-window and monthly mean precipitation analyses. The eastern hemlocks in the Coker stand seem to be less affected by precipitation from the previous year, possibly due to the deeper soils that have high water storage capacity, as well as the groundwater access this stand likely is able to access given the stand's proximity to the small stream. The Devil's Courthouse stand of Carolina hemlocks seem to have greater dependency on year-of precipitation for its annual average growth, and precipitation from the previous year seems to have little effect on the growth of the population.

Previous studies have shown that trees acclimated to higher elevations see an increase in growth as temperature increases (Way et al. 2010); however, our higher elevation stand of Carolina hemlocks at Devil's Courthouse shows a slight negative trend with mean monthly temperature, and the Coker stand shows an extremely weak relationship with temperature overall. These data suggest that the exposed, rocky-habitat stand of young Carolina hemlocks on Devil's Courthouse may be more vulnerable to changes in temperature than the sheltered, old-growth eastern hemlocks in the Coker stand. The sheltered ravine that houses the stand on Coker (Costa 2012) may stabilize temperature in a way that the unsheltered stand at Devil's Courthouse may not.

Our results suggest that the stand of Carolina hemlocks that we sampled are more sensitive to changes in rainfall and temperature than the stand of eastern hemlocks on the Coker trail. Although we hypothesized that both eastern and Carolina hemlocks would show a correlation between increased rainfall and growth, we only found this to be true for the Carolina hemlocks in our study. We speculate that this can be attributed to eastern hemlocks growing in deeper soils and often in close proximity to streams, providing them with ample water to draw from that is less sensitive to changes in precipitation. We surmise that Carolina hemlocks, which frequent nutrient-poor, dry slopes, and exposed balds, are more vulnerable to environmental stressors such as droughts and temperature extremes. Carolina hemlocks are already considered endangered, and their range only lies within the southern Appalachian Mountains (Levy and Walker 2014). As our climate warms and weather patterns become more volatile, Carolina hemlocks may face even steeper survival challenges than those already posed to hemlocks by the HWA. Looking forward, we hope that these results are synthesized into a greater effort expended on Carolina hemlocks' treatment for the HWA and general conservation endeavors, despite not being an old-growth stand. We hope that future researchers can reference our master tree-ring chronologies and overall results to further our knowledge about dendrology and hemlocks.

ACKNOWLEDGEMENTS

We would like to thank Joel Scott and Dr. Chris Oishi from the Coweeta Hydrologic Lab for their generous help and mentorship throughout this entire study. We also extend our thanks to Jason Love, Dr. Rada Petric, and Mike McMahan of the Highlands Biological Station for their consistent support and guidance throughout this semester. We thank the USDA Forest Service Nantahala Ranger District, including Gary Kauffman, Maria Dunlavey, and Troy Waskey, for granting us permission to core Carolina hemlocks at Devil's Courthouse. We also extend our thanks to Dr. Jim Costa for granting us permission to core trees along the Coker Trail at Highlands Biological Station, Western Carolina University.

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Old growth Tulip Poplar (*Liriodendron tulipifera*) on the Boogerman Trail, Great Smoky Mountains National Park. Pictured (l-r): Vy Pham, Noa Meiri, Alex Hubbs, and Gus Winiker.

QUANTIFYING POLYETHYLENE TEREPHTHALATE AND LOW-DENSITY POLYETHYLENE MACROPLASTIC DETECTION RATES DURING TRASH CLEANUPS

CHLOE HALL AND GRACE KINDER

Abstract. This study was conducted to examine the detection rates of plastics in different southern Appalachian terrains. In the three different terrains of varying vegetative growth, biodegradable replacements for polyethylene terephthalate and low-density polyethylene plastics were distributed evenly. Participants were then asked to pick up as much littered plastic as they could find under a time constraint. Detection rate percentages were developed based on the amount of litter picked up by each individual in each terrain. We observed higher detection rates in terrains not covered in dense vegetation. No major difference in the detection based on the coloration of the substituted plastics was observed. In addition to simulated studies, public cleanups were also conducted to apply our simulated detection rates to non-simulated data. We determined that the most amount of litter per meter was missed in wooded areas. Few studies have been conducted on the effectiveness of trash cleanups and the ability of volunteers to detect trash in different terrains. Based on our estimates, there may be large amounts of macroplastics remaining in an area even after trash cleanups are conducted. We hope our findings can be used as a baseline for future studies and can be used to better inform future environmental stewardship initiatives.

Key words: detection rates; environmental stewardship; macroplastics; polyethylene terephthalate; polyethylene; trash cleanups; vegetation

INTRODUCTION

The accumulation of plastic debris on the surface of the planet is one of the most severe anthropogenic impacts of the past century on the natural world (Jambeck et al. 2015). Mass production of plastics began in the 1950s as a post-WWII global economy demanded innovation (Plastics Industry Association 2021). Studies have shown increasing rates of plastic accumulation in natural environments adjacent to urban centers, of plastic fragmentation, and of plastic dispersal into natural systems (Barnes et al. 2009). Plastic debris can be divided into different sizes and characteristics due to fragmentation, with each type causing a unique set of ecological impacts. Macroplastics, by definition, are large pieces of plastic waste, >5 mm, that vary in color, texture, and chemical composition. Plastic materials have an estimated longevity rate of hundreds to thousands of years after they are disposed of (Beltrán-Sanahuja et al. 2020, Beltrán-Sanahuja et al. 2021). Littered macroplastics can threaten natural systems by serving as a choking hazard for wildlife, emitting toxic chemicals, and breaking down into microplastics that can be digested and infiltrate aquatic and atmospheric systems (Bucci et al. 2020). Moreover, salamanders and small mammals can become trapped inside discarded bottles and die (Brannon and Bargelt 2013).

Plastics are developed from different natural elements such as oil, gas, and various plants which refine to form ethane and propane. When heated, they become ethylene and propylene and form seven different plastic types (Geyer 2017). These seven types include Acrylic or Polymethyl Methacrylate (PMMA), Polycarbonate (PC), Polyethylene (PE, which in itself can be divided into four subsections depending on its density level), Polypropylene (PP), Polyethylene Terephthalate (PET), Polyvinyl Chloride (PVC), and Acrylonitrile-Butadiene-Styrene (ABS).

Commonly used plastics include PET and LDPE, which are the two macroplastics examples focused on in this study (Bolgar et al. 2015). Common forms of PET plastic that are available to consumers are often rigid containers such as food and beverage containers (Tamburini et al. 2021). The rigid form of these PET plastic containers differs from that of the flimsy LDPE plastic. Common forms of LDPE include food wrappers and grocery bags (Wunderlich 1964; Roy et al. 2011), which are notably more pliable allowing them to potentially be more easily hidden under debris and away from cleanup volunteer's eyes. Biodegradable alternatives are being produced and used to help combat the overabundance of commonly used plastics such as PET and LDPE. These biodegradable alternatives use naturally occurring polymers (Priyanka et al. 2014). Due to the increasing accumulation of plastic debris in the natural world, many civilian conservation organizations resort to volunteer-based trash cleanup initiatives to manage this issue. However, problems in public trash cleanup initiatives have been found, such as the volunteer population lacking the appropriate training for the focus cleanup area which in turn leads to a decrease in thorough cleanup efforts. Volunteers, while still eager to participate in cleanups, mainly focus on larger pieces rather than the multitude of little pieces (Krasny et al. 2015).

The objective of this study is to analyze the effectiveness of trash cleanups by determining PET and LDPE macroplastic detection rates during simulated cleanup scenarios in varying terrains. Additionally, we sought to determine macroplastic detection rates in real-life cleanup scenarios as compared to our simulated cleanup scenarios. We predict that the highest amount of plastic will be detected in open, low vegetation terrains during the simulations and public cleanups. To the best of our knowledge, the detection rates for macroplastics have never been determined before. Detection rates will allow us to estimate the total amount of trash discarded in the environment. Furthermore, we discuss the implication and potential uses of these detection rates and how they could be used in the realm of conservation and ecology.

METHODS

Site Selection

Our study took place in the Blue Ridge physiographic province of western North Carolina. The varying mountainous terrains common in western North Carolina and identified as typical locations for macroplastic litter were: 1) open areas consisting of short grasses or other low-growth vegetation ≤15 cm tall, 2) tall vegetation areas consisting of undergrowth ≤1m tall, and 3) wooded areas with mature forest stands. The same three terrains (open, tall vegetation, and wooded) were used for determining our sample sites in both the simulation and public cleanups.

The terrain sites for the simulation cleanups were conducted in Acidic Cove Forests surrounding Lindenwood Lake at the Highlands Biological Station, Macon County, North Carolina (Fig. 1). Abundant Dog Hobble (*Leucothoe fontanesiana*) growth was noted in the tall vegetation simulation site, and mature Eastern Hemlock (*Tsuga canadensis*) and Eastern White Pine (*Pinus strobus*) stands in the wooded simulation site.

Simulation Studies

To calculate detection rates for PET and LDPE macroplastics in the determined terrains, simulated trash cleanups were conducted. Within each terrain, we marked a 15 meter long transect with visible flags. Each of the participants was asked how far to the right and to the left they would go to collect trash if only told to travel forward in a linear transect in a limited amount of time. The width of our study transects was calculated from the average estimated collection distance of our eight participants and determined that our simulation transects would be 10 meters wide.

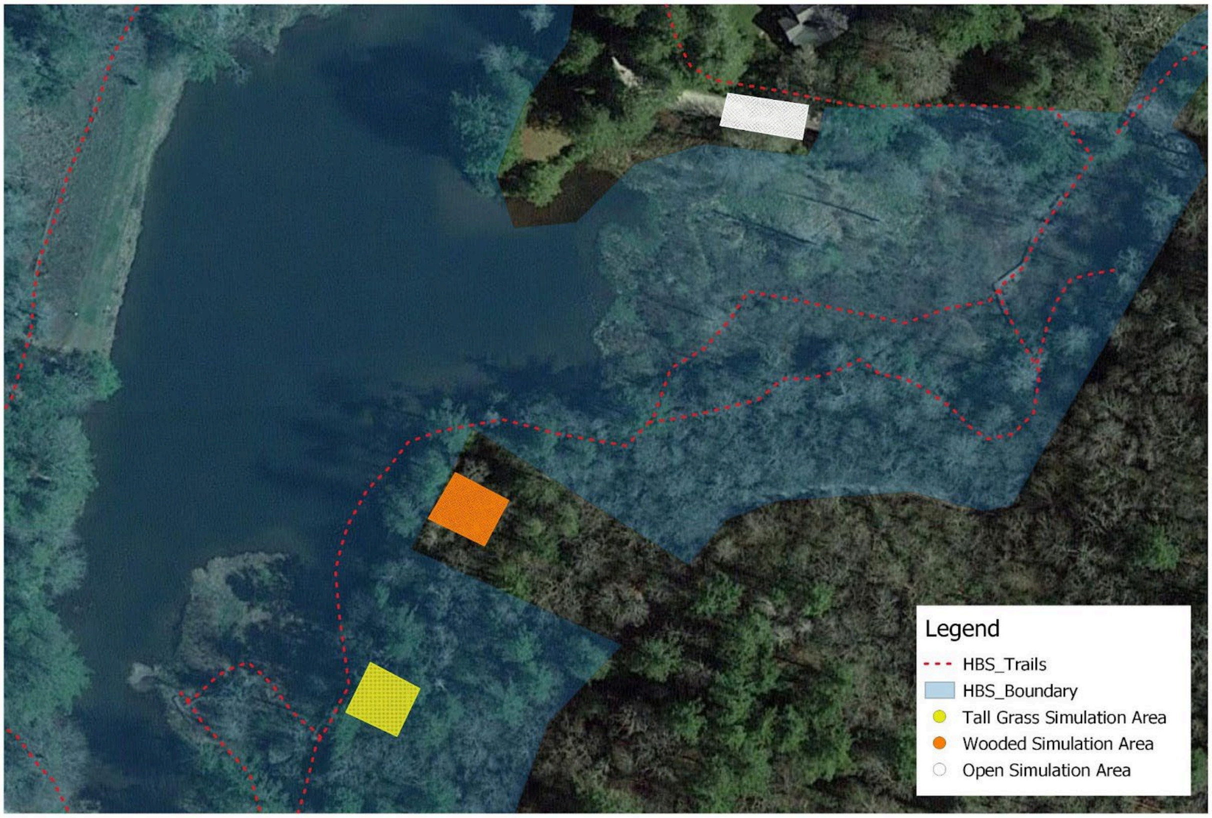

FIG. 1. Aerial map of Highlands Biological Station (HBS) with cleanup simulation sites outlined in comparison to the station boundary. The HBS boundary is shown in blue. The varying simulation terrain sites are shown in orange, yellow, and white.

In each of the three marked transects, we randomly dispersed 20 pieces of the selected biodegradable surrogates, 10 pieces for PET (mean length = 24.4 cm, range = 4.0 – 8.0 cm) and 10 for LDPE plastics (mean length = 15.0 cm, range = 4.0 – 15.0 cm). The selected PET biodegradable surrogate was a compostable to-go food container and the selected LDPE biodegradable surrogate were compostable trash bags (Fig. 2). Each volunteer was given a large trash bag for collection and was allotted 10 minutes per transect in each of the three terrains to collect the trash. We instructed participants to collect as much trash as visible to them and to end collection once the time was called. Participants were not informed of the total number of pieces dispersed in the transect nor of the physical attributes of the biodegradable surrogates. The total amount of surrogate pieces collected by each participant in each transect was recorded. Any remaining surrogate pieces were collected by us to prevent environmental contamination.

Public Cleanup Studies

After our detection rates were determined, we examined PET and LDPE collection rates in public trash cleanups hosted by Mainspring Conservation Trust, a local conservation organization. The first public cleanup took place at Hiwassee Lake and provided us with wooded and open terrain. The second public clean-up took place on a cut-out on route 64 from Highlands to Cashiers and referenced our tall vegetation terrain. At each cleanup, we marked out 60 square meter areas in each of the three focus terrain types for volunteers to work in. We then provided a subset of volunteers with specific collection bags, safety vests, and trash grabbers. We assigned

FIG. 2. This photo shows the two substitute littered plastics used in the simulated studies. The light green biodegradable plastic is a substitute for LDPE while the light brown biodegradable plastic is a substitute for PET.

each volunteer a specific transect to work in during their hour-long shift. We informed each volunteer of common trash items that fall into the categories of PET and LDPE plastics categories for easy identification. To test the validity of a multi-pass cleanup method for a more thorough cleanup, we conducted an additional pass through each of the three terrains at the public sites. Once each volunteer had completed their initial cleanup shift, we worked our way through the same transect again and collected any additional PET and LDPE litter we could identify. The plastics collected during the second pass were stored in a separate trash bag outside of the other forms of trash at the cleanup sites. Once cleanup shifts were completed, we collected the designated trash bags for analysis.

Participants

We selected participants for both the surrogate studies and public cleanups from a local population of students who could attend both the simulated and public cleanups. The 8 participants used for the simulation study were aged between 19-22 and were of varying ethnicities and genders. They were all in good health and able to see, identify, and pick up plastics during the study. We gained consent for participation from all selected volunteers and informed them that no personal identifying information would be shared.

Data Analysis

Basic estimates of detection rates (p) were calculated by averaging the number of pieces collected (n) divided by the total number of pieces (N):

$$\bar{p} = \frac{n}{N}$$

We compared the cumulative PET and LDPE detection rates from all three simulation sites to determine if the color of the substitute pieces or terrain impacted detection. Overall detection rates for both types of plastics in each of the three terrains were calculated and compared. To apply our detection rates to public cleanups and to determine the amount of trash missed during cleanups, the collected plastics from the public cleanups were counted to determine the total collection amount in the public sample terrains and then divided by the appropriate detection rate. The total amount of plastics collected in each terrain was then subtracted from this result to get the amount of trash missed in the transect. We then divided the amount missed in total by the transect length to determine the amount of PET and LDPE litter missed per 60 square meters. We also compared the PET and LDPE pieces collected in each terrain after a first and second cleanup pass through the area.

RESULTS

PET and LDPE Detection Simulations

Based on the data recorded from the 24 simulated cleanups in varying mountainous terrains, there was no major difference in the cumulative detection rate of the green LDPE substitute pieces as compared to that of the brown PET substitute pieces (Fig. 3).

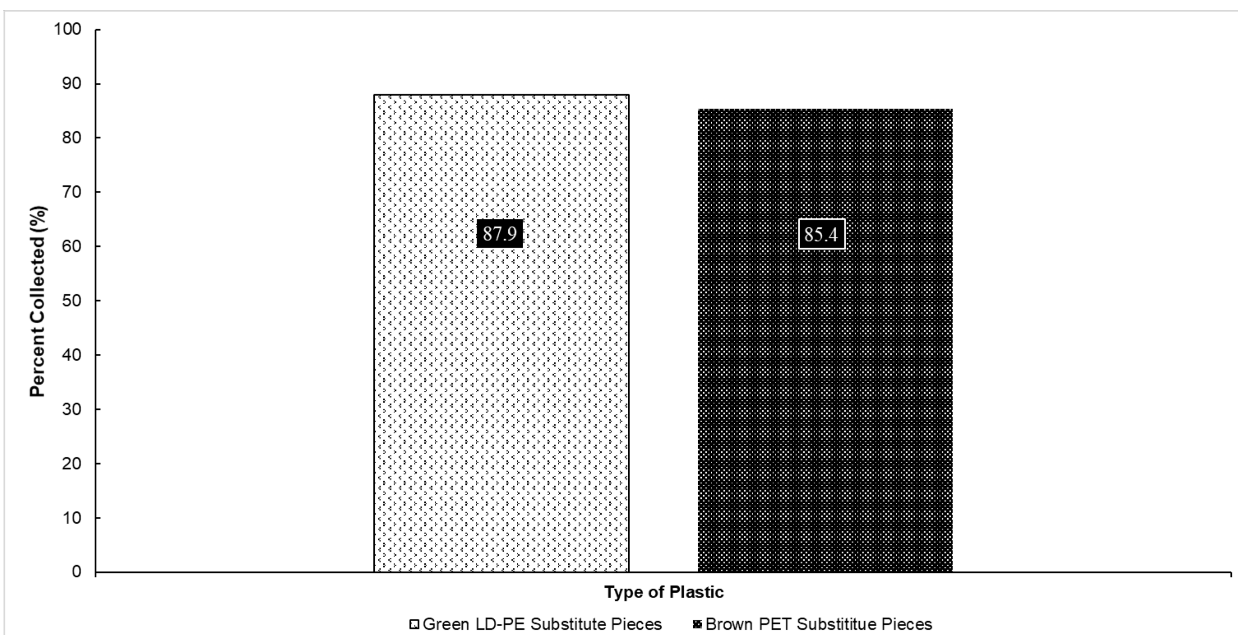

FIG. 3. There was no meaningful difference in overall detection rate between the green LDPE substitute pieces and brown PET substitute pieces.

The highest cumulative macroplastic detection rate of PET and LDPE biodegradable substitutes occurred in the open terrain at a rate of 93.8 % overall detection (Fig. 4). The lowest cumulative macroplastic detection rate occurred in both the tall vegetation and wooded terrains with detection rates of 83.1% overall detection.

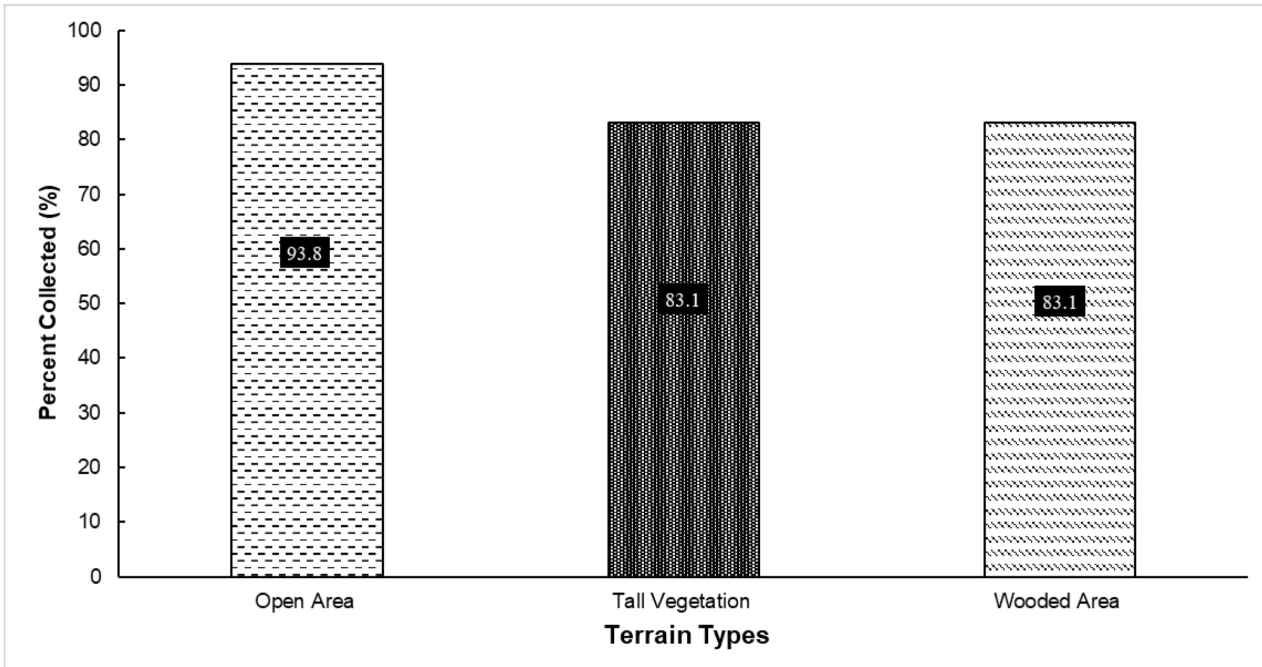

FIG. 4. The cumulative detection results from the open area simulation had a higher detection rate than the tall vegetation and wooded results.

Public Terrain Studies

Based on our previously determined simulated detection rates and the data collected from the public trash cleanups, PET and LDPE litter is overlooked the most in wooded terrains during cleanups (missed pieces / 60 m^2= 3.5) (Table 1). The least amount of trash missing per meter occurred in the open sample areas (missing pieces / 60 m^2=2.6). Higher amounts of PET and LDPE litter were collected in all three terrains during the first cleanup pass (Fig. 5).

TABLE 1. A summary of public cleanup collection data from 60 m^2 transects.

Public cleanup site	Simulated detection rate (%)	PET and LDPE pieces missed
Open	93.7	2.6
Tall vegetation	83.1	3.1
Wooded	83.1	3.5

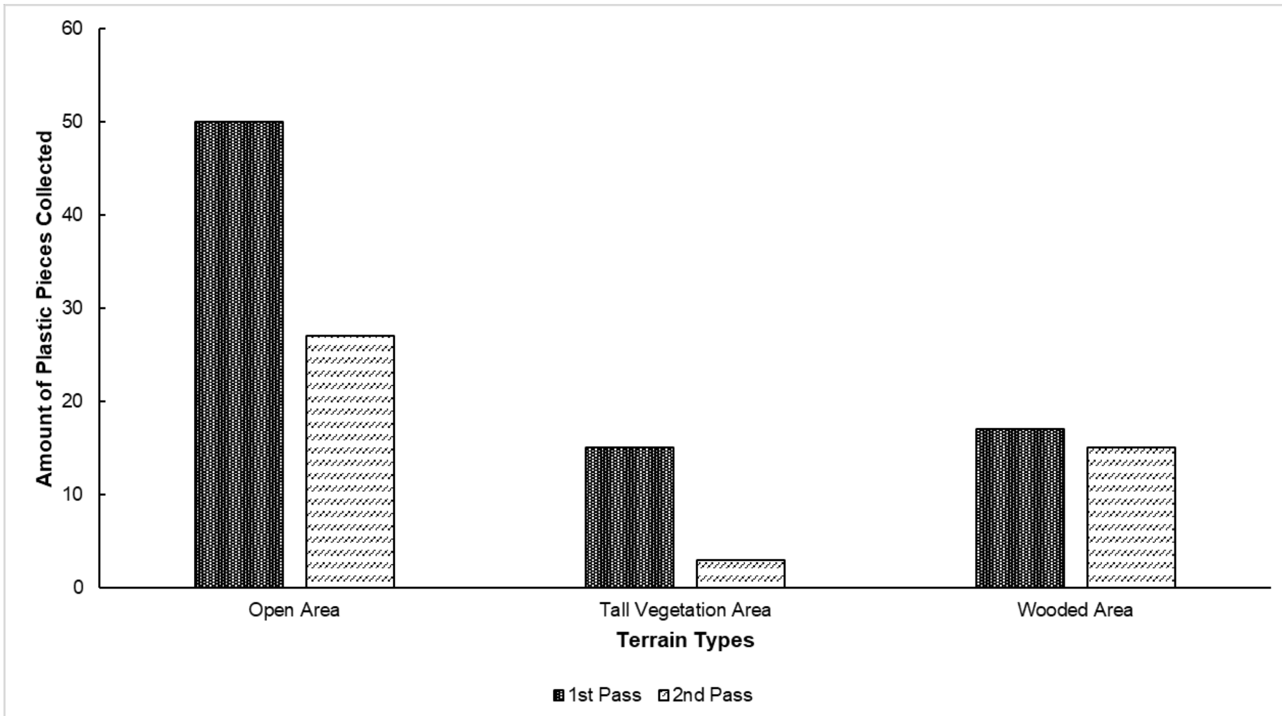

FIG. 5. Amount of LDPE and PET pieces collected during the public cleanups was higher during the 1st pass through the terrain.

DISCUSSION

The novel nature of this study provided a unique set of confounding variables we had to face when conducting our research. First and foremost, our study supports the idea that incomplete detection occurs at trash cleanups. However, we believe that we overestimated detection probabilities. One major potential cause of overestimation is that our multi-pass depletion test was only run twice, therefore lacking a substantial data set. When multi-pass depletion tests are used in ecology, three passes are normally conducted (Hanks et al. 2018). Additional study variables that could have led to an overestimation of detection rates include the simulation study participants which consisted of a small age demographic ranging from 19-22 years old. Because of this, the data found in these simulated studies only reflect that of young, healthy, and able-bodied individuals. In addition, the simulation studies participant pool was limited in population to eight total participants. This was due to our finite access to available volunteers that were willing and physically able to perform a series of simulated trash cleanups in a multitude of terrain types at a given time and place. Because of this minimal study population, the data represented does not include a wide range of individual volunteer types and is likely an overestimation of PET and LDPE detection rates. Furthermore, the simulation studies participant pool was acquired directly from the Highlands Biological Station's UNC-IE particpants. Consequently, all volunteers were environmentally inclined individuals who are theoretically more aware of the plastic litter abundance issue. This could have affected their motivation or ability to detect the simulation plastics in each terrain and effectively skew the results when compared to other real-life cleanup scenarios. Based on these confounding variables, we can assume that our determined detection rates are potentially an overestimation and that there are likely more plastics being overlooked during cleanups than we determined.

In comparison to the real-life cleanup scenario, the simulation transect was significantly smaller with fewer littered plastics. In addition, there was a clear time limit to the simulated scenario whereas there was no time limit in the real-life cleanups. Therefore, the simulation participants may not have experienced the same fatigue as public volunteers. With larger areas to cover, ultimately resulting in larger amounts of littered waste, the fatigue could have affected the individual's detection rates in the real-life scenario. As seen in the results, the open terrain detection rate in public vs. simulated was very different. The public cleanup for that terrain type had a lower probability of detection when compared to the open terrain simulation. This could be because the public open terrain transect was conducted in a larger area with large rocks, logs, and other factors not found at the simulated study which may have inhibited volunteers from looking for and detecting plastics in that area.

The final limitation found in our study was the change in weather events over the course of the two months when the simulated study took place. Because our simulated experiment took place over September and October, the participants experienced a range of weather events. Some volunteers experience large amounts of rain and cold wind that persisted throughout each of their cleanups. As a result, the participant's ability to identify and pick up plastics may have been hindered due to these weather conditions. In addition to the rain, leaf off occurred during these months which created an abundance of leaf coverage on the ground. This made it more difficult for participants to identify plastics in given terrains with such dense ground coverage. These weather hindrances were noted. However, they are not reflected in the data. Additionally, other causes for incomplete detection may include age, vision, and/or physical condition of the volunteers, terrain/slope, etc.

Our data allowed us to draw conclusions on the relationship between color, vegetation, and plastic litter detection. Our initial simulation trials indicated that cleanups conducted in open areas yielded higher overall detection rates. Additionally, the open terrain was flatter and no vegetation or woody growth hindered participants. Our results also indicated that the wooded and tall vegetation simulation sites yielded lower overall detection rates. Denser tree canopy coverage and vegetation can decrease light levels and can make plastic detection within the vegetation more difficult.

The color of the two substitute plastics used in the simulations seemed to have little difference in terms of detection rate. Both of the biodegradable plastic substitutes used in the simulations were fairly large and light colored. The similarity in color when placed in a darker, vegetative environment may explain the lack of a difference in detection rate based on color. This coloration trend leads us to believe that vegetation density of the cleanup site impacts detection rates more than coloration of the trash does.

We determined that volunteers missed on average 2.6 pieces per 60 square meters in open areas, 3.1 pieces per 60 square meters in tall vegetation areas, and 3.5 pieces per 60 square meters in wooded areas. This trend supports our initial claim that the most trash can be found in open, low-vegetation areas (as shown by our simulation detection rates). While these missing piece trends may not seem significant on the scale of one meter, this trend can be scaled up to help better inform public cleanup efforts by providing an impactful visualization of the amount of trash being missed. For instance, if a cleanup initiative was conducted along a one-meter-wide path in the tall vegetation zones alongside US-64 from Highlands N.C. to Cashiers N.C. (a 17.1 km stretch of road), approximately 883 pieces of PET and LDPE litter could be overlooked during cleanup. By scaling up these observed detection relationships, these trends become more relevant and impactful.

On a global scale, southern Appalachia is one of the most biodiverse regions in the world (Simon et al. 2005). With this fact in mind, the discrepancies in the litter cleanup process pose a unique set of ecological implications for this region. Overall lower detection rates in densely vegetated terrains could cause these types of areas to be left with significant litter after cleanup has been conducted. Macroplastic litter can serve as a choking hazard for wildlife, and they can emit toxic chemicals into the surrounding environment (Bucci et al. 2020). Plastic litter can also degrade into microplastics due to weathering and exposure (Patel 2021, Zongguo et al. 2021). When microplastics are exposed to a natural system, they can further infiltrate the environment by entering aquatic and atmospheric systems (Ryan et al. 2009). Microplastics have even been detected in organic life such as mussels, plant life, and human infants (Ragusa et al. 2021). The ability of microplastics to infiltrate all aspects of the natural world heightens the threat of undetected macroplastic litter on the environment.

With these ecological implications in mind, we can further conjecture the importance of this study's results on the overall public trash cleanup process. Informing cleanup volunteers that detection fatigue may occur in densely vegetated terrains could help to make volunteers more diligent in their cleanup efforts. Additionally, environmental stewardship organizations could dedicate extra cleanup efforts to these problem terrains to help increase the likelihood of complete litter detection and clean-up. One possible method that could be implemented to increase trash detection is a multi-pass methodology. Multi-pass depletion is a common method for determining the population of a focus species and has proven successful in improving detection (Hanks et al. 2018). The formula $E = 1 - (1-p)^n$ where effectiveness (E) is influenced by the detection probability (p) and the number of passes (n) is used to determine the effectiveness of multi-pass depletion. If two passes were conducted in a cleanup area with a plastic detection rate of 60%, then we could estimate that after the second pass, 84% of the total trash present in the area has been collected.

CONCLUSION

We hope that the development of this pilot study on the specific relationships between plastic litter and terrains can be used to better inform future studies on similar topics and future cleanup initiatives. Increasing awareness about the tendency to overlook littered plastics and the tendency to become fatigued when working in densely vegetated terrains is a key step in increasing the effectiveness of environmental stewardship. Expanding the focus terrains, participant sample size, the demographics of the participants, and the types of plastic used in the study could help to better inform the relationship between litter detection and terrains in future studies. With this starting point, we hope to create more awareness for volunteers' cleanup capabilities and shed light on how lead conservation organizations can better inform volunteers of the most effective cleanup methods.

ACKNOWLEDGMENTS

We would like to thank Jason Meador from Mainspring Conservation Trust for his guidance and mentorship throughout this project. Without his assistance, access to Mainspring clean-up events, feedback, and supplied materials, this project would not have been possible. In addition, we would like to thank Jason Love at the Highlands Biological Station for his assistance with our project and for allowing us to conduct our surrogate studies on the HBS campus. Finally, we would like to acknowledge our ever-supportive peers who joined us rain or shine (often-time rain) to participate in our study and who taught us that collecting trash can be fun. Without the combined assistance of all these wonderful people, our project would not be what it is today.

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Students explore downtown Clayton, GA where Stekoa Creek, a tributary of the Chattooga River, passes through. (l-r): Daniela Zarate, Hannah Obenaus, Vy Pham, Chloe Hall, Gus Winiker, Alex Hubbs, Noa Meiri, Scout Allen, Eva Kinney, and Grace Kinder.

A COMPARISON OF LAND COVER AND THE DISTRIBUTION OF THE COMMON MUDPUPPY (*Necturus maculosus maculosus*) IN THE UPPER LITTLE TENNESSEE RIVER WATERSHED, NORTH CAROLINA

EVA KINNEY AND ALEX HUBBS

Abstract. The Common Mudpuppy (*Necturus maculosus maculosus*) is a fully aquatic salamander found throughout eastern North America, yet little is currently known about the species distribution throughout the southern Appalachians. One key watershed that runs through this area is the Little Tennessee River drainage, which runs through diverse land cover types, including forested and developed areas. In order to understand the impact these land cover types have on the Common Mudpuppy; dip netting and minnow trapping surveys were conducted in areas previously known to contain the species. GIS tools were then utilized to look at the relationship between sample sites and the surrounding land cover. We failed to capture any Common Mudpuppies using our survey methods. Using the historical capture data, there is no difference between land cover types in areas where Common Mudpuppies have been found and where they have not been found. Current landowner practices or legacy effects from past farming or logging ventures may affect the distribution of the species throughout the Little Tennessee River drainage.

Key words: eDNA; Common Mudpuppy (Necturus maculosus maculosus)*; GIS; land cover; salamander; watershed.*

INTRODUCTION

The Common Mudpuppy (*Necturus maculosus maculosus*) is a large and entirely aquatic salamander found in eastern North America (Nelson et al. 2017). This species is commonly found in river tributaries in the region surrounding the Great Lakes within the United States and Canada (Eycleshymer 1906, McDaniel et al. 2009). The range of the Common Mudpuppy also extends south into Mississippi, Alabama, and Georgia and west into northern Louisiana across the Mississippi River (Nelson et al. 2017). In the southern Appalachian region, mudpuppies have a similar distribution to Eastern Hellbenders (*Cryptobranchus a. alleganiensis*), with organisms found within the Great Smoky Mountains National Park (Nickerson et al 2002, Dodd 2004). However, little is known about the species' distribution in western North Carolina other than a few county records within the New River, French Broad River, and Little Tennessee River drainages (Williams and Corey 2007, Beane et al. 2008, Williams and Corey 2008, Williams et al. 2011).

The Common Mudpuppy is a long-lived species, living up to 30 years in the wild, with a four-stage amphibian life cycle, involving an egg, larval, juvenile, and adult stages (McDaniel et al. 2009, Haines 2021). Females breed in the late fall/winter and lay eggs in late spring or early summer. Larvae hatch at about 50 days and will remain in larval form until November, when their egg sack is fully absorbed, and they enter into the juvenile form (Pauley 2004, Haines 2021). Once in the juvenile stage, it can take up to six years for mudpuppies to reach maturity (Haines 2021). Juveniles can be distinguished by their external red gills, striped black and brown pattern, and four toes on each foot (Petranka 1998, Dodd 2004, Mitchell and Gibbons 2010). Mudpuppies are unique in that the species is neotenic and paedomorphic, with adults retaining the red external gills (Mills and Hill 2016). Besides their red gills, adult mudpuppies have spotted patterns over the back (Petranka 1998, Dodd 2004, Mitchell and Gibbons 2010). Moreover, it is possible to distinguish individual salamanders based on their spot and scar pattern, which includes scars or wounds on any part of the body as well as other physical anomalies (e.g., Gamble et al. 2008). However, it should be noted that not all individuals will have a scar, especially if they are younger.

Habitat preferences differ between juvenile and adult mudpuppies. The adult Common Mudpuppy can commonly be found living under and around submerged boulders in the deeper parts of streams, creeks, lakes, and rivers (Petranka 1998, Fischer 2020). They burrow in the sand under these boulders to shelter themselves from predators, breed, and feed. Their diet consists of crustaceans, insects, fish, annelids, and mollusks (Petranka 1998, Collins 2017). Adults can also be found near large, submerged logs, as these fallen trees are very similar to the boulders that they are commonly found around (Fischer 2020). Juveniles live in submerged packs of leaf litter or dense root wads on the shore bank (Martof et al. 1980). These types of habitat provide ample protection from most predators of the young salamanders.

Many different organisms live alongside the Common Mudpuppy in its various habitats. Some of the organisms that are found in the leaf packs alongside the mudpuppies include dragonfly (Odonata) nymphs and crayfish (Cambaridae); large mudpuppies can prey upon these as well (Sollenberger 2013). Some adult mudpuppies may even take shelter inside crayfish burrows, adding an interesting dynamic to the species' habitat use (Collins 2017). Other types of juvenile salamanders can be found living beside young mudpuppies as well. This includes other aquatic salamander species and some terrestrial species which have aquatic larval stages, where the juvenile salamanders have gills and live in the protection of the water (Pierson and Miele 2019). Fish are commonly found swimming near the habitat of the adult mudpuppies. There are many different types of fish that live in the water systems with the salamanders, including chubs (*Cyprinidae*), sunfish (*Centrarchidae*), catfish (*Ictaluridae*), and sculpins (*Cottidae*) (L. Williams, North Carolina Wildlife Resources Commission, pers. comm.). These fish use the boulders and rocks as protection, in much of the same way as the Common Mudpuppy does.

Stream salamanders are commonly used as an indicator for stream disturbances as they have relatively stable populations and respond quickly to environmental changes (Weaver and Barret 2018). Many salamander species around the world are facing population declines due to several external factors, including habitat loss and degradation, pesticide use, disease, and invasive species (Chellman et al. 2017). Common Mudpuppies are not exempt from these declines. In states with better-studied Common Mudpuppy populations, such as Indiana, Illinois, and Ohio, populations were found to be in decline due to siltation and poor water quality (Hoffman et al. 2014). To determine these effects on the Common Mudpuppy in the southern Appalachians, the Little Tennessee River watershed was selected for sampling.

The Little Tennessee River begins in northern Georgia, running north through western North Carolina and ending in eastern Tennessee (Miller and Mackin 2013). The river and its tributaries run through the Blue Ridge Mountains in western North Carolina, through different landscape types found in a highly weathered and forested area (Jackson et al. 2021). In the early 1800s, settlers arrived, and native Cherokee tribes were forcibly removed (Jackson et al. 2021). These settlers clear cut the forest in the mid to late 1800s, resulting in residences, farms, and the expansion of roads into the area in the 1900s (Bolstad and Gragson 2008, Kirk et al. 2012). In the 1980s and 1990s, the region experienced a spike in development, where the exurban area increased from 54% to 65% over 20 years (Kirk et al. 2012). These developments have affected water quality through increased sedimentation and nutrient concentration, caused habitat fragmentation through the construction of roads, and diminished forest quality (Kirk et al. 2012, Jackson et al. 2021). This diversity of land use surrounding potential habitat locations allows us to determine how Common Mudpuppy populations are being affected by these external factors.

To determine this relationship, specific sample locations were selected using previous environmental DNA (eDNA) positives for the Common Mudpuppy within the watershed. Every living organism sheds DNA at some point in their life span, such as skin, hair, and scales (Barnes et al. 2014). EnvironmentalDNA is a novel species detection method that utilizes this shed DNA to detect a desired species in different areas, including Common Mudpuppies (Collins et al. 2019). Environmental DNA is sampled in streams and rivers because the DNA is moved throughout the stream and allows for detection that minimizes habitat disruption (Deiner et al. 2016). The North Carolina Wildlife Resources Commission has conducted eDNA surveys for the Common Mudpuppy, collecting positive samples within several streams in the Little Tennessee River watershed (L. Williams, North Carolina Wildlife Resources Commission, unpublished data).

We used two sampling methods, aquatic dip nets and minnow traps, to try and locate Common Mudpuppies in streams where eDNA has been detected in the past. We then utilized GIS and land cover data to look at any relationships between land use and streams that have been identified as containing Common Mudpuppies compared to streams where we failed to detect them. We hypothesize that streams where eDNA or individuals have been found will contain more forests and less developed land compared to streams where mudpuppies have not been detected.

METHODS

Study Area and Sampling Sites

In order to conduct a comprehensive survey on the Common Mudpuppy population, we used survey methods to look for both juveniles and adults in the Little Tennessee River watershed within Macon County, North Carolina, which is located in the Blue Ridge physiographic province. We surveyed streams and rivers that had either a positive eDNA sample or a successful Common Mudpuppy capture or had suitable habitat (Fig. 1). The North Carolina Wildlife Resources Commission identified 20 positive sample surveys in four different sub-watersheds within the study area since 2011 (L. Williams, North Carolina Wildlife Resources Commission, unpublished data). Suitable habitat for Common Mudpuppies is described as leaf litter packs, woody debris such as log jams and root wads, or the presence of large boulders within the stream channel (Chabarria et al. 2018).

Survey Methods

We used dip netting to try and capture juveniles and baited minnow traps to locate adults (Gendron et al. 1997, Collins 2017). When conducting the survey, we looked for study areas that contained multiple sampling sites and recorded the GPS coordinates with a handheld Garmin GPSmap 62s. Sampling was conducted between August 23, 2021, to November 2, 2021, where dip netting was used at 29 sample sites and minnow traps were used at 24 sample sites.

When in the juvenile stage of the life cycle, Common Mudpuppies can be found in leaf packs or root wads within streams. In order to sample these habitat types, we used D-ring dip nets (Collins 2017). These nets were used to collect a portion of the sampled habitat, including the leaves making up the pack and anything living within it. This sampling was done in minute intervals by multiple collectors and then picked through to find Common Mudpuppies.

We deployed minnow traps in the streams to find adult Common Mudpuppies (Gendron et al. 1997). These traps are made of metal mesh and are shaped like buckets, except the bottom of the buckets consists of a cone with a hole at the top. The trap works by hooking two of the “buckets" together to make a cylinder shape. The animal then smells the bait and enters through the hole in the cone, where it then becomes trapped inside. We used chopped chicken liver to bait the traps, as this has been the most successful bait used to catch Common Mudpuppies in North Carolina (L. Williams, North Carolina Wildlife Resources Commission, pers. comm.). We placed the traps in locations with suitable habitats, which include large rocks that are submerged in the deeper parts of the river and submerged logs (Haines 2021). To place the traps in the river, we first hooked a rope to each trap and then attached 1-2 bricks to the rope, which weighed the traps down and kept them in place where the river current was swift. Then, we walked them out to the spot where we wished to place them, or we threw them into the river near the potential Common Mudpuppy habitat. After the traps were in place, we tied the rope to a tree or some exposed roots, and then tagged each trap with a number. The traps were set overnight to allow for a collection time of roughly 24 hours (Gendron et al. 1997).

Land Cover Analysis

To discern the land cover types for the eight sample watersheds, we used the geographic information system application QGIS. Through this software we added our survey point data with county and watershed shapefiles to generate maps that displayed the survey sites within the watersheds, as well as the land coverage of Macon County, as a whole and for each of the sample watersheds. First, we generated a map of Macon County with the eight watersheds and all survey sites (Fig. 1). The sites were input as point vectors and divided into three categories, each of which was selected and exported as a new shapefile. The three categories were dip nets, minnow traps, and minnow traps and dip nets. A map was then created showing the land cover of the eight watersheds where we surveyed for the Common Mudpuppies, using the MRLC National Land Cover Database 2019 as the land cover raster (Figs. 2-9). We created vector files for each of the watersheds, which we did by creating new polygon shapefiles. Watershed land cover raster files were made by using the clip tool to combine the land cover raster data with each watershed's vector file. We then reclassified the land cover symbology of the newly formed layer to delete the excess symbology colors that do not appear within the watershed.

The land cover analysis for each of the creeks and rivers was limited, as the shapefiles that we created for each of the watersheds were not of the entire watersheds, but only of the sections of the watershed that were nearby and directly upstream of the sampling sites. We created the watershed vectors by either stopping where the sampled tributary entered another body of water, or when we had gone at least a kilometer upstream from the sample site.

RESULTS

Survey Methods

Using the Common Mudpuppy positive sample site information, 53 sample surveys were conducted in eight sub-watersheds (Fig. 1, table 1). Of these surveys, 24 minnow trap surveys were conducted (n = 1 trap night/site) and 29 surveys were dip netting. These surveys failed to capture any salamanders.

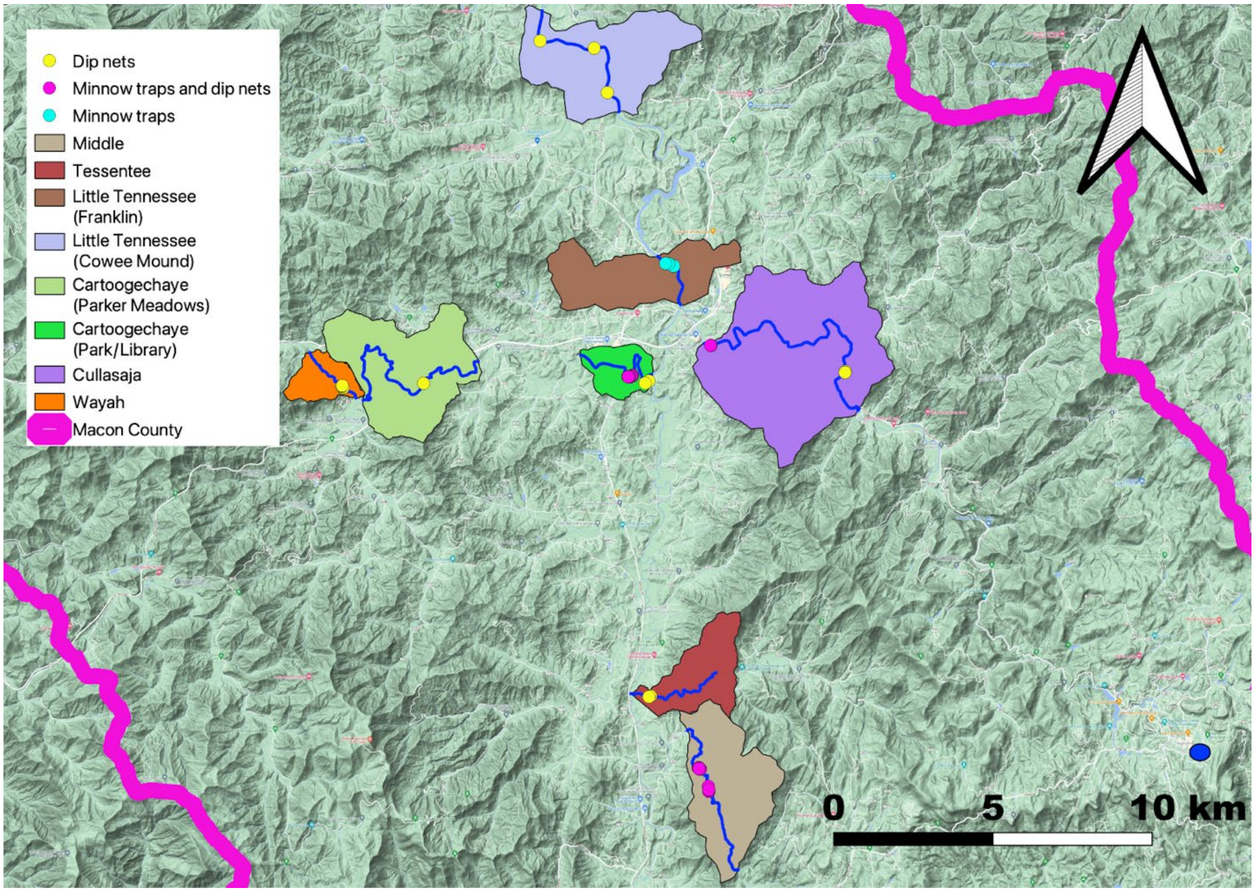

FIG 1. Macon County overlaid with the watersheds within the Little Tennessee River drainage and depicting all of the Common Mudpuppy survey sites sampled. The blue dot in the lower right corner represents Highlands, NC.

Land Cover Analysis

Through the GIS tools, land cover distributions were determined for each of the eight sample watersheds. Some of the land cover types were grouped to represent a general land type. The developed category represents open space, low intensity, medium intensity, and high intensity development. The forest category represents deciduous, evergreen, and mixed forest, and shrub. Agriculture is made of pasture/hay and cultivated crops land cover. Wetlands comprises woody wetlands and emergent herbaceous wetlands. The water and barren land covers were not grouped with other land cover types.

From the GIS analysis of the land cover percentages, seven out of the eight watersheds had forest cover as the highest land cover, with a mean of 70.4% (range = 47.1 - 90.8%). Development was the highest land cover at the Little Tennessee River at the Franklin Greenway, at 65.4%. Only four of the land cover types were in each watershed: forest, mean cover was 65.1%, development, mean cover was 23.8%, agriculture, mean cover was 10.0%, and grassland, mean cover was 0.64% (Fig. 10).

TABLE 1. Sub-watersheds with survey site number and type.

Stream Name	Sites Sampled	Survey Type
Cartoogechaye Creek (Lower)	10	Minnow Trapping
	6	Dip Netting
Cartoogechaye Creek (Upper)	2	Dip Netting
Cullasaja River	6	Dip Netting
	1	Minnow Trapping
Lake Emory	4	Minnow Trapping
Little Tennessee River (Lower)	3	Dip Netting
Middle Creek	5	Dip Netting
	9	Minnow Trapping
Tessentee Creek	2	Dip Netting
Wayah Creek	5	Dip Netting

Middle Creek was found to be predominantly forest, with 90.8% of the land being covered in trees or shrubs, 5.49% developed land, 3.62% farmland, and 0.07% grassland (Fig. 2, Fig. 10). There were two distinct sample watersheds from Cartoogechaye Creek, one which was at the Parker Meadows Complex, which is predominantly forest, with the land cover being 66.9% forest or shrubs, 18.4% developed land, 0.37% grassland, 14.2% farmland, and 0.14% wetland (Fig. 3, Fig. 10). The second watershed from Cartoogechaye Creek was at the Macon County Recreation Park, which is primarily forest as well, with the land cover being 47.1% forest, 38.1% developed, 14.0% farmland, 0.59% grassland, and 0.19% barren land (fFg. 4, fFg. 10). The Cullasaja River watershed is mainly forest land cover, with 65.6% being covered in forest, 24.4% developed, 9.36% agriculture, 0.50% grassland, 0.12% barren, 0.04% water, and 0.01% wetlands (Fig. 5, Fig. 10). The Wayah Creek watershed was also predominantly forest land cover, at 77.5%, 13.4% agriculture, 7.52% developed land, 1.46% grassland, and 0.17% wetlands (Fig. 6, Fig. 10). Little Tennessee River comprises two of the sample watersheds, one of which was from below Lake Emory, which had a forest land cover percentage of 67.8%, followed by 16.9% developed, 13.2% agriculture, 1.15% water, 0.85% grassland, 0.05% wetlands, and 0.03% barren (Fig. 7, Fig. 10). The second of these watersheds is at the Little Tennessee River Greenway, has predominantly developed land cover, at 65.4%, followed by 27.8% forest, 4.86% agriculture, 1.04% wetlands, 0.55% grassland, 0.29% water, and 0.10% barren land (Fig. 8, Fig. 10). The last of the watersheds was at Tessentee Creek and is comprised mainly of forest land cover, at 77.3% forested, 14.5% developed, 7.46% agriculture, and 0.72% grassland (Fig. 9, Fig. 10).

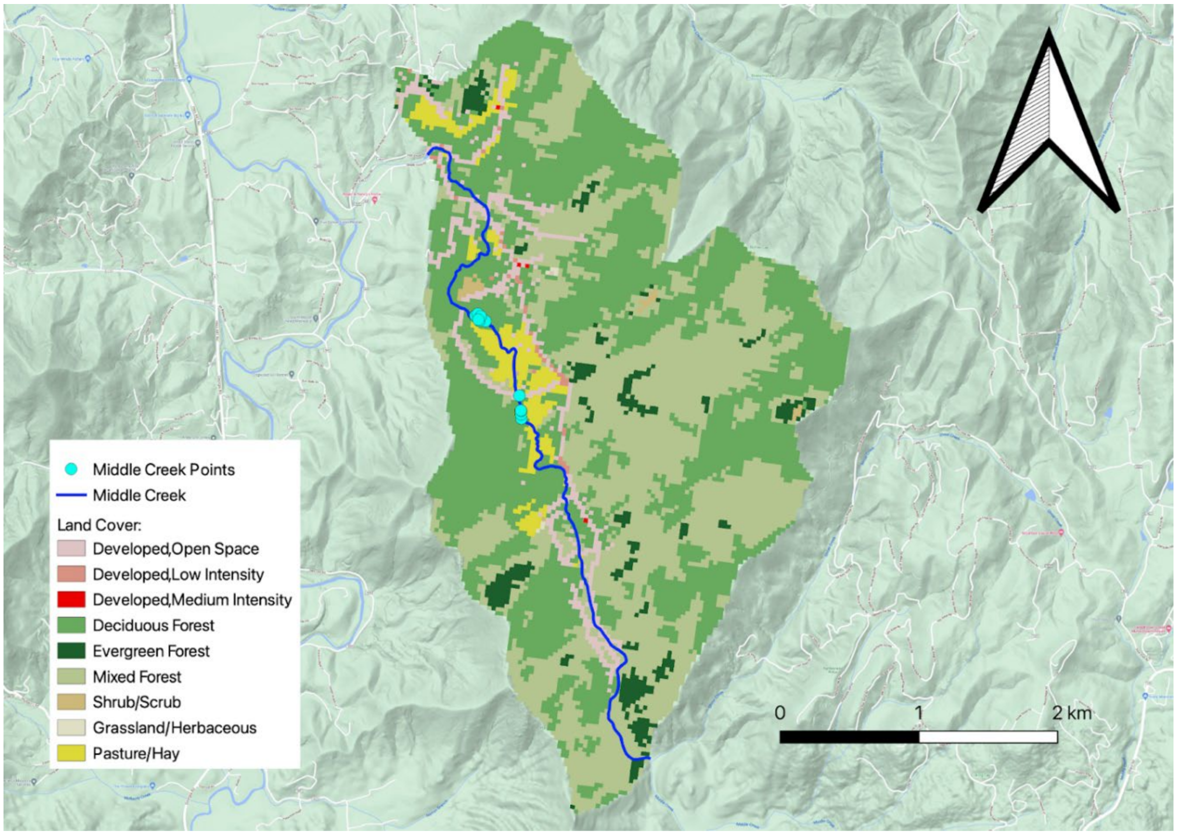

FIG 2. Land cover types of the Middle Creek watershed.

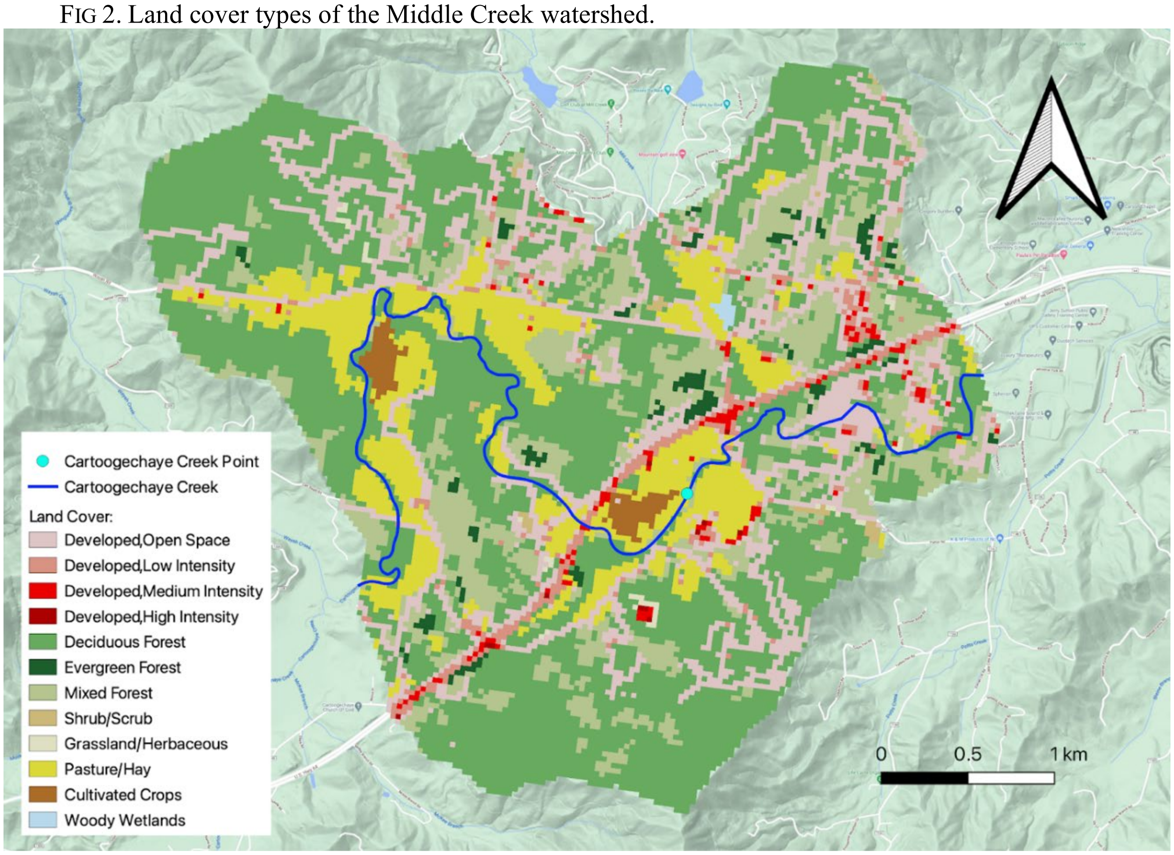

FIG 3. Land cover types of the Cartoogechaye Creek watershed at Parker Meadows.

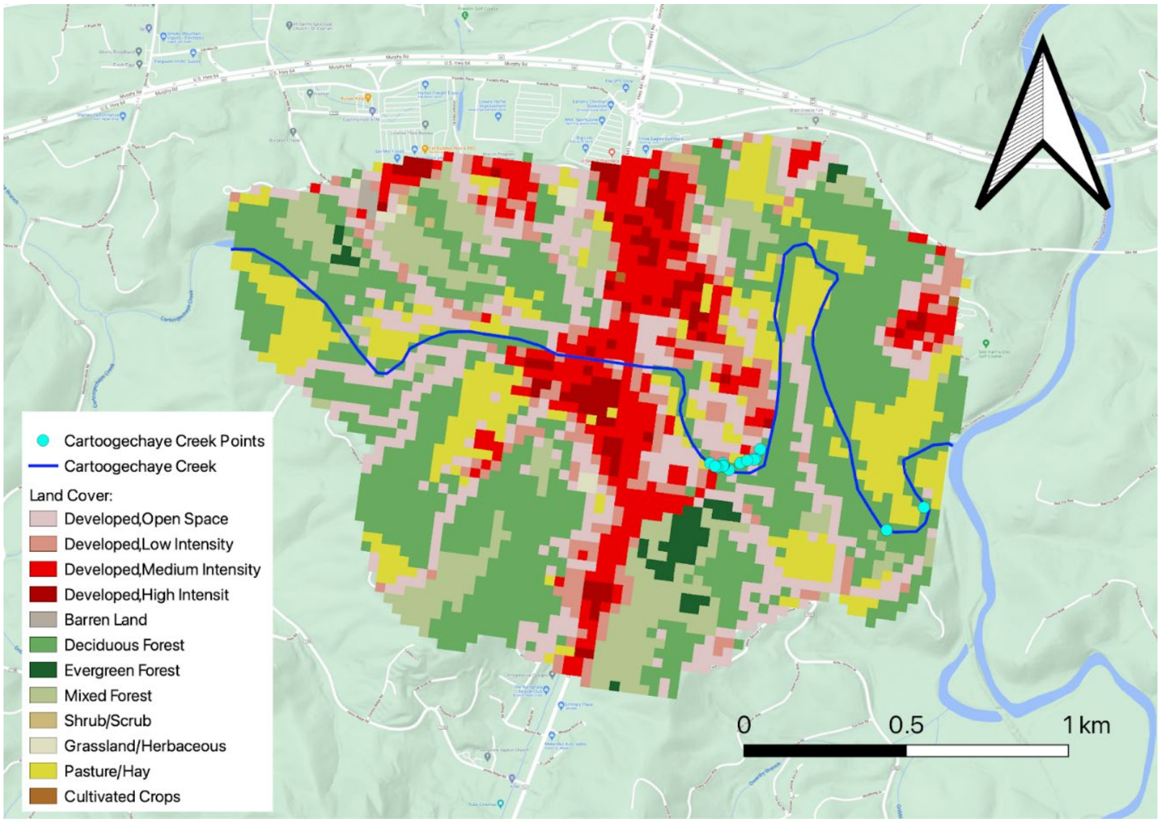

FIG 4. Land cover types of the Cartoogechaye Creek watershed at the Macon County Recreation Park.

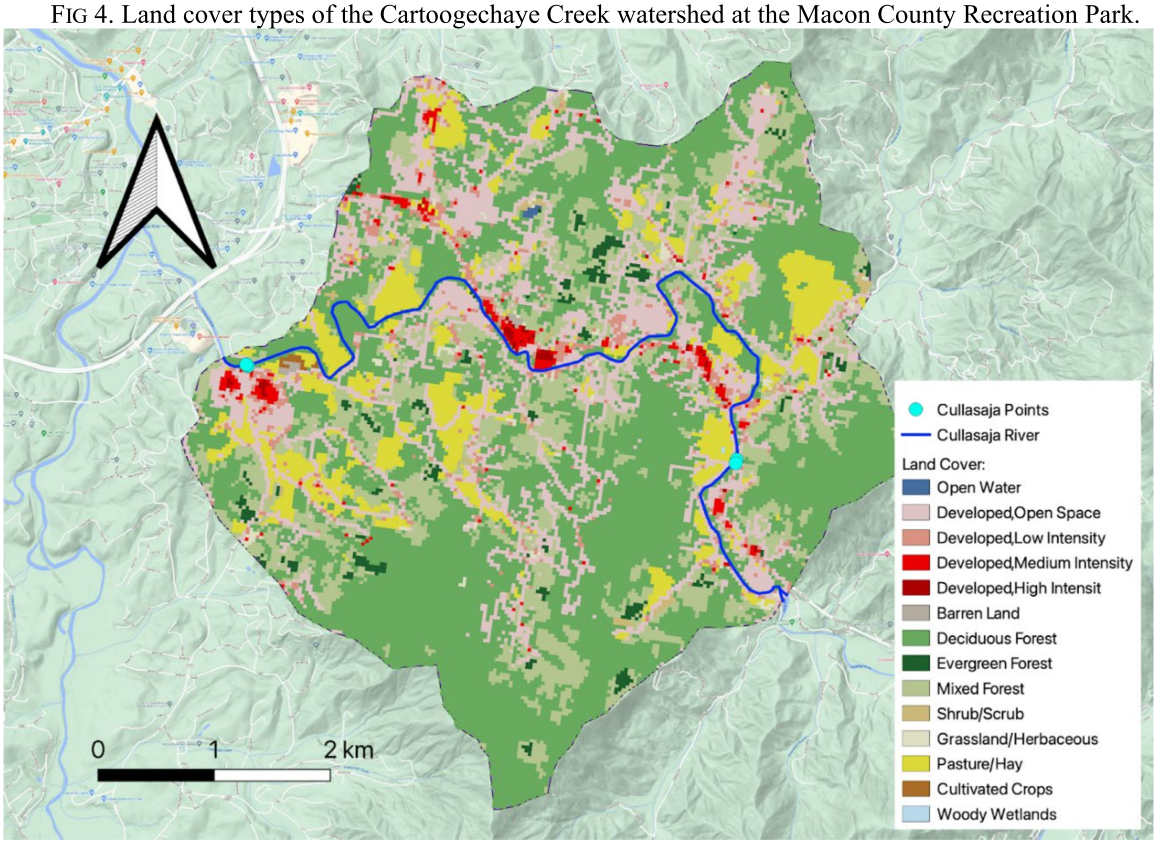

FIG 5. Land cover types of the Cullasaja River watershed.

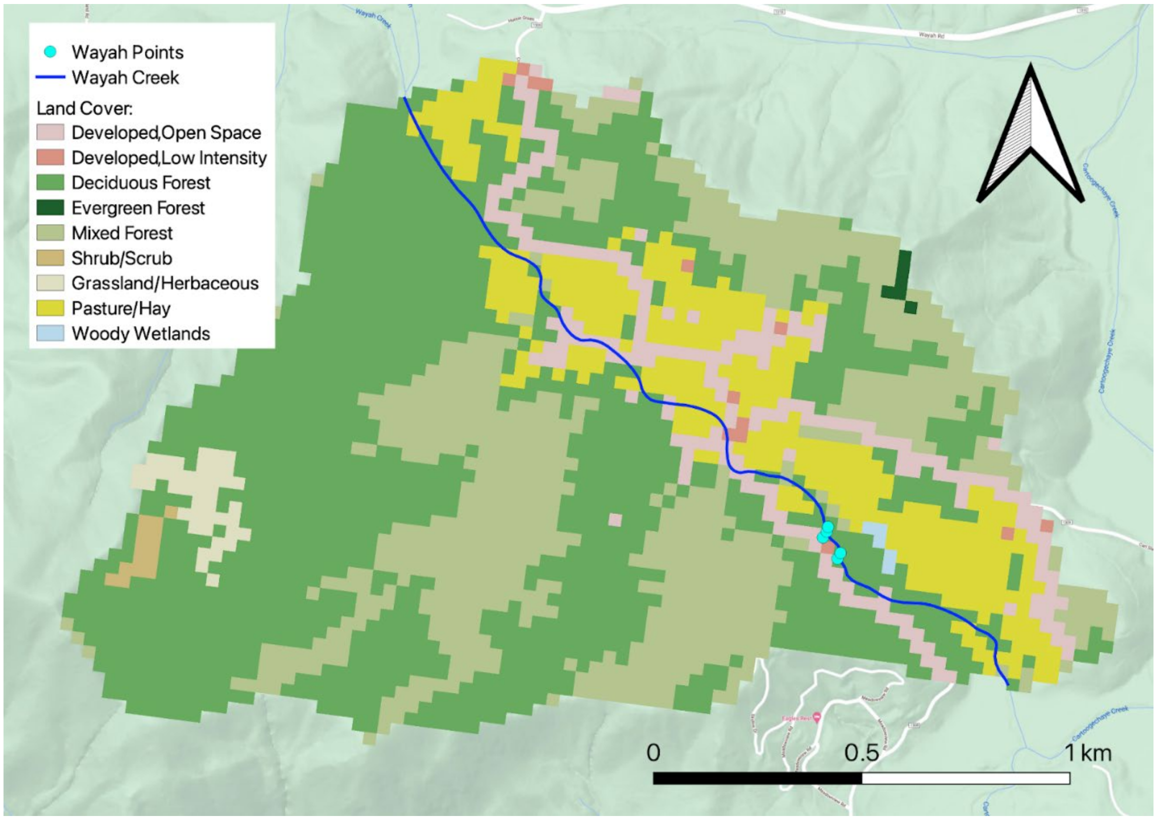

FIG 6. Land cover types of the Wayah Creek watershed.

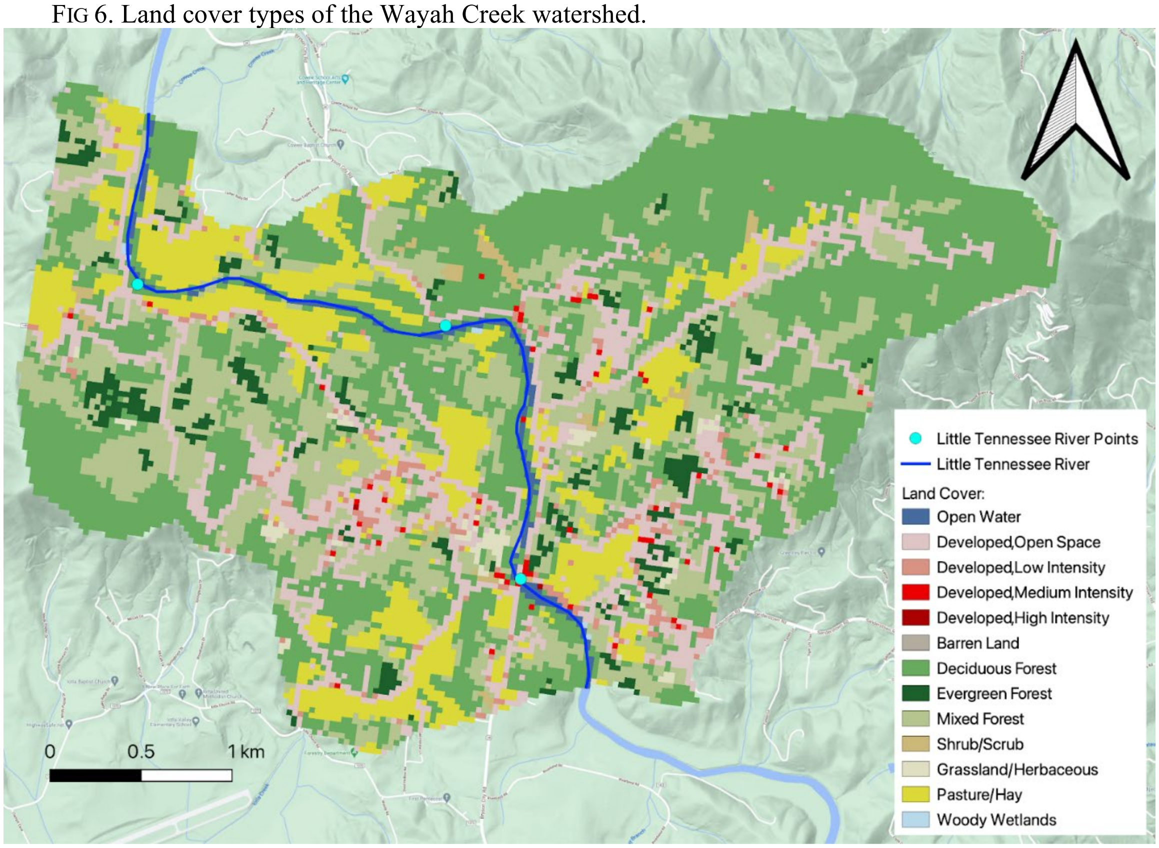

FIG 7. Land cover types of the Little Tennessee River from below Lake Emory to the Cowee Mound.

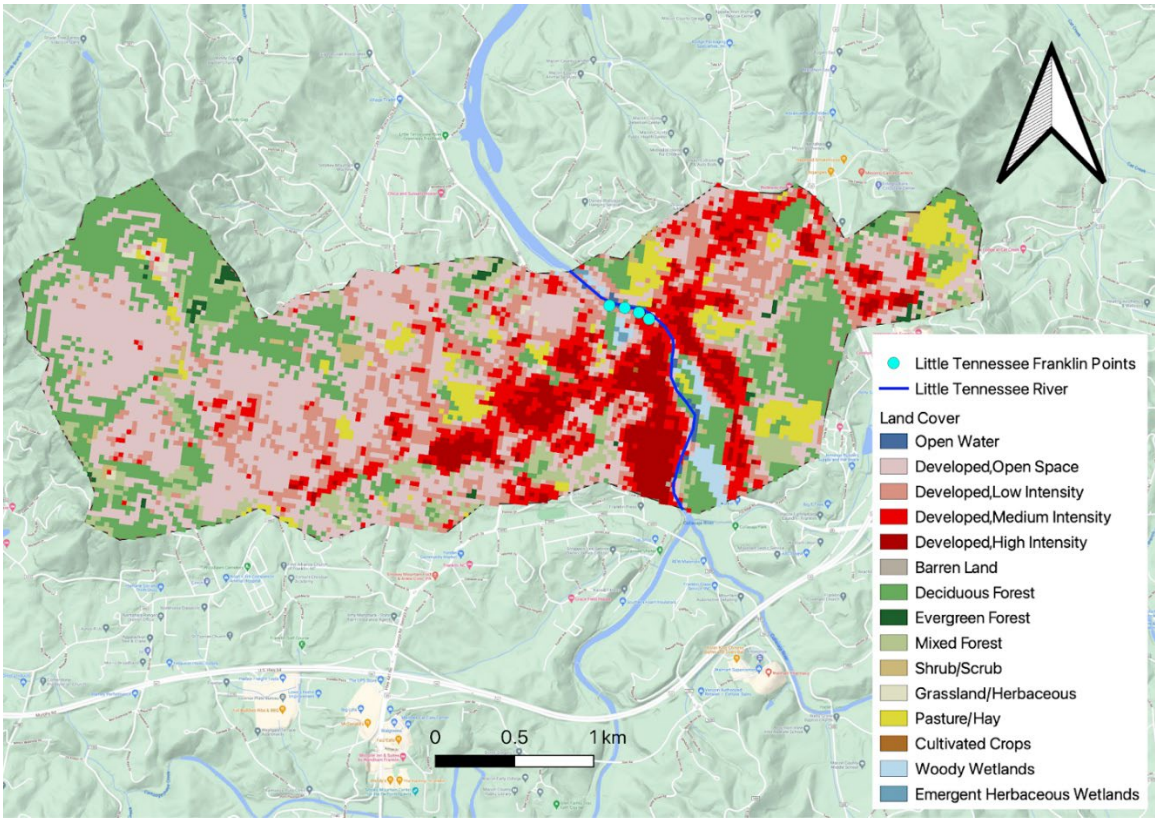

FIG 8. Land cover types of the Little Tennessee River watershed at the Little Tennessee River Greenway.

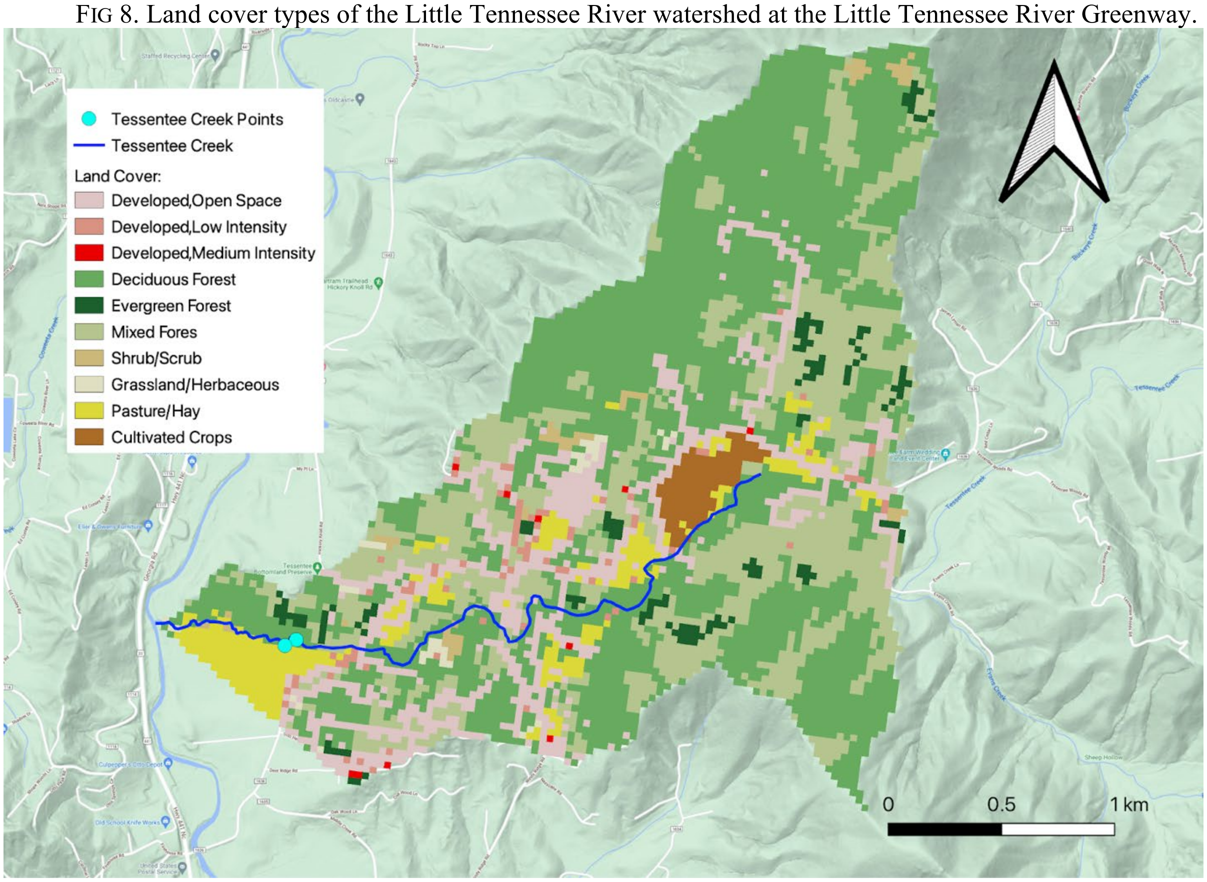

FIG 9. Land cover types of the Tessentee Creek watershed.

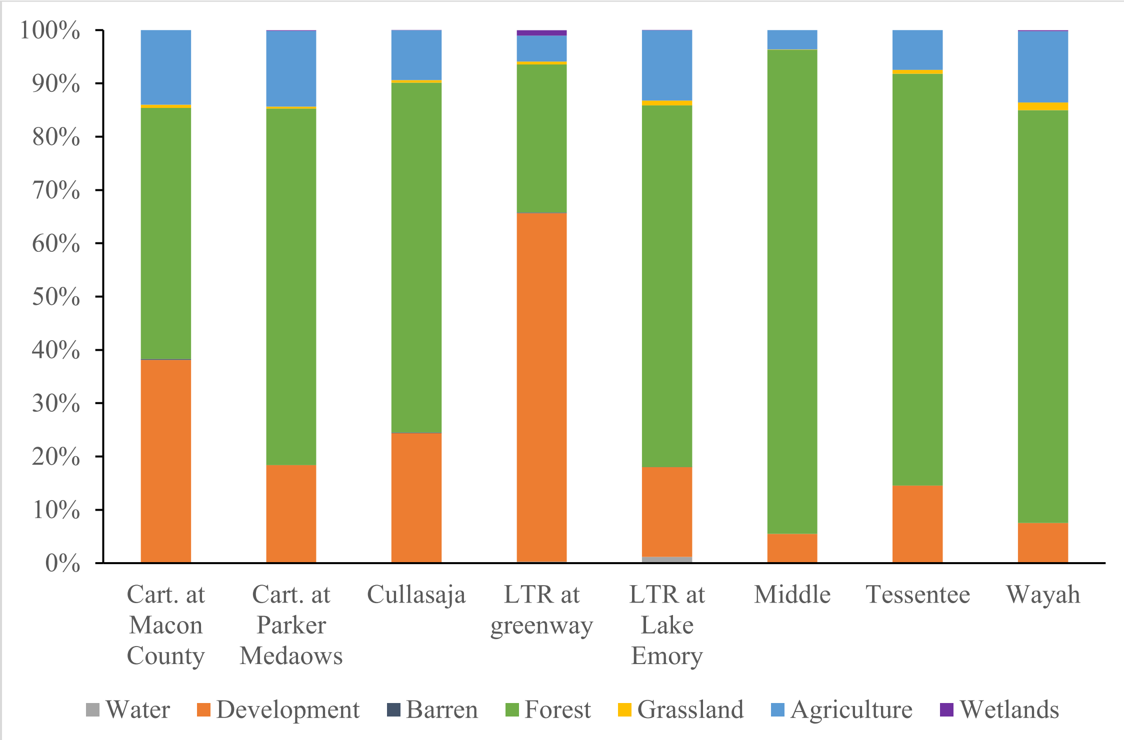

FIG 10. Land cover percentages for each watershed. The abbreviation Cart. represents Cartoogechaye Creek and the abbreviation LTR represents the Little Tennessee River.

DISCUSSION

Forest, development, and agriculture were in the top three land cover types for all watersheds. Similar land cover percentages were found by Webster et al. (2012) for watersheds in the Little Tennessee River Basin.

The survey sites used for this study were all located in areas where evidence of Common Mudpuppies had previously been found or in areas with the ideal habitat for the species (Petranka 1998). Despite this, no Common Mudpuppies were found through our sampling efforts. We found no correlation between the predominant land cover types and the streams that had evidence of Common Mudpuppies and those that did not (Fig. 10). Due to the lack of any key differences in land cover type, the influencing factor between being a Common Mudpuppy positive or negative stream might result from outside stressors.

One of the potential impacts on Common Mudpuppies that cannot be seen through GIS analysis is direct human impacts on water quality. In rural landscapes, such as this study area, water quality is controlled by near-stream and basin-wide land uses (Jackson et al. 2021). One of these water quality controlling factors is basin-wide use of impervious surfaces, which have been linked to decreases in salamander abundance due to increased flooding (Weaver and Barrett 2018). Some near-stream influences are local landowner practices including illegal water discharge, poor waste and manure management, pesticide use, and fertilizer application (Webster et al. 2012, Jackson et al. 2021).

A second phenomenon with potential implications on Common Mudpuppy distribution is legacy effects. Historically, our study area had small local milldams, farming for corn, cotton, tobacco, and wheat, and logging in the 1950s and 1960s (Webster et al. 2012, Surasinghe and Baldwin 2014). These previous land uses lead to increases in impervious surface and decreases in forest cover and vegetation complexity, which in turn caused chemical and biological process changes in streams (Surasinghe and Baldwin 2014). Some of these chemical changes can impact stream water quality, such as increases in dissolved inorganic nitrogen or elevated levels of phosphorus and potassium in the riparian zone (Jackson et al. 2021). These effects can last well after the affected habitat has been restored to its pre-disturbance state. These large-scale and long-term disturbances limit the recovery of biodiversity in streams for decades (Harding et al. 1998).

This study did have several limitations. When analyzing the eight sample watersheds, some of the watersheds were nested within other watersheds (i.e., Wayah Creek was nested within the Cartoogechaye Creek watershed). When the land cover percentages were discerned for each watershed, the land cover was analyzed for the stream reach that we sampled, which in turn left out the headwaters. Most of the headwaters for the streams, especially the larger rivers, like the Little Tennessee and the Cullasaja, are forested which skewed this analysis towards the valleys. This outcome resulted in the land cover analysis containing a larger percentage of agriculture and development for streams that most likely have good water quality because the headwaters are in forested mountain watersheds. In the future, the entirety of the watersheds should be analyzed to have a better understanding of the land cover types for the sample areas. Future analysis could also compare the land cover right beside the stream reach to the land cover that is 100 m adjacent to the stream. Water quality could also be tested during sampling to understand the health of each watershed in relation to presence of Common Mudpuppies.

This study also had some limitations when using the minnow traps. Typically, when using these traps to look for Common Mudpuppies, baited traps will sit in the river for a period of seven days and the bait is changed every three days. When this is done the traps are checked every day, but Common Mudpuppies are often found on the last day of trapping (L. Williams, North Carolina Wildlife Resources Commission, pers. comm.). Due to time constraints with this study, traps were only set for a period of 24 hours. If this study is replicated, traps should be set for longer periods of time to allow for a higher chance of trapping a Common Mudpuppy. Another unfortunate constraint was the timing of the trap surveys, since it was outside of the breeding season, and waters were warmer in September and October compared to the ideal time of December for trapping.

The Common Mudpuppy has proved elusive not only in this study but also in other surveying efforts. This could potentially be due to the species rarity in North Carolina or uneven distribution within watersheds. Due to low detection rates from current sampling, survey efforts need to be increased. One method that can be used to accomplish this is eDNA. Environmental DNA is a great tool for understanding the distribution of species where the target species is difficult to detect (Pilliod et al. 2014). It appears that the Common Mudpuppy has low densities in the region, making eDNA a useful tool to use for surveying. This tool allows for detection rates that traditional surveying methods, like dip netting or trapping, cannot always achieve (Pilliod et al. 2014). One of the potential issues with eDNA detection is that Common Mudpuppies do not shed as much DNA into water bodies as other stream dwelling salamanders, i.e., Hellbenders (L. Williams, North Carolina Wildlife Resources Commission, pers. comm.). This issue could be solved by shortening the distances between collection locations for eDNA and increasing the number of sampling efforts during the breeding and nesting season, when Common Mudpuppies would be more active. Environmental DNA has other problems, such as misidentifying the species that DNA belongs to or the sensitivity of the identifying mechanism, but it is still useful for elusive animals (Furlan et al. 2016). These problems with eDNA can also be minimized as the understanding of this detection method grows. One of the ways to avoid uncertainties in the sample eDNA is through species specific assays for amplifying DNA, which has been developed for *Necturus* spp. (Collins et al. 2019).

Salamanders are key to understanding stream disturbances and are key species to use in the southern Appalachians, the global biodiversity hotspot for the taxon (Weaver and Barrett 2018). While this study found no obvious links between the predominant land cover types and the occurrence of Common Mudpuppies, unsustainable land use is the main cause of biodiversity loss worldwide (Surasinghe and Baldwin 2014). Protecting riparian buffer zones, along with in-stream habitats, is crucial for salamander conservation because stream-dwelling salamanders rely on both (Cecala et al. 2018, Jackson et al. 2021). Current efforts are focused on habitat preservation, but protection and expansion of riparian buffer zones is necessary, as their current use in watershed management is not effective for stream salamander conservation (Willson and Dorcas 2003). Conversion of the forests in these habitats has led to negative impacts on distribution and diversity of stream-dwelling salamanders (Cecala et al. 2018). Overall, to protect salamanders, organisms that have little resilience to environmental changes, not only is local stream and riparian zone protection necessary, but whole watershed management is key to species survival (Cecala et al. 2018).

ACKNOWLEDGMENTS

We would like to thank our mentors Lori Williams, North Carolina Wildlife Resources Commission, and Jason Love, Highlands Biological Station, for their guidance through this project. We would like to thank Mainspring Conservation Trust, Dick and Gil Heywood, Bill Roof, and Mysa Crawford Mathews for permitting us to conduct the surveys on their properties. Common Mudpuppy surveys were conducted under North Carolina Wildlife Resources Commission Endangered Species Permit 21-ES00639 and Highlands Biological Station IACUC Number 21-09.

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Alex Hubbs and Eva Kinney searching for Common Mudpuppies in Middle Creek.

COLOR COMPARISON OF TWO PHENOTYPES OF MALE BLUE RIDGE TWO-LINED SALAMANDERS

VY PHAM AND RACHEL LOPEZ

Abstract. Two phenotypes of male Blue Ridge Two-Lined Salamanders (*Eurycea cf. wilderae*) are classified by a polymorphism in physical traits. "Searching" males are identified by mental hedonic glands, premaxillary teeth, and cirri while "guarding" males are identified by their muscular jaws. Although it has been speculated that these two forms differ in color, this has never been formally studied. The study of color polymorphism between the two male phenotypes and females can contribute to better understanding differences in their sexual selection and reproductive behaviors. We conducted two different surveys in streams and on trails adjacent to streams during rainfall events to closely examine the habitats of each male phenotype and females. Photographic data collection and color analysis were used to compare the color similarities and differences between the "searching" males, "guarding" males, and mature females. We found that the two male phenotypes are spatially segregated since all "searching" males were found on trails while "guarding" males were primarily found in the streams. We also concluded that there were differences in coloration between the males since "searching" males show a lighter shade of brown when compared to darker "guarding" males, but no significant indication had been made for the color correlation between each male phenotype and females in their shared habitats. The data may indicate that each phenotype adapts to their breeding habitats through their color polymorphisms to increase their reproductive rate.

Key words: Eurycea cf. wilderae*; "guarding" male; "searching" male; female; color comparison; trait; polymorphism; aquatic habitat; adaptation; terrestrial habitat; sexual selection; reproductive behaviors.*

INTRODUCTION

Reproductive success plays a significant role in natural selection and ensures DNA and advantageous traits are passed down to the next generations. Different species adapt to different reproductive behaviors, and selection pressure challenges each species to establish its adapted behavior to increase survival and reproductive rate which leads to divergent evolutionary dynamics (Halliday 1990, Fabre 2020). Alternative reproductive tactics is a form of evolutionary divergence that organisms apply to adapt for higher chances of reproductive success (Moore and Jessop 2003). Color polymorphisms are widely observed in amphibians and much evidence shows that this polymorphism is maintained by selection (Moreno 1989). This is seen in Blue Ridge Two-lined Salamanders (*Eurycea cf. wilderae)*, with females and the two male tactics (Sever 1979). This study is focused on the color similarities and differences between the two male phenotypes and the *E. cf. wilderae* females according to their choice of habitats to analyze how the polymorphic color differences contribute to their reproductive behaviors.

Salamanders (Caudata) are primarily found in riparian habitats of temperate forests (Crawford and Semlitsch 2007). They live in or near water or find shelter on moist ground and are typically found in brooks, streams, ponds, and other moist locations such as under rocks. *Eurycea cf. wilderae* is distinguished from other salamanders within its range by its striking yellow, brown, or orange body and broad dark stripes (Petranka 1998); and both female and male *E. cf. wilderae* share similar coloration (Sever 1979). The first phenotype, the "searching" males, are differentiated with mental hedonic glands, premaxillary teeth, and elongated cirri; ideal traits for locating and courting females (Sever 1979, Pierson 2019). "Searching" males tend to have a smaller body size, and it is possible that the variation is ontogenetic which is related to shifts in reproductive behavior with age (Bruce 1988). These salamanders are called “searching” males because they are likely to explore terrestrial habitats for females during the breeding season. The second phenotype has much larger and more muscular jaws and is called the “guarding” male due to their mating behaviors. The “guarding” males are adapted for “aggressively guarding females” in aquatic habitats, then guard the females (and perhaps the eggs) after mating (Rainey et al. 2021). These polymorphic and habitat differences contribute to the distinct difference in each male and female type’s behavior which affects their ability to successfully mate. By surveying polymorphism differences between the two male types and the mature females of *E. cf. wilderae*, further discoveries can be made to understand the species’ sexual selection and reproductive behavior.

In a previous study, Sever (1979) compared the distinct physical differences between the two male phenotypes and mature females by examining data from the salamander's mental hedonic gland, premaxillary teeth, head size, temporal musculature, and cirri. Habitat and distribution were studied by Sever (1979) to assess the ecological effects on the difference between the two male types. It was concluded that “searching” males possess mental hedonic glands, cirri, and reduced temporal musculature, whereas “guarding” males have a strongly developed temporal musculature, but lack mental hedonic glands and cirri. Also, most mature females share the same characteristic feature with the “guarding” male by having temporal musculature. *Eurycea cf. wilderae* do not exhibit any indicators of these features pre-metamorphosis as juveniles. Another study by Pierson and Miele (2019) observed scars on male salamanders' tails consistent with mate-guarding behavior. It also found guarding males present at communal nests. The presence of these males at nests may be coincidental and caused by mate-guarding near nest sites, but repeated observations of the same male at the same nest suggest that both parents, not just the female, could be guarding these nests. Features that differ between the two phenotypes and sexes of salamanders like cirri, jaw size, and mental hedonic glands can affect the sexual selection, but no research has been done on how the difference in the coloration of these two male phenotypes and mature females can affect sexual selection. A previous study conducted by Acord et al. (2013) observed the mating patterns of striped and unstriped Eastern Red-backed Salamanders (*Plethodon cinereus*). They found that striped and unstriped females were more likely to mate and associate with striped males, suggesting that striped males are more attractive to females. Another study on color and sexual selection of cichlid populations concluded that color may be important when females are choosing between males of a similar phenotype (Pauers and Mckinnon 2012). This conclusion can be looked at through the lens of *E. cf. wilderae’s* reproduction since searching and guarding males look similar despite them technically being two separate phenotypes.

In this study, we used terrestrial and aquatic surveys at Highlands Biological Station in western North Carolina to investigate two ideas. First, we tested the theory that during the breeding season, searching and guarding males of *E. cf. wilderae* are strongly spatially segregated, indicating that these alternative reproductive tactics have strongly divergent reproductive phenologies. The two phenotypes' different features contribute to their spatial segregation since their characteristics are well-suited for specific environments. We aim to solidify the theory that searching males mostly inhabit wooded/leafy areas and that guarding males mostly inhabit streams. Second, we evaluated the possibility that searching and guarding males have quantifiable differences in color pattern; and the color similarity between each male phenotype and females is due to their similar habitats.

METHODS

Site Description

Between August 24th and November 2nd, surveys were conducted at the Highlands Biological Station in Macon County, North Carolina. This area is part of the Blue Ridge physiographic province. Specifically, our study took place at Station Creek, a shaded second-order stream that flows through an acidic cove forest dominated by deciduous hardwoods with scattered Eastern Hemlock (*Tsuga canadensis*) and a dense understory of Rosebay Rhododendron (*Rhododendron maximum*) and Dog Hobble (*Leucothoe fontanesiana*) (Bruce 1988). We surveyed alongside and in the stream that flows into Lake Lindenwood at the elevation of 1,100 m. The site is considered a temperate rainforest that receives an average of 212.5 centimeters of rain per year, and the temperature varies between -5 to 25 degrees Celsius (HBS 2010).

Sampling

Sampling was done by using two types of surveys which were dependent on the behavior of each male type of salamander: 1) daytime surveys within the stream; and 2) nocturnal surveys on trails along the stream during and after heavy rain. Since most *E. cf. wilderae* are located under rocks, leaf packs, and other organic debris along the stream, during daytime, we used the dip net method to collect samples when searching within the stream. This method was most effective to collect the "guarding" male type of *E. cf. wilderae*. When executing this method, we set the rim of the net against the surface a little downstream from the water flow and used one hand to stir the stream substrate and other upstream debris which caused the salamanders to flow into the net. During the nighttime, the hand catching method was a more effective way to collect salamanders on trails along the stream. This method was used to capture the "searching" males who usually live on land and search for females during courtship season. Both hands must be moist before contacting *E. cf. wilderae* due to their absorbent, and sensitive skins. We placed one hand in front of the *E. cf. wilderae* and use the other hand to gently nudge the salamander toward the other hand to create a small trap. The survey varied throughout the week according to the weather, and we dedicated one to two days toward collecting samples every week. Headlamps were used at night when searching for salamanders on the trails and on some specific plants. Dog Hobble was a plant that was closely observed during sampling due to a higher chance of finding "searching" males. Due to the small collection of "searching" males, we collected additional samples from *E. cf. wilderae* research at Johns Mountain, Georgia which was conducted by Dr. Todd Pierson from Kennesaw State University.

Data Collection

Once a salamander was found, we placed it in a moist plastic bag to examine the ventral part of the body closely to determine its specific sex. Also, we examined either the cirri or the enlarged jaw muscles to classify whether it was "searching" male or "guarding" male. Several photos were taken during this process for clarification using an Olympus Tough TG-6 Waterproof camera. To capture the color, we set up a color palette inside a big, clear container to prevent *E. cf. wilderae* from escaping. The salamander was then released from the bag next to the palette inside the container. We took pictures of the salamander from the top, including the 24-color block palette in the photo. A black jacket was used to cover the container when taking the picture to reduce the glare on the salamander's skin from the reflection of the light and water droplets. All of the pictures were uploaded to Google Drive for archiving prior to color analysis.

Statistical Analyses

We used R and RStudio to quantify the color contrasts of the different phenotypes of male *E. cf. wilderae*. First, we standardized all of the photos to correct the coloration of the salamander. We used functions in the packages “colordistance,” “patternize,” and “imager” to read in the image and map the true colors of the squares on the color catcher palette. The software normalized the color of the salamander based on the true standard colors of the palette. Once we normalized the color of the salamander, the picture was cropped in Adobe Photoshop to show just the space on the salamander’s back. We defined the back as the region between the two stripes, behind the front legs and in front of the back legs. We also cropped out any reflections or glare on the salamander’s back. We then used the second code set to create plot pixels, color histogram, and a distance matrix for the 27 *E. cf. wilderae* samples. The collage of salamanders’ cropped backs was designed using the function “plotImage " that combined all of the cropped backs’ photos into a PDF file. We then used the “plotPixel" function to create a collection of 3D red-green-blue (RGB) plot pixels of all *E. cf. wilderae* samples. We also created a color histogram of all 27 samples to classify the color of each *E. cf. wilderae* by utilizing the function “getImageHist.” Afterward, we used the function “getColorDistanceMatrix" to create distance matrices that showed the similarity and contrast between the colors on the collected salamanders’ backs. Unlike the samples from Highlands Biological Station, we did not create the distance matrices for the 5 “searching” male data points from Johns Mountain, Georgia.

RESULTS

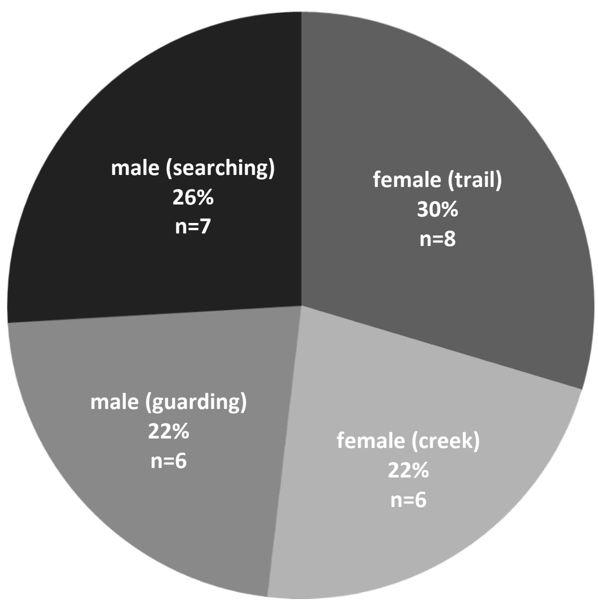

FIG. 1. The pie chart shows the percentage of each sex groups in our collected *E. cf. wilderae* samples. The male salamander groups occupied 52% of the total data, in which, guarding male group covered 22% and 26% for searching male group. The females are divided into 2 groups which are according to their mating habitats: on trails or in the creek.

Sample Collection

During the survey, we collected a total of 22 individuals from different sex groups (Fig. 1). We added 5 "searching" males from similar *E. cf. wilderae* research from Dr. Todd Pierson at Kennesaw State University to increase our "searching" male sample size. A total of 13 males were studied in this survey, with 6 "guarding" males and 7 "searching" males. We observed that "searching" males were highly active on the trails along the stream during and after heavy rain, especially on the surface of the leaves of Dog Hobble, while the "guarding" males were abundant in the stream. In the courtship season, 100% of our "searching" male samples were collected on trails, and 100% of our "guarding" male samples were collected in the stream. While searching for the two male phenotypes of *E. cf. wilderae*, we found a large number of female *E. cf. wilderae* on trails and in the stream. Female *E. cf. wilderae* made up 52% of the total sample size, of which 8 *E. cf. wilderae* females were found on trails and 7 were found in the stream.

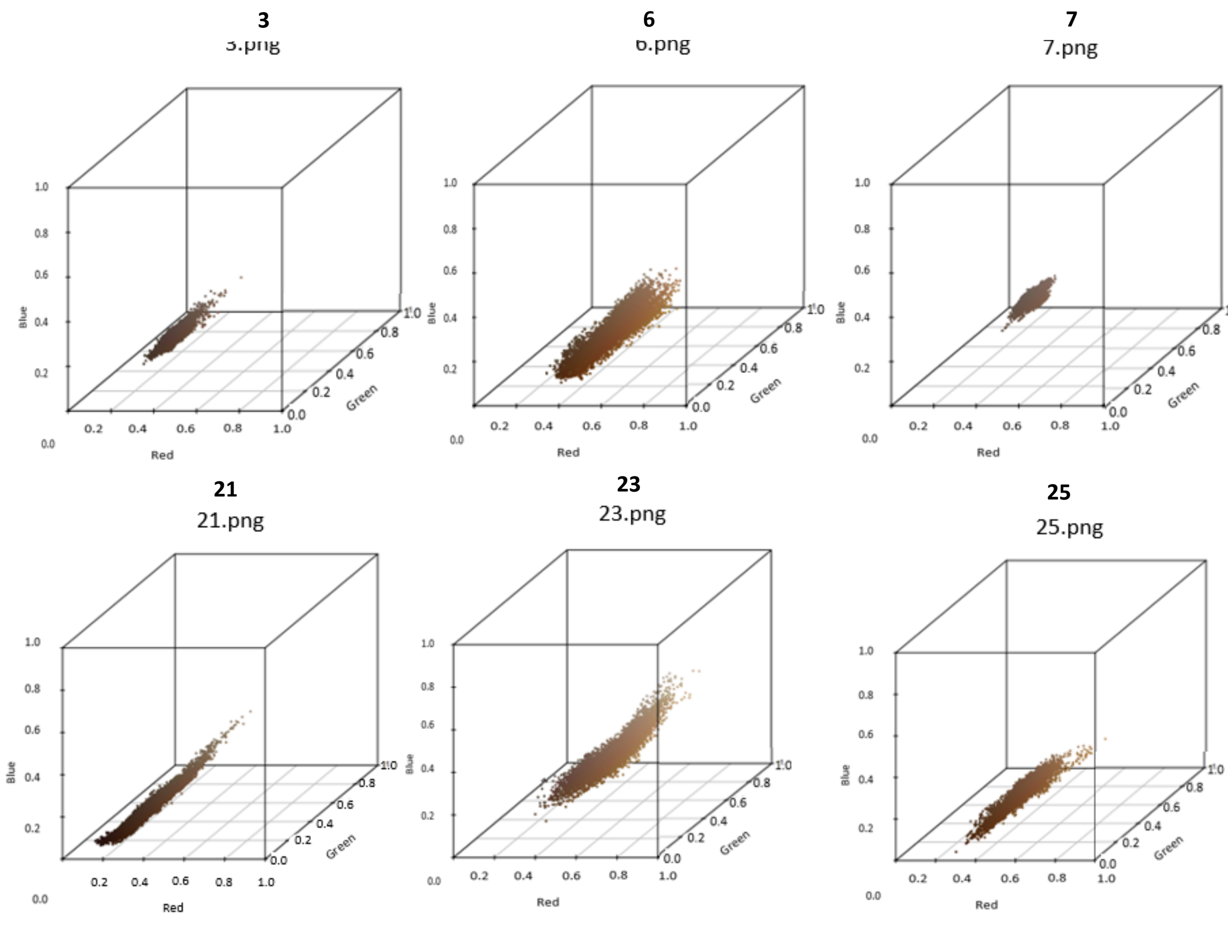

Fig. 2. 3D RGB plots of pixels for 6 "guarding" *E. cf. wilderae* males which expresses the color gradient of each sample in the range of blue (height), red (width), and green (length). This indication shows the amount of red, blue and green color pigments in each sample's cropped back.

Two Male Phenotypes Comparison

The 3D plots of color pixels explain the amount of RBG (red, blue, green) pigments that each sample's color contained (Figs. 2 and 3). Guarding male graphs #3, #7, and #21 all have red values at or below 0.2, which demonstrates a much darker brown shade while guarding male #6, and #25 has red values at 0.2 or below 0.4, which indicates a more reddish-brown shade (Fig. 2). The "searching" male group showed a slightly lighter brown shade when compared to the "guarding" male group. According to the "searching" males' pixel collection, the majority of "searching" male samples ranged from a red value at 0.0 to approximately 0.6, which shows a considerably wider color range and a much lighter shade (Fig. 3). The exception of "searching" male #5 showed a shorter color range between 0.2 and 0.4 (Fig. 3). This result shows that the colors of the "guarding" male group varied in a wide range of color shades from dark brown-gray to light mustard, which is much darker than the "searching" male group that varied from medium brown shades to light brown shades (Figs. 4 and 5).

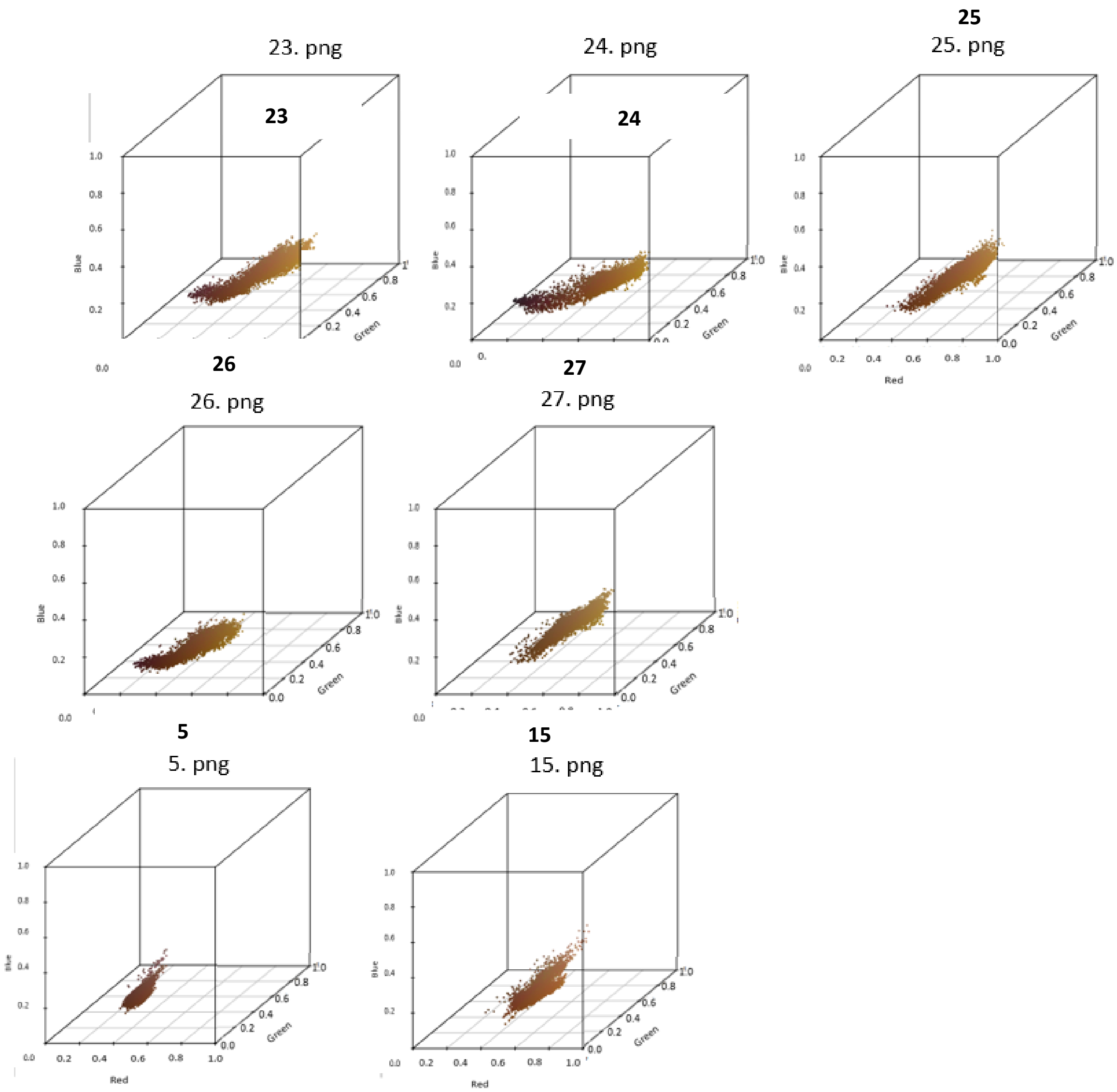

FIG. 3: The RBG pixel of 7 "searching" *E. cf. wilderae* males that shows the amount of red, blue and green color pigment in each cropped back photo. This pixel collection contained the "searching" males #5 and #15 were collected at Highlands, NC and #23-#27 were from Johns Mountain, GA.

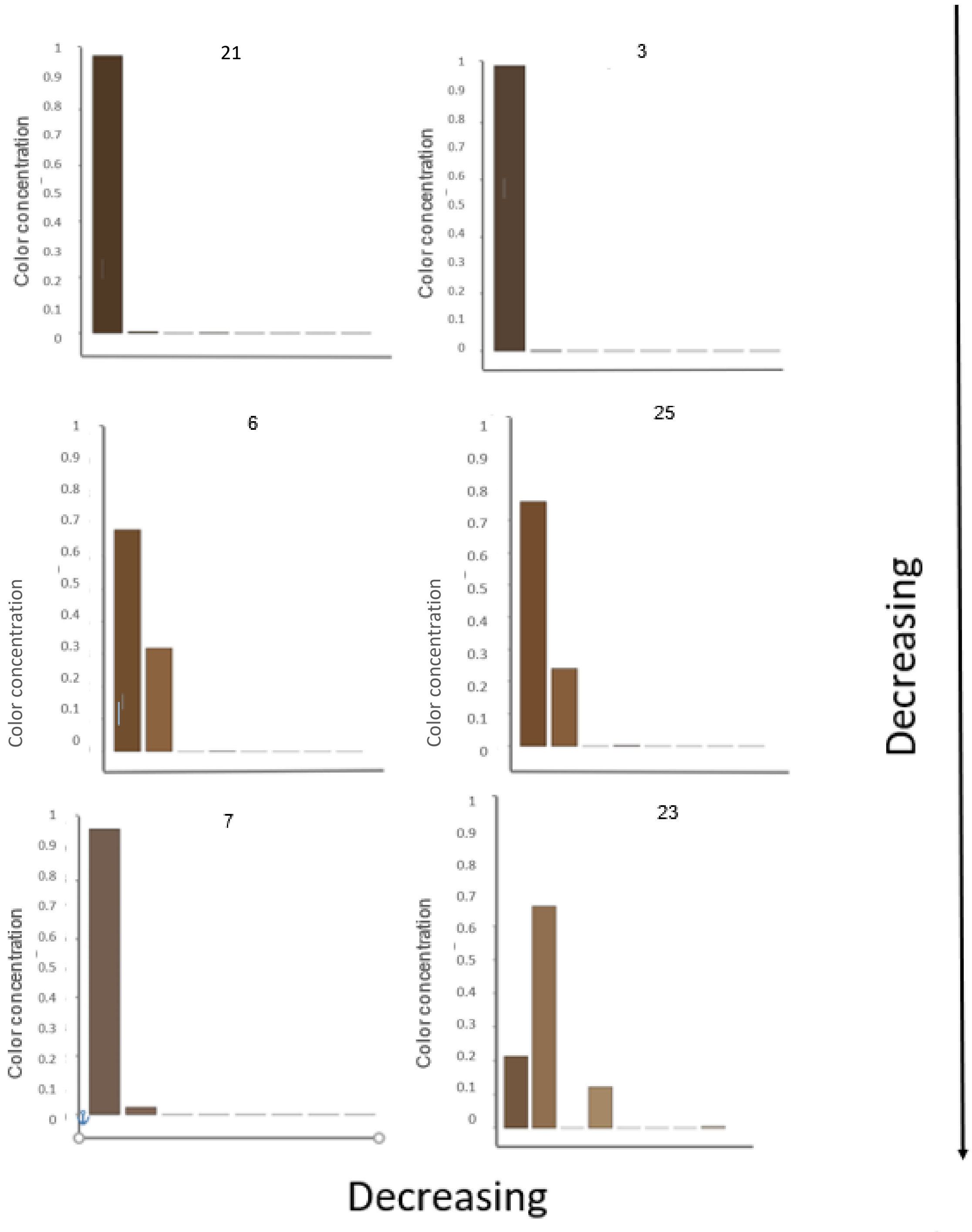

FIG. 4*:* The collection of 6 guarding males' color histograms that are arranged in decreasing trends of color shades from dark brown (guarding male #21) to mustard brown (guarding male #23).

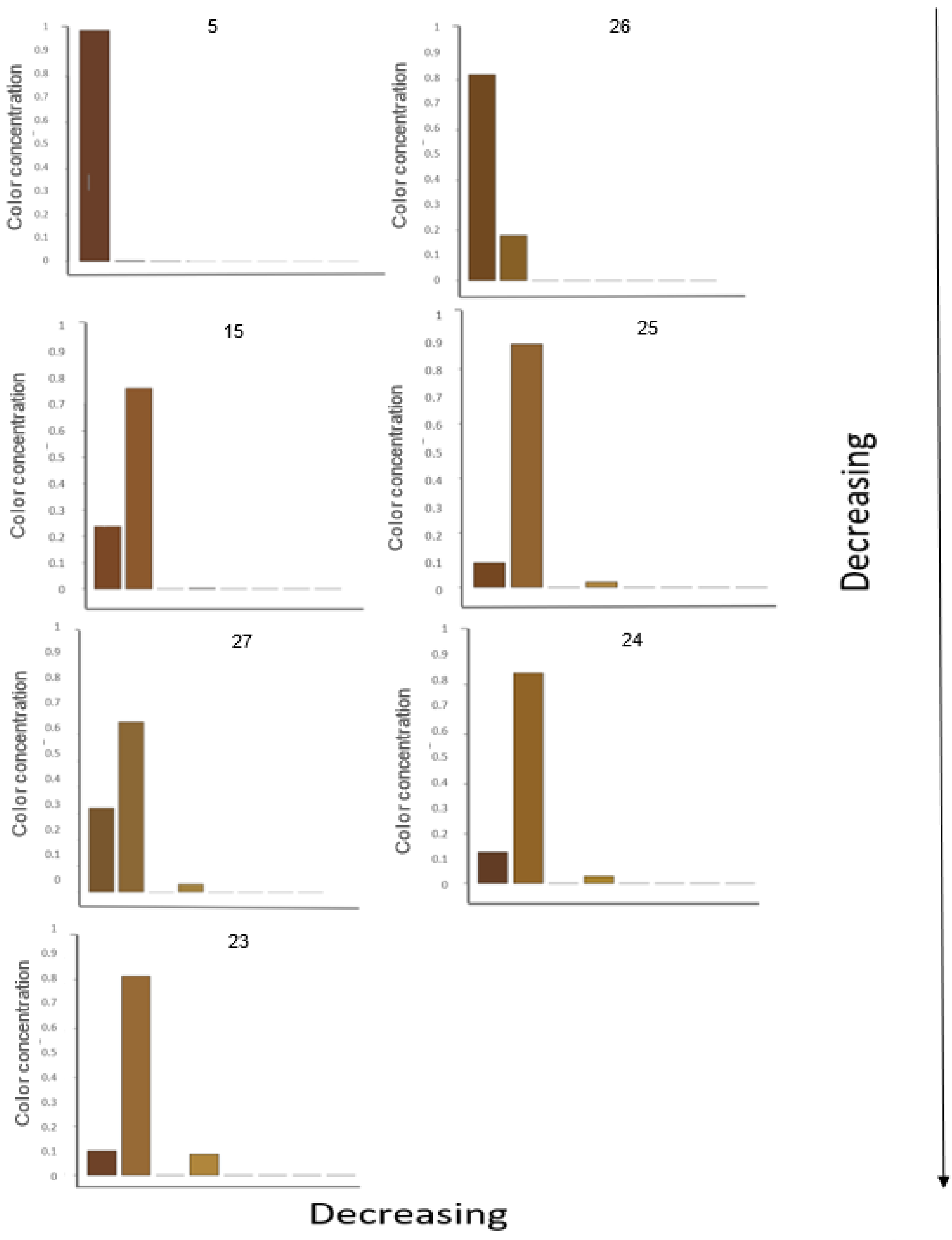

FIG. 5. The color histograms of 7 “searching” *E. cf. wilderae* males that are arranged in decreasing trends of color shades. These varied in the range of dark brown shades and to light gray-brown shades.

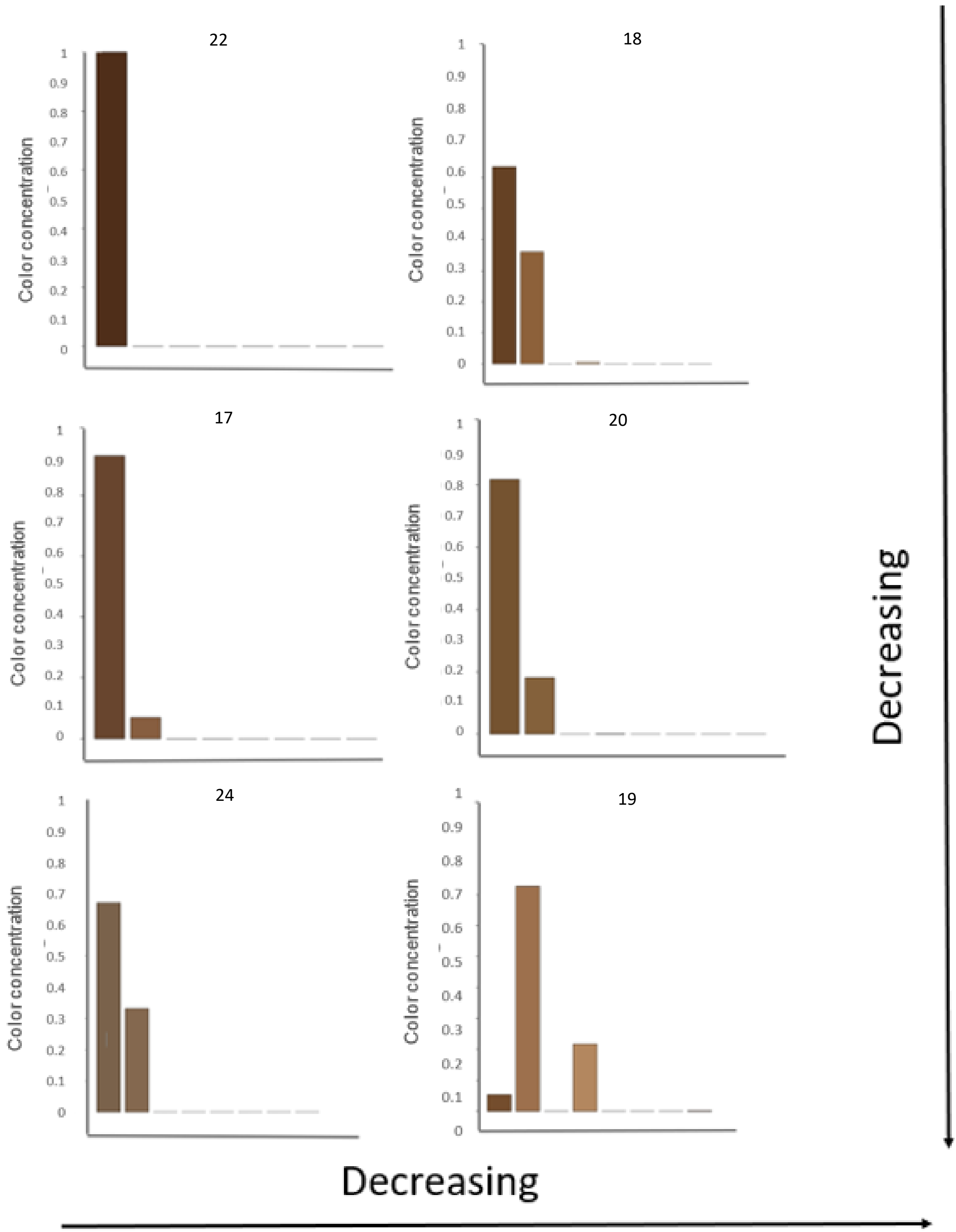

FIG. 6. The histogram collection of *E. cf. wilderae* females found in stream that are arranged in decreasing trends of color shades.

Comparison of color between sexes in aquatic habitats

Figure 4 showed the colors varied widely, from dark gray-brown shades (guarding #21) to lighter, mustard brown shades (guarding #23). Between the two groups, “guarding” males #6 and #25 looked identical to female #18 since they had the same dark medium brown shade. The female #24 had a medium brown shade, which was identical to guarding male #7 (Figs. 4 and 6). Out of all the brown shades, reddish-brown was the most abundant. Three out of 6 (50%) of the female group (#18, #17, and #20) and 2 out of 6 (33.3%) of the “guarding” males (#6 and #25) shared the same color shade (Figs. 4 and 6). The females that we found on trails also showed a wide range of shades but were slightly lighter when compared to the “guarding” male groups (Fig. 7). To strengthen the relationship between the “guarding” male group and the female group, the distance matrix data was used to show similarity and difference between each sample’s photo (Fig. 7). The blue color represents a “low color distance,” or homologous between photos. The darker the “blue” color between two photos, the more similarity that they share. The “pink” color represents “high color distance,” or differences between photos. When comparing a photo from the female group to all photos of “guarding” males, we found that these photos were in the blue range patterns. We continuedly compared each photo between groups of guarding males and female and found similar blue patterns between them. One exception was photo #19, which showed the most contrasting color between the groups of guarding males and females (Fig. 7).

Comparison of color between sexes in terrestrial habitats

The females found on trails showed much darker brown shades when compared to the females found in the creek. Female #1 was a dark brown shade (Figs. 6 and 8). But the terrestrial female group varied in a wide range of brown shades from extreme dark brown to light, peanut brown (Fig. 8). The histogram graphs demonstrated that the “searching” males were significantly lighter than the females, with “searching” males’ color ranging from dark medium brown to light mustard brown. Specifically, female #1 and female #2 were extremely dark when compared to other samples in the two compared groups (Figs. 8 and 9). Both female #16 and #14 looked the most identical to male #29 with medium brown shades while female #10 had a similar shade to “searching” male #30 (Figs. 8 and 9). Although there were some similarities between the two groups, the data did not show a strong color similarity between “searching” males and females found on trails.

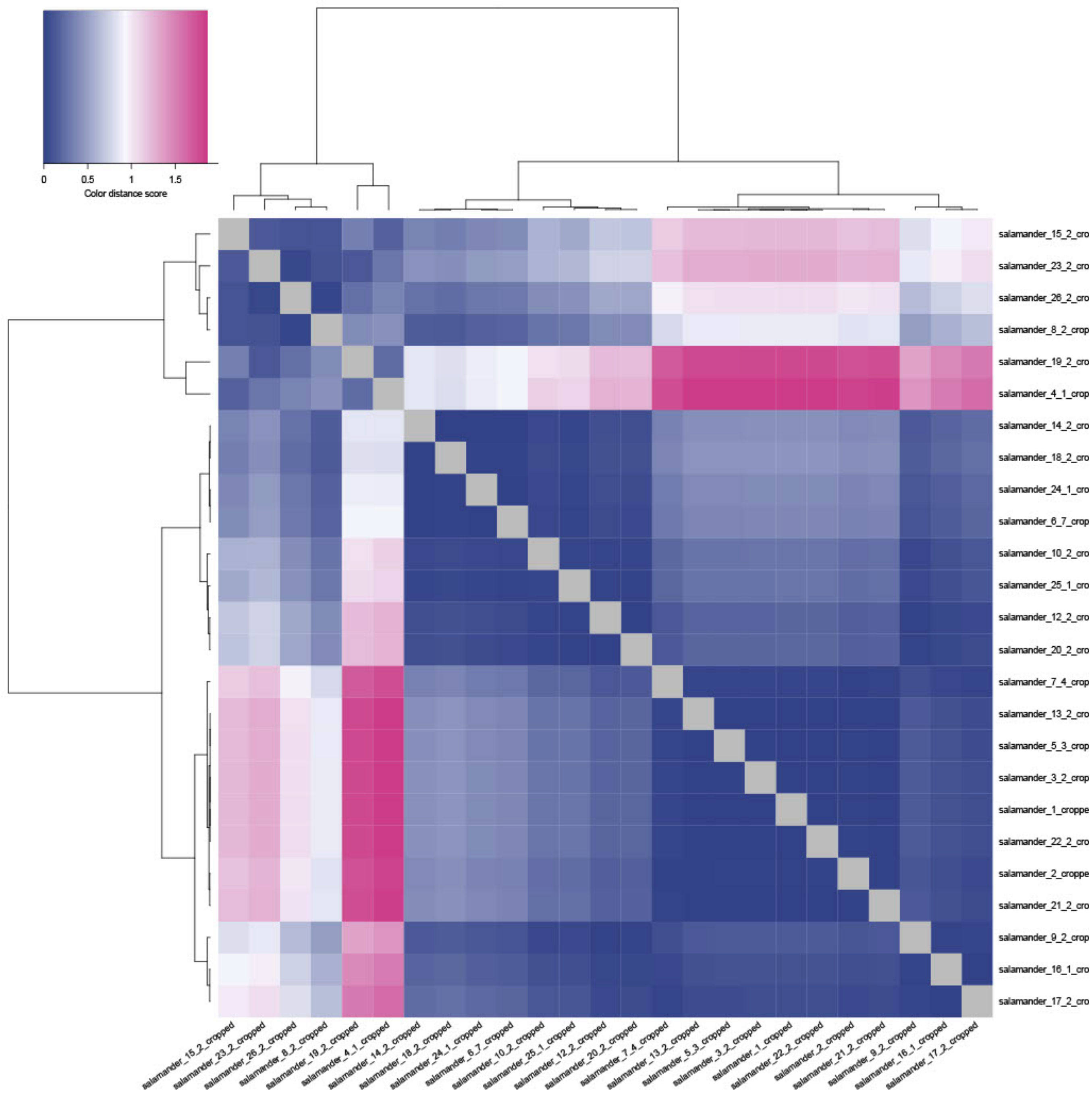

FIG. 7. The distance matrix shows the similarity and difference between each photo of *E. cf. wilderae* samples. The blue color represents similarity, the darker the blue between the two photos, the more similarity that they share. The pink color represents difference, the darker the pink, the more significant difference between the two photos. This distance matrix does not include the 5 "searching" male samples from Kennesaw State University.

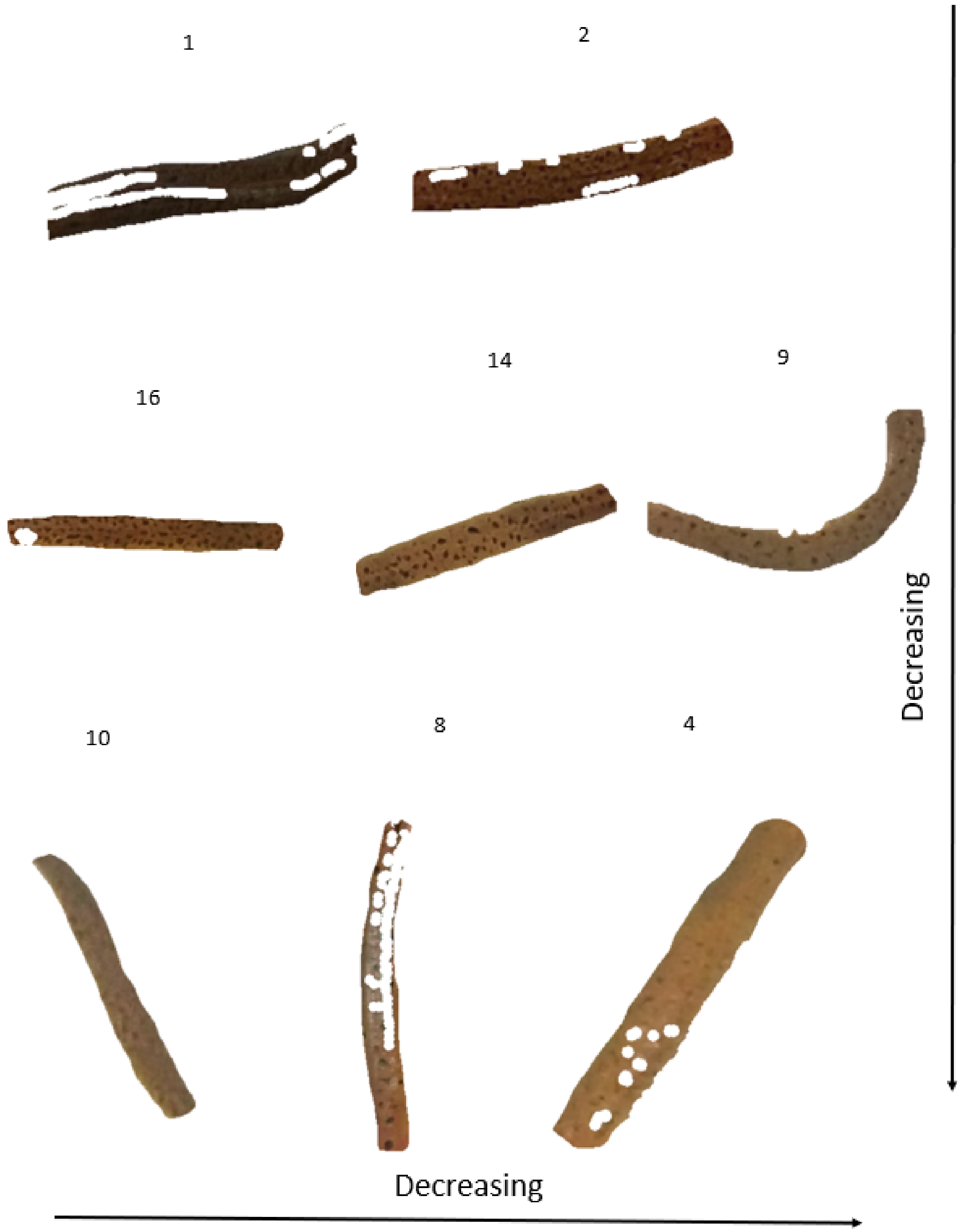

FIG. 8. the collage of the cropped backs of *E. cf. wilderae* females that were found on trails. The arrows demonstrate the decreasing trends from darkest to lightest brown shades.

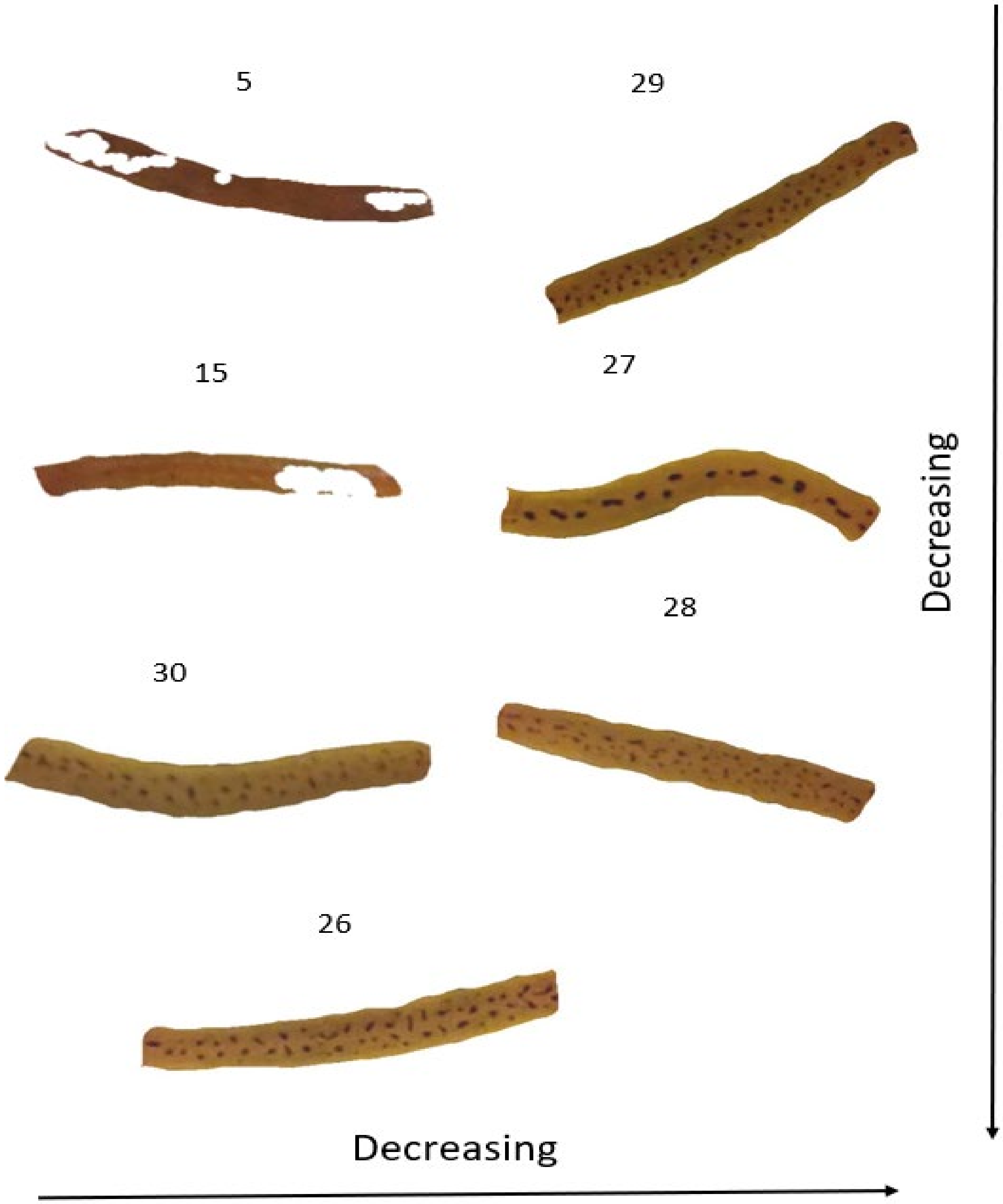

FIG. 9. The collage of 7 “searching” *E. cf. wilderae* males’ backs (cropped) are arranged in a decreasing trend from darkest to lightest shades.

Discussion

Habitat Segregation between Phenotypes during Breeding Season

Our results strongly supported the first theory that "searching" and "guarding" males of *E. cf. wilderae* are spatially segregated. The results showed that 100% of our "guarding" males were found in the streams, and 100% of our "searching" males were found on trails, which denotes a separation in habitat. The same pattern was recorded in Pierson's study (2019) when he found 100% of searching males on trails and 100% of "guarding" males in the stream. He observed that "searching" males tend to court females in terrestrial habitats before and during migrations to the streams; in contrast, "guarding" males wait for females to arrive in streams and secure the aquatic nesting sites. This proved that the two *E. cf. wilderae* males perform different reproductive phenologies in mating season (Sever 1979). The idea of spatial segregation between the male phenotypes of *E. cf. wilderae* was also mentioned in Crawford (2016) which suggested that Blue Ridge Two-lined Salamanders (*Eurycea cf. wilderae)* preferred to stay in a moist environment, but they could favor a less suitable dry habitat when there was the presence of predators or competitors in the moist environment. This was one possible reason why "searching" males were mostly found on forest floors during breeding season due to the presence of a "guarding" male, a more aggressive male phenotype in the streams. Arnold (1977) suggested that males tend to be more aggressive when females are around and, in a study conducted by Deitloff et al. (2014), Brown-backed Salamander (*Eurycea aquatica)* males with larger head size and the associated jaw musculature tend to show aggressive behaviors towards other male *E. aquatica*.

Polymorphic Color Differences between Male Phenotypes and Sexes

In Sever (1979) and Pierson (2019), it was concluded that there were several features such as cirri, mental hedonic glands, and muscular jaws that differentiate searching and guarding males. We noticed these same features when identifying the phenotypes in the field but used standardized color as another differentiating factor to solidify the separation between the two phenotypes. As predicted, we found a pattern between the colors of "searching" and "guarding" males. Qualitatively, the guarding males were noticeably duller, in contrast with the searching males which showed brighter shades of brown. The results substantiated our statement that lighter-colored searching males inhabit wooded leafy areas and duller guarding males inhabit streams. Similar color observation also appeared in *Plethodon cinereus* which contain two different color phenotypes: striped and unstriped (Pfingsten and Walker 1978). Each phenotype of *P. cinereus* is associated with a particular ecological condition. The striped *P. cinereus* is associated with cooler, wetter habitats and the unstriped phenotype prefers warmer, drier habitats (Moreno 1989, Anthony et al. 2008, Evans et al. 2020). This polymorphic coloration is widely observed in nature and represents a type of adaptation via natural selection (Nosil 2004). This demonstrates that each phenotype can effectively camouflage into their natural habitats to increase their survival rates (Cott 1940, Ruxton et al. 2004) and their reproduction rates. An alternative explanation for the color differences between the two phenotypes of *E. cf. wilderae* is the need to blend in with the different habitats that the "searching" males and "guarding" males occupy during courtship season.

A study conducted by Anthony et al. (2008) found positive assortative mating between sexes of *P. cinereus* such that male and female salamanders sharing the same color morphologies are more likely to be found together. But this positive assortative mating can be influenced by other mechanisms apart from color morphology, such as intrasexual competition for territory or food (Creighton 2001). When analyzing the distance matrix, we noticed that "guarding" males and females found in streams have very similar coloration. However, we did not find any similarities between searching males and females found on trails. Thus, our prediction that there is a color similarity between each male phenotype and female in the same habitat was not supported by our findings. Pauers and Mckinnon (2012) suggested that females preferred males with the same phenotypes within their own populations, but they could not show strong evidence to support that color traits played a role in assortative mating among populations.

Limitations and Future Direction

Some limitations that we faced during the research process included fast-moving water after rain, agitating sediments which made it harder to find salamanders in the stream. Another challenge was variation in lighting altering our pictures. Sunlight caused glare on the salamander's back and reflections on the water, and lighting in the forest is not always uniform which made taking standardized pictures difficult. This limitation affected our data which made it challenging to provide fair, unbiased color analysis. As autumn arrived, we found it harder to find salamanders as fallen leaves created leaf packs, which provided an easy and effective hiding spot for them. We also worked around time constraints, uncooperative weather, and had to work to gain practice finding salamanders. A more pressing limitation of our study was our small sample size. We found 22 salamanders which is not ideal. In a subsequent study, a larger sample set would be incredibly beneficial.

A future study including *E. cf. wildera*e could replicate Pauers and McKinnon's (2012) experiment with cichlids. The study could conduct mating trials to study nonrandom matings between the two male phenotypes and females. This would provide novel information about how the phenotypic differences between the two phenotypes affect their mating success. Further investigation should be done on color similarities between guarding males and females found in streams, and if these similarities affect mating. This brings up the question of if these two male phenotypes change habitats throughout their lifetime, going from streams to woods and vice versa, or if they stay year-round in the habitat that is more beneficial to their physical features. It would also be valuable to research if the coloration of the salamander's changes over their lifetime, and if those changes are due to habitat or mating seasons.

Our results have implications for polymorphism research and sexual selection. We have contributed more data on how differences between the two male phenotypes affect their habitation patterns, and we can formulate new questions to find out how these differences affect mating patterns and reproductive success. Our data provides evidence to explain the theory that Blue Ridge Two-lined salamanders are spatially segregated, and each phenotype adapts to the ecological condition during the breeding season to increase their survival and reproductive rates. We also tested and standardized methods for data collection and analysis so that our data can be used by other researchers studying the color and/or salamanders. Our study presented a standardized method for capturing color data of salamanders and a new catch/release method to reduce stress on the salamander and prevent euthanization in a lab. Since this is a novel study on the coloration of *E. cf. wilderae*, we encourage further studies to continue and improve our data on *E. cf. wilderae* to better understand the species and their reproductive behaviors. We hope that our preliminary data and standardization of methods are useful for other researchers wishing to expand upon our study.

ACKNOWLEDGMENTS

We would like to thank Dr. Todd Pierson, Kennesaw State University, for his dedicated guidance on this project. He committed his time and knowledge to teach us new scientific skills and to provide tips and feedback for the success of this project. We would also like to thank our professors and staff, Jason Love, Dr. Jim Costa, and Dr. Rada Petric from Highlands Biological Station. They created great opportunities for us to gain field work experience and obtain useful knowledge for our future careers. The authors carried out this research under permits held by lead-PI Dr. Todd Pierson, including North Carolina Wildlife Resources Commission animal collecting permit 21-SCO1448 and Highlands Biological Station IACUC number 21-08.

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A Red-spotted Newt (*Notophthalmus viridescens viridescens*) in a rock pool, West Fork of the Chattooga River.

INFLUENCE OF URBANIZATION AND WATER QUALITY ON SOUTHERN APPALACHIAN INSECTIVOROUS BATS AND ARTHROPODS

RACHEL MAUNUS AND NOA MEIRI

Abstract. As urbanization and concomitant pollutants rise in conjunction with the human population, freshwater sources are increasingly becoming compromised by urban development and chemical contamination. Degraded water quality reshapes the diversity and abundance of arthropod communities, which may in turn influence the composition of insectivorous bat communities. Bat populations are similarly sensitive to changes in water quality and urbanization, making it essential to understand how changing land cover and land use influences freshwater resources, aquatic arthropods, and bat community dynamics. In this study, we investigated nocturnal arthropods and insectivorous bats at 32 water bodies along a rural-exurban-urban gradient using light traps and acoustic recorders in the southern Appalachian Mountains of western North Carolina, USA. We took temperature, turbidity, and dissolved oxygen measurements at each site to assess water quality. We found no correlation between urbanization level and the water quality metrics we used. Total bat activity was the highest in areas with a medium disturbance level; however, individual species responded uniquely, and sometimes oppositely, to disturbance. Moreover, arthropod wet weight was highest in areas of low disturbance, demonstrating that both bat activity and arthropod biomass are affected by disturbance, but there was no significant correlation between activity and biomass at varying disturbances. The results of this study highlight the importance of a species-specific approach to bat conservation and points to the need for future research on the intricacies of the relationship between bat activity, arthropods, water quality, and anthropogenic disturbance.

Key words: arthropods; bats; Chiroptera; exurbanization; pollutants; southern Appalachians; species diversity; urbanization; water quality

INTRODUCTION

Presently, over half of the world's human population resides in urban areas, and urban infrastructure is expected to triple in size between 2000 and 2030 (Seto et al. 2012). Increases in human population and urbanization alters landscapes and coincides with increased contaminants (Grimm et al. 2008). Following rain and storm events, these contaminants concentrate in adjacent water bodies as well as percolate into groundwater and soil (Phillips and Bode 2004). The accumulation of agricultural chemicals has many documented health concerns for aquatic ecosystems, including the decimation of insect populations and bioaccumulation in larger trophic organisms (Chopra et al. 2011).

Insectivorous bats are major predators in riparian and forested ecosystems (Ballinger and Lake 2006), providing key ecosystem services including pollination, seed dispersal, and most importantly, pest control (Kunz et al. 2011). However, North American bat species are at risk. Bats depend on freshwater sources such as lakes and ponds to hunt and drink (Salvarina 2016). The encroachment of development on forested areas and water bodies has been found to cause declines in insectivorous bat activity (Dixon 2011). Because insectivorous bats can consume a volume of arthropods equalling their body weight each night, there is a short, direct path of potential contaminant bioaccumulation in bats (Kunz et al. 2011). Another factor causing bat species decline is white-nose syndrome (*Pseudogymnoascus destructans*), a fungal disease which has killed millions of bats in North America since its introduction from Eurasia in 2007 (Frick et al. 2015). The culmination of white-nose syndrome, habitat loss, and water quality degradation threatens the survival of many bat species.

Furthermore, the urbanization of water bodies decreases the availability and abundance of insects, specifically insects in the order of Diptera and Trichoptera, negatively impacting primary food sources for bats (Fenoglio et al. 2020, Straka et al. 2020). Degraded water quality alters basal resources which shifts invertebrate communities towards species with higher tolerance for turbidity and mesotrophic conditions (Jackson et al. 2021). The overall degradation of water quality due to chemical contaminants has been used to predict the presence of bats, as certain species respond rapidly to landscape disturbances and are useful bioindicators (Li and Kalcounis-Rueppell 2017). Similarly, changes in aquatic insect abundance and diversity are correlated to a change in the bat community (Li and Kalcounis-Rueppell 2017).

The goal of this study is to examine the relationship between water quality, arthropod abundance and diversity, and bat activity in freshwater systems across a rural-exurban-urban gradient in the mountains of western North Carolina. The southern Blue Ridge Mountains are a biodiversity hotspot experiencing rapid exurbanization, referring to light-density developments such as second homes and subdivisions. For instance, while public roads have not increased significantly, our study area in the southern Blue Ridge Mountains have experienced a 361% increase in private road development and expansion between 1954 and 2009 (Kirk et al. 2012), providing us with a unique opportunity to examine the effects of exurban expansion on bat activity and their prey. Thirteen insectivorous bat species are found across the area (Loeb et al. 2009), three of which are endangered, and seven of which have been affected by white-nose syndrome. These species use echolocation to hunt for a variety of nocturnal arthropods (Coleman and Barclay 2013).

While research has been conducted on the impact of urbanization on insectivorous bats, this study is specific to the species present in the southern Blue Ridge Mountains and the intersection of water quality and exurbanization. This region is a biodiversity hotspot for bats, making this study unique to further understanding the effects of land cover changes on bat activity. Likewise, this research contributes to bat monitoring efforts which help us gain better insight for bat conservation needs.

We visited natural and man-made freshwater bodies rated on a rural-exurban-urban gradient. There we measured basic water quality metrics, arthropod wet weight over time, and acoustic bat activity. We hypothesize that water quality, arthropod biomass and richness, and thus bat activity will differ between areas of low, medium, and high disturbance ratings.

METHODS

Site Description

Bat monitoring, arthropod collection, and water quality data took place within nine sub-watersheds in the North Carolina and Georgia sections of the southern Blue Ridge Mountains during the summer of 2021. The major watersheds were the upper Little Tennessee, Chattooga, and Horsepasture watersheds. Thirty-two lakes and ponds were selected across a rural-exurban-urban gradient. The key urban centers included Highlands, NC, Cashiers, NC, Franklin, NC, and Dillard, GA. This area is broadly classified as a temperate deciduous forest, consisting of various ecological mosaics at the local scale. Forest community composition varies by elevation and aspect (Whitaker 1956).

Sites were selected using Google Maps by identifying all lakes and ponds within a 32.2 km radius of the Highlands Biological Station in Highlands, North Carolina. Owners or land managers were contacted for permission to sample, and the final list of 32 lakes and ponds was ultimately affected by their consent. We overlapped the coordinates of the sites with National Land Cover

Data (MRLC National Land Cover Database 2019), including impermeable surface cover, canopy cover, and urbanization density in order to classify the sites according to their characteristics along an urbanization gradient. Each site was classified as low, medium, or high disturbance. There were 9 low disturbance sites, 13 medium disturbance sites, and 10 high disturbance sites. Sites vary between natural forests, exurban mountain top development areas, valley agriculture, urban development, and golf courses.

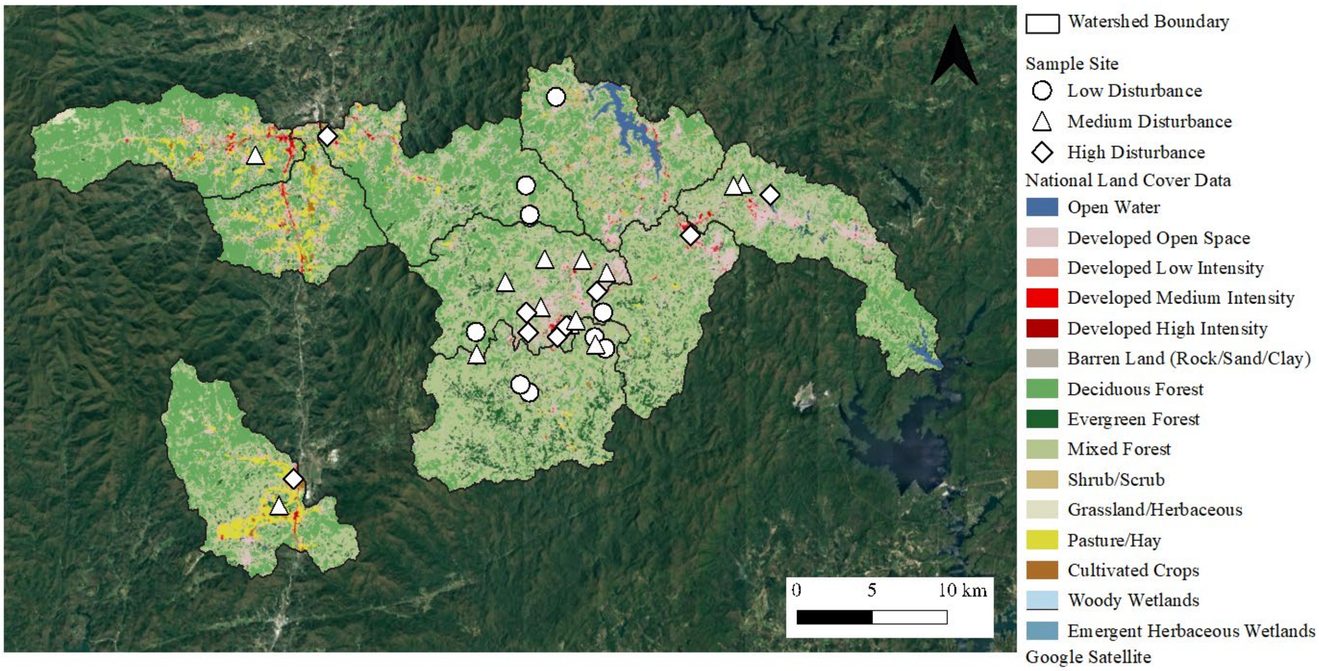

FIG. 1. Map of sample sites overlayed with National Land Cover Data and regional watershed boundaries. The southern Blue Ridge Mountains are characterized by deciduous and mixed forests, interspersed with exurban housing developments on mountainsides, farms in river valleys, and small towns scattered throughout the region.

Acoustic Surveying and Identification

Thirteen insectivorous bat species are found in the southern Blue Ridge Mountains: Rafinesque's Big-eared Bat (*Corynorhinus rafinesquii*), Big Brown Bat (*Eptesicus fuscus*), Eastern Red Bat (*Lasiurus borealis*), Hoary Bat (*Lasiurus cinereus*), Silver-haired Bat (*Lasionycteris noctivagans*), Gray Bat (*Myotis grisescens*), Eastern Small-footed Myotis (*Myotis leibii*), Little Brown Bat (*Myotis lucifugus*), Northern Long-eared Myotis (*Myotis septentrionalis*), Indiana Bat (*Myotis sodalis*), Evening Bat (*Nycticeius humeralis*), Tricolored Bat (*Perimyotis subflavus*), and Mexican Free-tailed Bat (*Tadarida brasiliensis*) (Loeb et al. 2009).

Bat echolocation calls were recorded using a Song Meter SM4BAT-FS ultrasonic recorder with a SMM-U2 microphone (Wildlife Acoustics Inc., Maynard, Massachusetts, USA) to detect bat passes and record echolocation calls for four hours following sunset. We secured the microphone three meters above the ground and pointed it towards the water body. All files were recorded to an internal SD card, and data was downloaded after collection. To identify bat calls to species, we used Kaleidoscope software, which classified the bat pass into noise, no ID, or one of the thirteen species detected in a given night. Only calls that the software identified with a confidence value of 0.6 or above were included in our species-specific data analysis, the rest of which were classified as “no ID.”

Arthropod Collection

We collected nocturnal arthropods using a blacklight trap, which consisted of a funnel leading to a plastic bag with a cotton ball soaked in acetone. For each site, we collected arthropods in 30-minute time intervals, for a total of eight discrete time points per night. The first time point began at sunset and data collection occurred for the next four hours. Acetone was selected to euthanize the arthropods without deteriorating their structure.

The bags of arthropods were left at least one night to dry. Once the acetone dried, we weighed each bag in grams, which included the mass of the bag and the cotton balls. Next, we extracted the arthropods from the bag and measured the arthropod wet weight in a petri dish in milligrams.

For select sample sites, we identified and counted the arthropods by Order. To identify each arthropod, we observed them under a dissecting scope and referenced dichotomous keys. We then consolidated all of the arthropods from the bag into a petri dish for future reference. We repeated this process for each of the eight bags per site. Future work will be done to identify the Orders of the arthropods from all the sample sites. Arthropods were preserved in the freezer to prevent deterioration. The most common nocturnal arthropod Orders in this area are moths and butterflies (Lepidoptera), true bugs (Hemiptera), true flies (Diptera), ants and bees (Hymenoptera), beetles (Coleoptera), caddisflies (Trichoptera), dobsonflies (Megaloptera), lacewings (Neuroptera), and spiders (Araneae) (Coleman and Barclay 2013).

Water Quality Data

At each body of water, we used an Aquatroll 600 to measure the following: turbidity, temperature, Dissolved Oxygen (DO) saturation, and DO concentration. We recorded ten measurements per parameter per site. We averaged the ten measurements into a single data point to represent the values for the site. The Aquatroll was calibrated three times throughout the sampling period.

Statistical Analysis

All data were analyzed using R Studio software version 4.0.2 to determine correlations between exurbanization, bat activity, arthropod abundance, and water quality with an alpha level of $p<0.05$ for the rejection criterion. Total bat activity and arthropod biomass were in violation of normality and variances and could not be normalized; therefore, we used Poisson distribution. We used General Linear Models (GLM).

Results

Data were recorded from 14 June to 19 September 2021. We recorded a total of 11,521 sound files from 32 sites. Of those, 2,209 were classified as noise, meaning that they were not bat calls; thus, they were removed from our dataset. We identified 9,311 sound files as bat calls and recorded and identified all thirteen of the insectivorous bat species found in the area (Appendix 1). The most common bat species was the Big Brown Bat which was detected at every site except two high disturbance sites. We only recorded Rafinesque's Big-eared Bat at one medium disturbance site. All three federally listed endangered and threatened species were found at low, medium, and high disturbance sites. All seven species known to be affected by white nose syndrome were also found across the three disturbance levels. Medium disturbance sites had the greatest diversity of bat species, with an average of 9.2 species represented. Low and high disturbance sites had an average of 8.2 and 6.9 species, respectively (Table 2).

TABLE 1. Detected presence (+) or absence (-) of bat species at each sample site in 2021. Big Brown Bat was the most common species identified, and Rafinesque's Big-eared Bat was the least common. All species of concern were found at all three disturbance levels. The greatest diversity of bats was found at medium disturbance levels.

Site Name	Disturbance	Rafinesque's Big-eared Bat	Big Brown Bat†	Eastern Red Bat	Hoary Bat	Silver-haired Bat	Gray Bat*†	Eastern Small-footed Myotis†	Little Brown Bat†	Northern Long-eared Myotis*†	Indiana Bat*†	Evening Bat	Tricolored Bat†	Mexican Free-tailed Bat
Turtle Pond	Low	-	+	+	-	-	+	+	+	-	+	+	+	+
Wilson Lake	Low	-	+	-	-	+	+	-	+	-	+	-	+	-
Eric	Low	-	+	+	+	+	-	-	+	+	+	+	-	-
Edwards Creek	Low	-	+	+	+	+	-	-	+	+	+	+	+	-
Greene	Low	-	+	+	+	+	-	-	+	-	+	+	+	+
Mollie	Low	-	+	+	+	+	-	-	+	-	+	+	+	+
Sonya	Low	-	+	+	+	+	-	-	-	-	-	+	+	+
Kristin	Low	-	+	+	+	+	-	-	-	-	+	+	-	+
Fawn Creek	Low	-	+	+	+	+	-	-	+	+	+	+	+	+
Lindenwood Lake	Medium	-	+	+	+	+	-	-	+	+	+	+	+	+
Lindenwood Lake	Medium	-	+	+	+	+	+	+	+	-	+	+	+	+
Ravenel	Medium	-	+	+	+	+	-	-	+	-	+	+	+	+
Cliffside Lake	Medium	-	+	+	+	+	-	-	+	+	+	+	+	+
Lonesome Valley Upper	Medium	-	+	+	+	+	-	+	+	-	+	+	+	-
Lonesome Valley Lower	Medium	+	+	+	+	+	-	-	-	+	+	-	+	+
Mirror Lake	Medium	-	+	+	+	-	-	-	+	-	+	-	+	+
Buck Creek	Medium	-	+	+	+	+	-	-	+	-	+	+	+	+
Big Creek	Medium	-	+	+	+	+	-	+	+	+	+	+	-	+
Biscuit Lake	Medium	-	+	+	-	+	+	+	+	+	+	+	+	+
Katie	Medium	-	+	+	+	+	-	-	-	-	-	+	+	-
Mountain Retreat	Medium	-	+	+	+	-	+	-	+	-	+	+	+	+
Rabun Gap Lake	Medium	-	+	+	+	+	-	-	+	-	-	+	+	+
Horse Cove	Medium	-	+	+	+	+	+	+	+	+	+	+	+	-
Harris Lake	High	-	+	+	+	+	-	-	+	-	-	+	-	+
Highlands High School	High	-	+	-	+	+	-	+	+	+	+	+	-	+
Golf	High	-	+	-	+	+	-	-	+	-	-	+	+	-
Sequayah	High	-	+	+	+	+	-	-	+	-	-	+	+	+
Club Lake	High	-	-	+	+	-	-	-	+	-	-	+	+	-
Cashiers Lake	High	-	+	+	+	+	+	+	+	+	+	+	+	+
Tudor	High	-	+	-	-	-	-	-	+	-	-	+	-	-
Fairfield	High	-	+	+	+	+	-	-	+	-	+	-	+	+
Walmart	High	-	+	+	+	+	-	-	-	-	-	+	+	+
Rabun Gap Field	High	-	-	-	+	+	-	-	-	-	-	+	-	+

* Federally listed endangered or threatened species
† Species that have been known to be affected by white-nose syndrome

Table 2. Average diversity of bat species detected by disturbance level.

Disturbance Level	Average Number of Bat Species Detected
Low	8.2
Medium	9.2
High	6.9

We found that bat activity was significantly higher at a medium disturbance level (GLM Estimate 0.40+/-0.03, $p<0.01$). The mean bat activity recorded per night was 32.6 calls at a low disturbance, 48.4 calls at medium disturbance, and 24.1 calls at high disturbance (Appendix 1). There was 39.2% more bat activity at medium disturbance sites than at low disturbance sites, and 67.1% more bat activity at medium disturbance sites than at high disturbance sites. Bat activity at low disturbance levels was 29.8% higher than bat activity at high disturbance levels (Fig. 2). Bat activity followed a significant trend across the eight discrete data collection time intervals. Compared to the first time interval, bat activity increased at time intervals two, three, and four, followed by a decrease in activity for time intervals five, six, and seven. Time interval eight shared a similar level of bat activity as time interval one (Fig. 3).

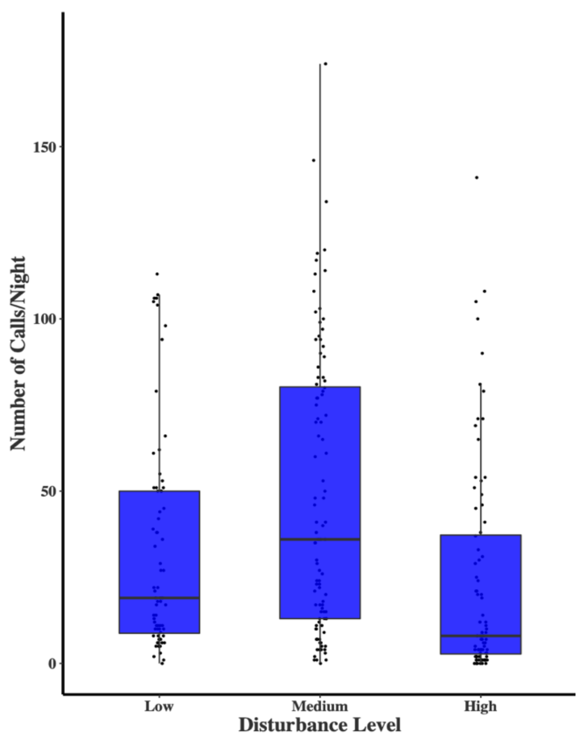

FIG. 2. Median and quartiles of bat calls per night in each disturbance level (low n=9, medium n=13, high n=10). Bat activity was highest at water bodies with medium disturbance levels (GLM Estimate 0.40+/-0.03, $p<0.01$) and lowest in high disturbance levels (GLM Estimate -0.30+/-, $p<0.01$). Circles represent individual data points.

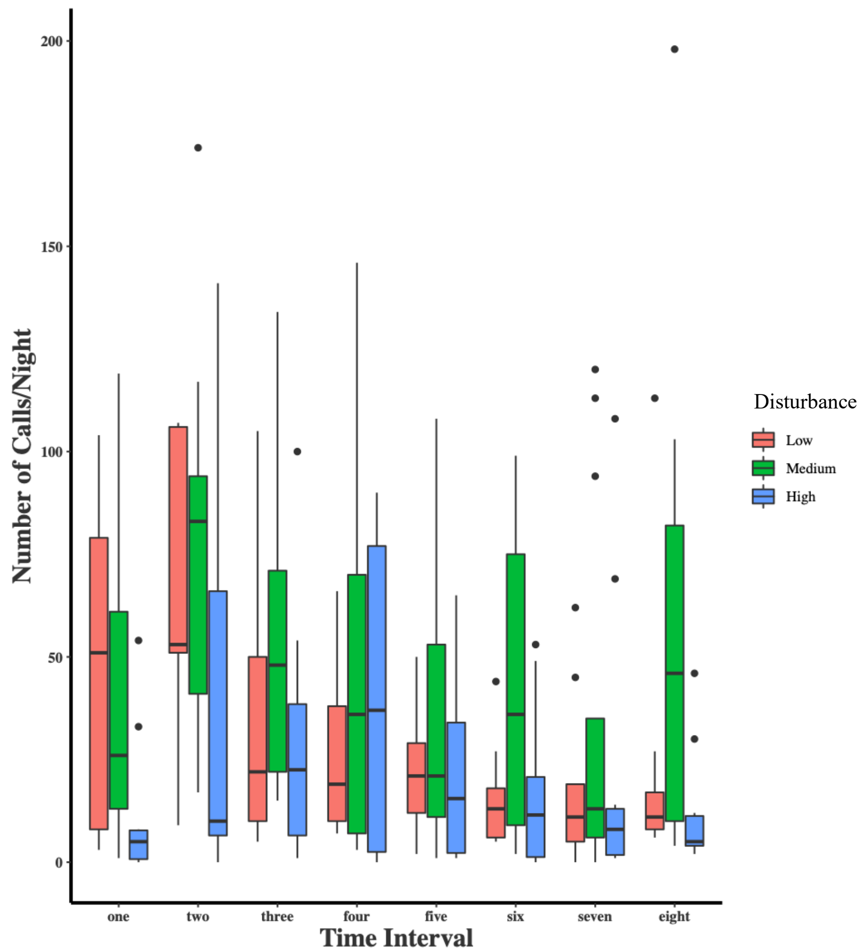

FIG. 3. Median and quartiles of bat calls per night at each data collection time interval, characterized by disturbance level (low n=9, medium n=13, high n=10). Bat activity at all time intervals except interval eight differed significantly from time interval one. Time intervals two, three, and four had more bat calls, and intervals five, six, and seven had fewer calls than time one.

When bat activity versus disturbance was separated by individual species, we found significant correlations (Fig. 4, Table 3). At low disturbance sites, Big Brown Bats were found at significantly higher rates compared to medium and high disturbances. Eastern Red Bat and Eastern Small-footed Myotis activity was significantly higher at high disturbance sites. Hoary Bat and Little Brown Bat calls per night were significantly greater at both medium and high disturbances compared to low disturbances. Silver-haired Bat and Gray Bat activity was lower at high disturbances. Indiana Bat, Mexican Free-tailed Bat, and Northern Long-eared Myotis calls per night did not differ significantly between disturbances levels. Evening Bats were found at higher rates in medium disturbance areas. Tricolored Bat activity was greater at medium disturbances and lower at high disturbances compared to low disturbances.

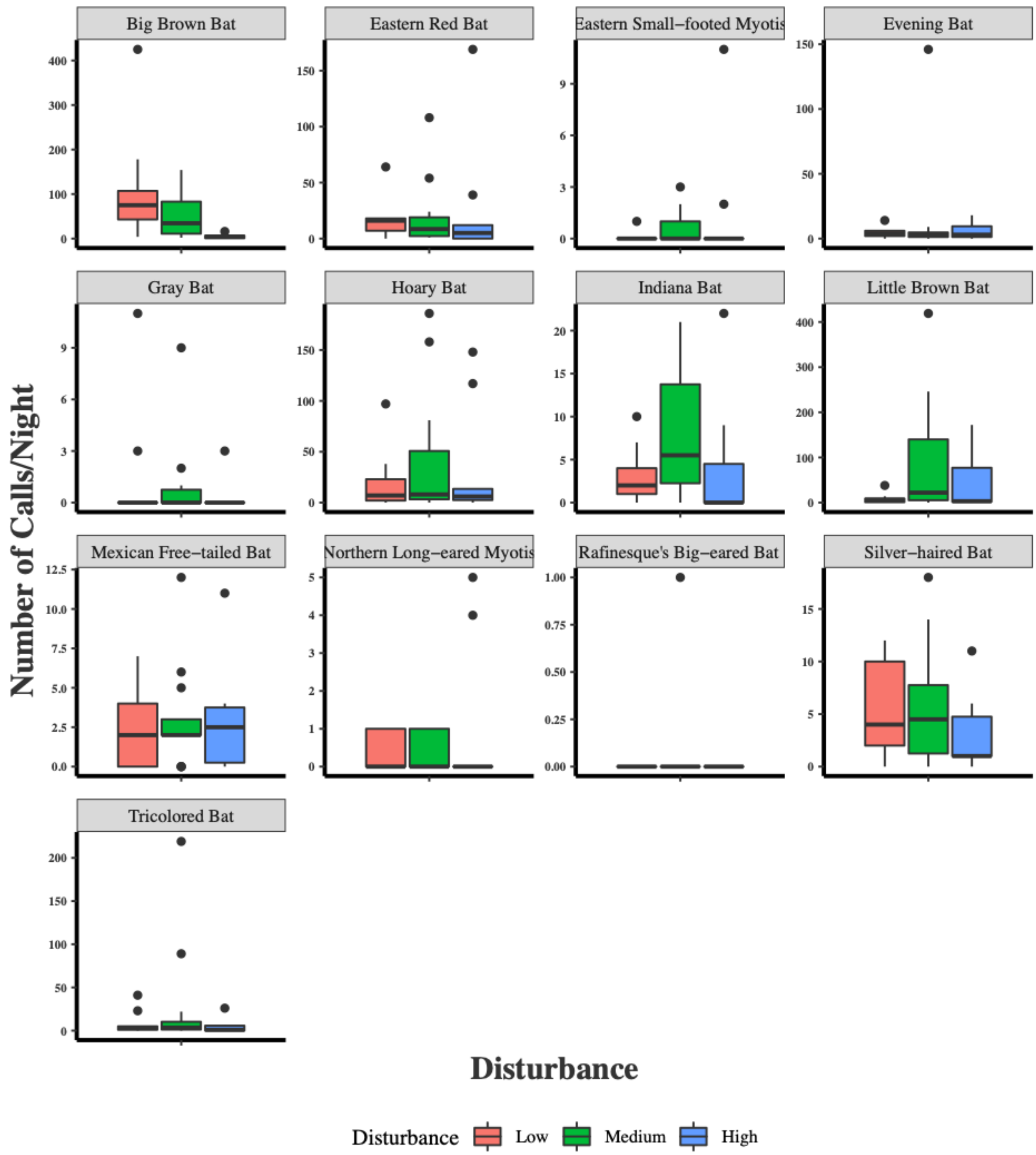

FIG. 4. Median and quartiles of species-specific bat calls per night, characterized by disturbance level. Individual species reacted differently, and sometimes oppositely, to disturbance type.

TABLE 3. GLM Estimate values for each bat species, testing for significant differences in bat calls per night by disturbance level compared to low disturbance level bat activity.

Species	Disturbance Level	Estimate	Standard Error	z value	Pr(>|z|)
Big Brown Bat	(Intercept)	4.72	0.03	149.81	<0.0001
	Medium	-0.68	0.05	-14.40	<0.0001
	High	-3.19	0.15	-21.18	<0.0001
Rafinesque's Big-eared Bat	(Intercept)	-21.30	8541.00	0.00	1.00
	Medium	18.66	8541.00	0.00	1.00
	High	0.00	11770.00	0.00	1.00
Eastern Red Bat	(Intercept)	2.83	0.08	34.85	<0.0001
	Medium	0.10	0.10	0.97	0.33284
	High	0.36	0.10	3.48	<0.001
Hoary Bat	(Intercept)	3.03	0.07	41.49	<0.0001
	Medium	0.65	0.08	7.64	<0.0001
	High	0.40	0.09	4.32	<0.0001
Silver-haired Bat	(Intercept)	1.67	0.14	11.60	<0.0001
	Medium	0.03	0.18	0.17	0.87
	High	-0.58	0.23	-2.47	0.01
Gray Bat	(Intercept)	0.44	0.27	1.65	0.10
	Medium	-0.52	0.39	-1.34	0.18
	High	-1.65	0.64	-2.59	0.01
Eastern Small-footed Myotis	(Intercept)	-2.20	1.00	-2.20	0.03
	Medium	1.64	1.06	1.54	0.12
	High	2.46	1.04	2.37	0.02
Little Brown Bat	(Intercept)	2.18	0.11	19.54	<0.0001
	Medium	2.31	0.12	20.07	<0.0001
	High	1.63	0.12	13.47	<0.0001
Northern Long-eared Myotis	(Intercept)	-1.10	0.58	-1.90	0.06
	Medium	0.25	0.71	0.36	0.72
	High	0.99	0.67	1.49	0.14
Indiana Bat	(Intercept)	-1.10	0.58	-1.90	0.06
	Medium	0.25	0.71	0.36	0.72
	High	0.99	0.67	1.49	0.14
Evening Bat	(Intercept)	1.54	0.15	9.98	<0.0001
	Medium	1.06	0.17	6.19	<0.0001
	High	0.16	0.20	0.80	0.42
Tricolored Bat	(Intercept)	2.16	0.11	19.07	<0.0001
	Medium	1.11	0.12	8.93	<0.0001
	High	-0.66	0.19	-3.50	<0.001
Mexican Free-tailed Bat	(Intercept)	0.94	0.21	4.50	<0.0001
	Medium	0.14	0.26	0.52	0.60
	High	0.09	0.28	0.33	0.75

We found that arthropod wet weight was highest in areas of low disturbance. The mean wet weight was 322.3 mg at low disturbance, 212.8 mg at medium disturbance, and 143.2 mg at high disturbance (Appendix 2). Both medium and high disturbance sites had significantly lower wet weights compared to high disturbance sites (Medium Disturbance GLM Estimate -0.42+/-0.01, $p<0.01$; High Disturbance GLM Estimate -0.81+/-0.01, $p<0.01$). There was 40.9% less wet weight at medium disturbance sites than at low disturbance sites, and 76.9% less arthropod wet weight at high disturbance sites than at low disturbance sites. Wet weight at medium disturbance levels was 39.1% higher than at high disturbance levels (Fig. 5). Further, arthropod wet weight differed between time intervals, with time point 1 containing the fewest arthropods compared to the rest. Wet weight was significantly highest at time intervals 2 and 3, then generally followed a decreasing trend (Fig. 6).

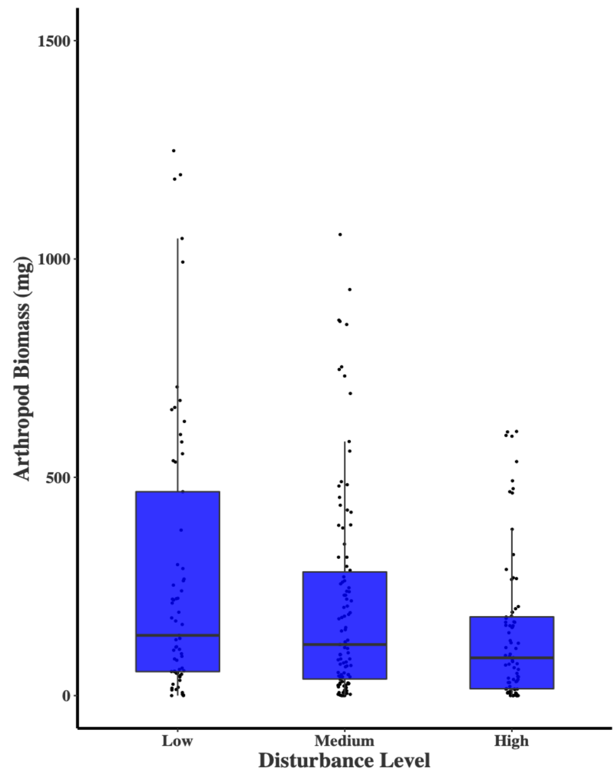

FIG. 5. Median and quartiles of collected arthropod wet weight (mg) in each disturbance level (low n=9, medium n=13, high n=10). Medium and high disturbance sites had lower biomasses compared to high disturbance sites (Medium Disturbance GLM Estimate -0.42+/-0.01, $p<0.01$; High Disturbance GLM Estimate -0.81+/-0.01, $p<0.01$). Low disturbance had the greatest range of biomasses (0-2251 mg). Circles represent individual data points.

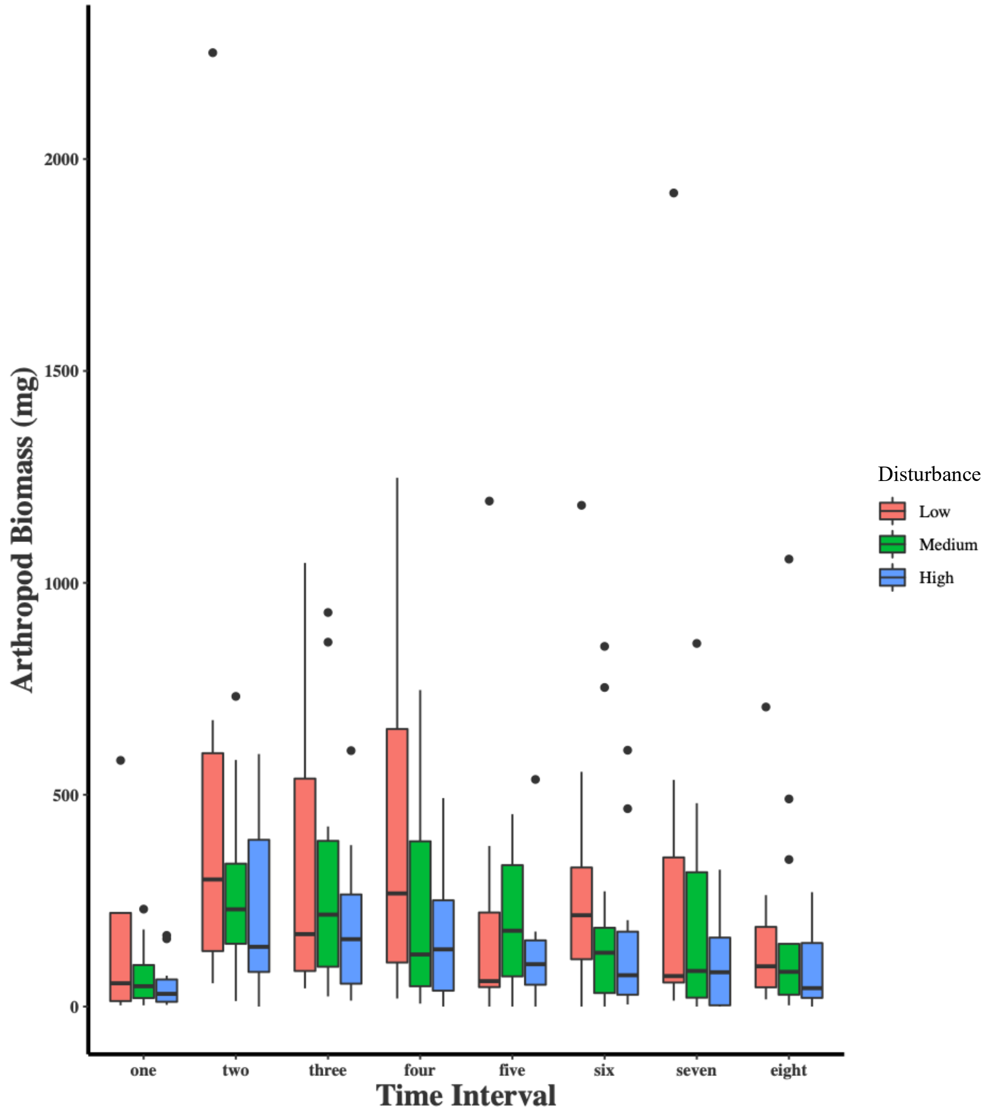

FIG. 6. Median and quartiles of arthropod biomass (mg) at each data collection time interval, characterized by disturbance level (low n=9, medium n=13, high n=10). Biomasses at all time intervals were significantly higher than at interval one. Biomass peaked at times two and three, then followed a decreasing trend compared to time interval one.

When a linear regression was run for biomass against disturbance level, there was a trend between the two variables (GLM Estimate 0.01+/-0.01, p=0.13) (Fig. 7).

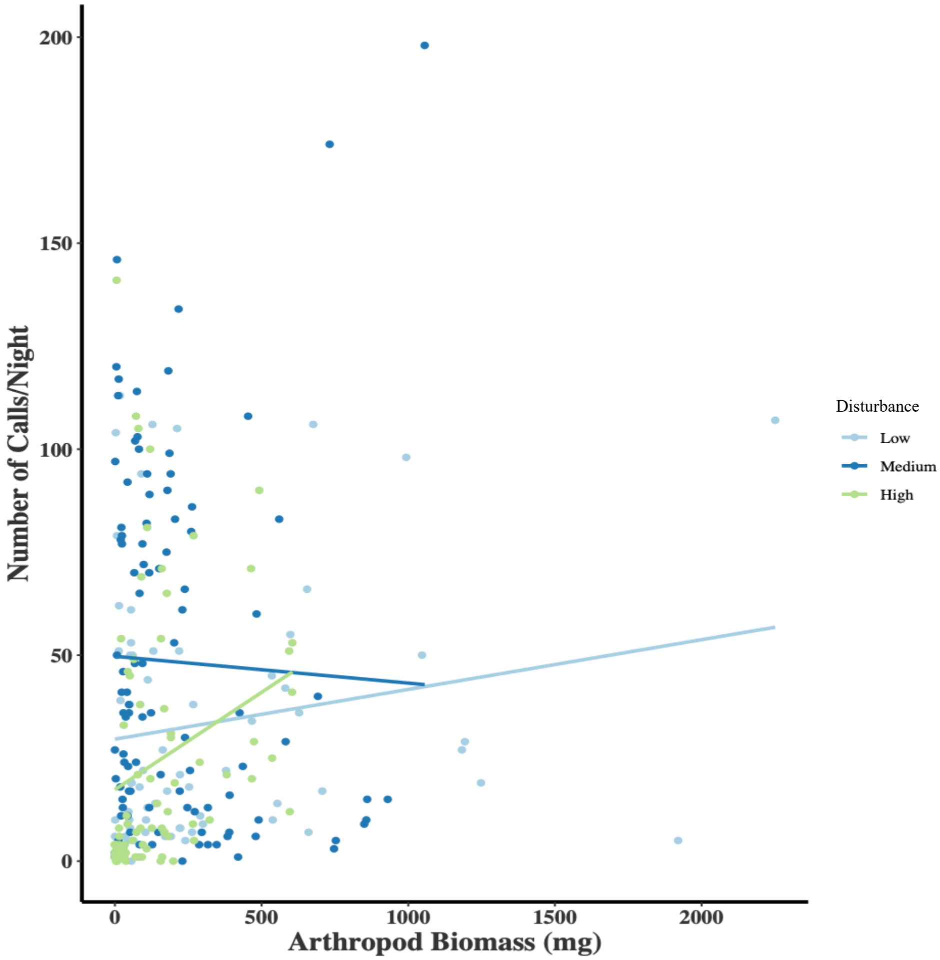

FIG. 7. Linear regression model of bat calls per night and arthropod biomass by disturbance level. There was no significant correlation between the variables, although there was a trend (GLM Estimate 0.01+/-0.01, *p*=0.13).

Arthropod identification to Order was completed only at four of 32 sites. Currently this is not a large enough sample size to show any trend or significance. For the sites analyzed, we recorded the total number of individuals as well as the individuals collected per Order (Table 4). Preliminary observational analysis shows a high abundance of arthropods at high disturbance levels and low arthropod abundance at low disturbance levels.

TABLE 4. Count of most common orders of arthropods by site and disturbance level.

Site Name	Disturbance	Trichoptera	Diptera	Hemiptera	Lepidoptera	Coleoptera	Hymenoptera	Other	Total Arthropods
Wilson Lake	Low	58	81	93	58	23	269	10	590
Lindenwood Lake	Medium	163	90	16	821	0	118	0	1208
Mountain Retreat	Medium	2	34	24	45	2	4	3	111
Cashiers Lake	High	50	728	11	392	1	1	2	1138

At 16 of the 32 sites, we analyzed the correlations between disturbance levels and DO concentration, DO saturation, and water temperature (Appendix 3). No statistical significance was found between any of the water quality measurements and disturbance level (all $p>0.55$). Turbidity measurements were analyzed for 10 sites. Statistical significance was found between medium disturbance levels and turbidity; however, when an outlier in the dataset was removed, no statistical significance remained.

DISCUSSION

Bat Activity and Disturbance

We found that total bat activity was correlated to disturbance level; areas of medium disturbance had the highest bat activity. It is possible that bat activity is positively influenced by urban features such as streetlights, which attract insects, thus attracting bats to forage in increased disturbance areas (Avila-Flores and Fenton 2005, Jung and Valko 2010). Jung and Valko (2010) found that, at a tropical forest-town interface, bat activity was the highest at streetlights and lowest in the forested site, with 18 of 25 bat species they detected frequently foraging around streetlights. This suggests that foraging near urban areas was advantageous for the bat species they detected. Similarly, urban environments present higher roost-site availability for some bat species that are able to take advantage of human-made structures such as houses and bridges to roost (Gaisler et al. 1998). Generally, our data is consistent with previous studies that suggest that insectivorous bats are able to adapt to anthropogenically disturbed environments. Compared to other mammals, it is possible that bats are less threatened, or even benefited, by urbanization because they possess the ability to cover large distances every night and move quickly between habitat types (Avila-Flores and Fenton 2005). This trait also allows them to react swiftly to changes in resource and roost availability (Rydell et al. 1996). However, this adaptability is species-specific and has a threshold. Our results show that areas of high disturbance had the lowest amount of bat activity. This is supported by a global meta-analysis which showed that high degrees of urbanization had strong negative effects on bat habitat use compared to intermediate disturbance habitats (Jung and Threlfall 2016).

The effect of disturbance and urbanization intensity becomes more nuanced when viewed at a species-specific level. We found that activity levels at low, medium, and high disturbances varied among the thirteen bat species we recorded. While phylogenetic similarity and functional ecology can explain the difference between species' persistence in disturbed environments, the global meta-analysis by Jung and Threlfall (2016) indicated that behavioral, morphological, and regional differences influence bats species' ability to adapt to urbanization. These differences were evident in our results. For example, we found that Mexican Free-tailed Bat activity did not differ between disturbance levels, which can be attributed to their broad niche, generalist foraging behavior, and use of human constructed roosting sites (Kunz et al. 1995, Feldhamer et al. 2003). In contrast, Silver-haired Bats were least common in high disturbance sites, perhaps due to their preference for roosting in foliage and tree bark and aversion for human-made structures (Patriquin and Barclay 2003, Perry et al. 2010). Other factors potentially influencing bat presence in urbanized environments include wing morphology and echolocation call design, both of which may limit species' foraging ability in cities (Norberg and Rayner 1987, Neuweiler 1984, Avila-Flores and Fenton 2005).

Arthropod Biomass, Disturbance, and Bat Activity

Arthropod biomass was highest in areas of low disturbance and lowest in areas of high disturbance. These results are consistent with a study by Threlfall et al. (2011) which found a negative relationship between insect biomass and housing density along an urbanization gradient. This relationship could be due to the abundance of impermeable surfaces in areas of high urbanization (Lagucki et al. 2017). These impermeable surfaces decrease primary productivity and therefore the abundance of bats' arthropod prey (Threlfall et al. 2011).

Despite these initial findings, arthropod biomass is a baseline measure of arthropod presence, and it does not give us adequate data to fully connect arthropod behavior to bat activity. One lepidopteran can be equivalent to the biomass of over one hundred dipterans, meaning that biomass and abundance cannot be understood interchangeably. Perhaps as a result, we found that arthropod biomass does not directly affect bat activity. However, there was a positive trend between biomass and activity, and both variables increased approximately an hour after sunset before following a steady decline. This implies that, within each disturbance level, an increase in arthropod presence occurred synchronously with an increase in bat activity.

An alternate explanation for why we did not find a significant relationship between bat activity and biomass is that urban areas could serve as an ecological trap for bats (Russo and Ancillotto 2015). An ecological trap is defined as a habitat "low in quality for reproduction and survival that cannot sustain a population, yet preferred over other available, high-quality habitats" (Donovan and Thompson 2001). In a study conducted on Little Brown Bats in the North American Prairies, Coleman and Barclay (2011) found that bats residing in high-density urban environments were in poor condition and produced fewer juveniles. This possibly resulted from greater competition for food, increased stress, higher disease transmission, and more pollution, indicating that this urban habitat acted as an ecological trap (Russo and Ancillotto 2015). We speculate that bats could be lured to more urbanized areas for reasons other than insect abundance, where they expend more energy on feeding than they would in low disturbance sites. This could explain why bat activity was highest in medium disturbance sites, whereas arthropod abundance was highest in low disturbance sites. Further research must be done in order to corroborate this hypothesis.

Water Quality and Disturbance

We found no significant relationship between urbanization and temperature, turbidity, DO concentration, or DO saturation. These results are consistent with other North Carolina based studies which have found that water quality degradation and urbanization are not correlated (Li and Kalcounis-Rueppell 2017). We found no relation between water quality and urbanization, potentially because urban pollutants such as industrial chemicals, municipal waste, and agricultural runoff are found to contaminate waterways in even non-urban areas (Brabec et al. 2002). At the landscape scale, evidence suggests that water quality degradation and urbanization are two separate processes, potentially influencing bat activity and arthropod diversity and abundance independently of one another (Li and Kalcounis-Rueppell 2017). Despite research by Li and Kalcounis-Rueppell (2017), other studies have found land use and land cover to be important indicators of water quality in the southern Appalachians (Wear et al. 1998). Moreover, in other studies, bat activity actually increased in areas of degraded water quality. Naidoo et al. (2011) suggest that this increase could be due to a simultaneous increase in insect biomass caused by eutrophication in the polluted waterbody.

Inconsistencies in these results could be rooted in different definitions of water quality and the parameters used to assess stream or lake heath. To support or reject these previous studies' conclusions, more thorough water quality measurements must be taken at each sample site. The four metrics measured for this study give an incomplete picture of water quality and the hydrological effects of urbanization. Tests for nutrients, such as phosphorus and nitrogen concentrations, and pesticide residues would be a more useful measure in parsing out the effects of urbanization on water quality.

Limitations and Further Study

The sites were tested over different temperatures throughout the summer and fall months, potentially influencing arthropod and bat abundance and diversity. The mean temperatures for each month, beginning in June and ending in September, were 18.9, 20.6, 20.0, and 16.4℃, respectively (US Climate Data). The seasonal variation in temperature also could have affected our water quality measurements. Dissolved oxygen concentration and saturation is highly dependent on temperature, and turbidity levels are partially a result of climatic events like rain storms and wind. Because water quality measurements at each site were only taken once, data were subject to variation which could have skewed determinations of true, overall water quality.

Additionally, as our research was conducted in a less developed region of the Southern Appalachians, areas classified as medium or high disturbance could be considered low disturbance in studies based in other geographic regions. This means that other research conducted on water quality and urbanization may not translate directly to our understanding of water quality in areas of high disturbance.

Because we did not find a significant correlation between arthropod biomass and bat activity, further study is necessary. In the future, we could analyze the bat calls to determine whether the bats were feeding at the sites or merely passing, and this could give us a better understanding on how the arthropod biomass and composition at each site affects bat activity. Future studies could be focused on bat population health in urban, exurban, and rural environments to investigate whether developed habitats serve as ecological traps for certain species.

In addition, we would like to identify each arthropod to Order. This would give our study a greater understanding of how anthropogenic disturbance affects arthropod diversity, and how that in turn affects bat activity and diversity along a disturbance gradient. It would also be useful to understand the pesticide use employed at each site in order to discern how pesticide use affects arthropod diversity and species composition. Golf courses, for example, which are areas of high disturbance, frequently apply pesticides, which would be consistent with low arthropod biomass.

Overall, our study showed that bats generally prefer areas of medium disturbance. Moreover, our findings told a more complete story when we specified the significance of disturbance on each species, indicating that bats can be more effectively understood utilizing a species-specific analysis. Our results can inform conservation efforts by discouraging a one-size-fits-all approach to managing the bat populations in a geographic area.

Acknowledgments

We would like to thank Dr. Rada Petric, Director of the Highlands Field Site, for allowing us access to her original research, for her mentorship, and for her help with this paper. We would also like to thank Jason Love, Associate Director of the Highlands Biological Station, for his support of the project. Lastly, thank you to Dr. Jim Costa, Director of the Highlands Biological Station, for his guidance in arthropod identification and for providing us with so many opportunities this semester.

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Getting a lesson on bear hunting from Roy McClure, Coweeta Hydrologic Laboratory. Starting from the left: Dr. Rada Petric, Chloe Hall, Vy Pham, Rachel Maunus, Daniela Zarate, Hannah Obenaus, Rachel Lopez, Scout Allen, Alex Hubbs, Gus Winiker, Dr. Chris Oishi, Dr. Jim Costa, and Roy McClure.

APPENDICES

APPENDIX 1. Descriptive statistics on bat activity and bat activity per time interval by disturbance level.

Variable	Time Interval	Disturbance	Sample Size	Minimum	1st Quartile	Median	Mean	3rd Quartile	Maximum	sd	se	ci
		Low	72	0	8.75	19	32.56	50	113	31.58	3.72	7.42
		Medium	104	0	13	36	48.44	80.25	198	42.86	4.20	8.33
Bat Activity		High	80	0	2.75	8	24.11	37.25	141	31.20	3.49	6.94
	one	Low	9	3	8	51	50	79	113	38.11	12.70	29.29
	two	Low	9	9	51	53	63.56	106	104	35.07	11.69	26.96
	three	Low	9	9	51	53	63.56	106	107	35.07	11.69	26.96
	four	Low	9	5	10	22	40.44	50	105	38.53	12.84	29.62
	five	Low	9	7	10	19	26.78	38	66	19.63	6.54	15.09
	six	Low	9	2	12	21	22.33	29	50	14.82	4.94	11.39
	seven	Low	9	5	6	13	16	18	44	12.59	4.20	9.68
	eight	Low	9	0	5	11	17.67	19	62	21.55	7.18	16.56
	one	Medium	9	6	8	11	23.67	17	113	34.12	11.37	26.22
	two	Medium	13	1	13	26	39.46	61	119	34.79	9.65	21.03
	three	Medium	13	17	41	83	76.62	94	174	42.15	11.69	25.47
	four	Medium	13	15	22	48	51.46	71	134	34.68	9.62	20.95
	five	Medium	13	3	7	36	48.46	70	146	45.90	12.73	27.74
	six	Medium	13	1	11	21	36.31	53	108	38.64	10.72	23.35
	seven	Medium	13	2	9	36	43	75	99	37.58	10.42	22.71
	eight	Medium	13	0	6	13	34.85	35	120	43.73	12.13	26.42
	one	High	13	4	10	46	57.38	82	198	56.70	15.73	34.26
	two	High	10	0	0.75	5	11.5	7.75	54	17.83	5.64	12.76
	three	High	10	0	6.5	10	40.6	66	141	49.88	15.77	35.68
	four	High	10	1	6.5	22.5	28.7	38.5	100	30.62	9.68	21.91
	five	High	10	0	2.5	37	40.3	77	90	37.40	11.83	26.76
	six	High	10	1	2.25	15.5	20.3	34	65	21.29	6.73	15.23
	seven	High	10	0	1.25	11.5	17	20.75	53	19.84	6.28	14.20
Bat Activity by Time Interval	eight	High	10	1	1.75	8	22.4	13	108	36.29	11.48	25.96

APPENDIX 2. Descriptive statistics on arthropod biomass (mg) and arthropod biomass (mg) per time interval by disturbance level.

Variable	Time Interval	Disturbance	Sample Size	Minimum	1st Quartile	Median	Mean	3rd Quartile	Maximum	sd	se	ci
		Low	69	0	55	138	322.28	467	2251	440.21	52.99	105.75
		Medium	102	0	38	117	212.78	283.25	1056	242.44	24.01	47.62
Arthropod Biomass		High	80	0	15.75	86.5	143.21	180.5	605	166.05	18.57	36.95
	one	Low	9	3	13	55	135.44	221	581	188.10	62.70	144.59
	two	Low	9	55	131	300	536.11	598	2251	678.92	226.31	521.86
	three	Low	9	43	84	171	359.56	538	1047	403.61	134.54	310.24
	four	Low	9	19	104	267	415.78	655	1248	411.92	137.31	316.63
	five	Low	9	0	46	60	236.67	222	1193	379.49	126.50	291.70
	six	Low	9	0	111.75	215.5	330.5	328.25	1183	380.89	134.66	318.43
	seven	Low	9	14	56.75	72	377.13	352	1920	647.70	229.00	541.49
	eight	Low	9	17	45.5	95	178	188	707	229.22	81.04	191.63
	one	Medium	13	3	20	48	67.62	98	230	70.44	19.54	42.57
	two	Medium	13	13	148.25	229.5	274	337.25	732	233.22	67.33	148.18
	three	Medium	13	24	94	217	295.08	391	930	293.87	81.51	177.59
	four	Medium	13	7	48	123	256.46	390	747	254.38	70.55	153.72
	five	Medium	13	0	71.25	179	206.83	333.75	454	156.37	45.14	99.35
	six	Medium	13	0	32	127	210	186	850	276.06	76.57	166.82
	seven	Medium	13	0	21	84	202.54	317	857	257.91	71.53	155.85
	eight	Medium	13	3	28.00	82.00	194.00	148	0	295.20558	81.8753	178.39095
	one	High	10	4	28	82	194	148	1056	295.21	81.88	178.39
	two	High	10	0	11.25	30	53.7	64	168	61.50	19.45	44.00
	three	High	10	14	81.75	141	229.1	393.5	596	232.34	73.47	166.20
	four	High	10	0	54	159	196.4	264.5	604	185.79	58.75	132.91
	five	High	10	0	37.5	135	179.7	250.75	492	181.58	57.42	129.89
Arthropod Biomass by Time Interval	six	High	10	5	51.5	100	133.4	156.25	536	153.73	48.61	109.97
	seven	High	10	0	28	74	161.9	176.75	605	207.69	65.68	148.57
	eight	High	10	0	3	81	107.4	162.75	323	116.62	36.88	83.42

APPENDIX 3. Descriptive statistics on temperature, dissolved oxygen concentration, dissolved oxygen saturation, and turbidity by disturbance level.

Date	Site Name	Disturbance	Turbidity (NTU)	Temperature (°C)	DO Concentration (mg/L)	DO Saturation (%Sat)
12 Jul 2021	Edwards Creek	Low	1.8	22.2	7.6	93.8
24 Jun 2021	Sonya	Low	1.4	21.3	9.2	115.4
5 July 2021	Fawn Creek	Low	NaN	20.2	8.1	95.6
23 Aug 2021	Lindenwood Lake	Medium	3.1	23.5	7.1	96.0
6 Jul 2021	Ravenel	Medium	NaN	23.0	7.9	100.6
25 Jun 2021	Mirror Lake	Medium	4.2	21.9	6.8	5.3
5 Jul 2021	Buck Creek	Medium	NaN	19.3	8.2	96.0
23 Jun 2021	Big Creek	Medium	1.8	23.4	8.1	105.8
2 Jul 2021	Biscuit Lake	Medium	21.2	21.4	6.8	88.5
19 Sept 2021	Mountain Retreat	Medium	2.9	20.4	7.6	96.9
7 Jul 2021	Rabun Gap Lake	Medium	NaN	22.2	8.0	93.3
12 Jul 2021	Horse Cove	Medium	1.5	23.5	8.7	110.4
6 Jul 2021	Golf	High	NaN	23.0	7.9	100.6
28 Jun 2021	Sequayah	High	2.3	25.0	8.2	110.6
10 Sept 2021	Cashiers Lake	High	2.8	21.7	8.2	-69.3
7 Jul 2021	Rabun Gap Field	High	NaN	23.9	8.6	109.8

HABITAT CHARACTERISTICS OF NEST BOX PLOTS FOR NORTHERN SAW-WHET OWLS (*Aegolius acadicus*) IN WESTERN NORTH CAROLINA

HANNAH OBENAUS AND DANIELA ZARATE

Abstract. Northern Saw-whet Owls (NSWO; *Aegolius acadicus*) are a common forest owl in North America. They mainly breed in northern parts of the US and southern parts of Canada. However, there is a disjunct population of NSWO in the southern Appalachian Mountains that is thought to be a remnant of the last ice age; this population resides year-round in western North Carolina and eastern Tennessee. Although NSWO are common owls, only general information about their habitat requirements is known, and even less is known about the disjunct population in Southern Appalachia. Only a few studies have focused on this disjunct population, and only two of those focused on habitat. We conducted habitat surveys for 13 NSWO nest boxes placed in the mountains of Southern Appalachia to see if there are any habitat variables that influence nest box occupancy. We found no significant difference between the habitat variables we tested and nest occupancy.

Key words: Aegolius acadicus; *Northern Saw-whet Owl; western North Carolina; habitat; nest boxes; survey*

INTRODUCTION

The Northern Saw-whet Owl (NSWO; *Aegolius acadicus*) is one of the most common forest owls in North America. It is a small owl the size of a robin, weighing between 65-151 g, that is found year-round in northern regions of the United States and southern regions of Canada (Whalen and Watts 2002, Ray 2014). These owls often migrate south during winter months, some going as far south as Florida, with females making up the majority of migrating NSWO (Neri et al. 2018, Soehren et al. 2019, Wall et al. 2021). There are two year-round populations: 1) the Allegheny Plateau of West Virginia and Maryland, and 2) the southern Appalachian Mountains of western North Carolina and eastern Tennessee. These two populations are believed to be disjunct colonies from the last glacial maximum that occurred 18,000 years ago (Milling et al. 1997). During this glacial maximum, ice sheets covered the northeastern US but stopped before reaching the Allegheny Plateau and southern Appalachian Mountains. High elevation mountains in these areas can support boreal flora and fauna, like the spruce-fir forests, that are typically found in the North. Therefore, northern species like the NSWO were able to survive in these areas. As the ice sheets began to retreat, some NSWO remained in these southern areas while others returned north creating two isolated disjunct populations (Tamashiro 1996). It is not known if NSWO from the southern Appalachia populations also migrate south during winter or if they remain residential but relocate to habitats at lower elevations (Soehren et al. 2019). Although these owls are common, not much is known about them because of their elusive and nocturnal behavior. Unless there are active monitoring programs in place, it is difficult to obtain even general NSWO data (Stedman 2003). Northern Saw-whet Owls are only vocal during mating season, making them difficult to survey when using playback recordings outside of the mating season (Bent 1937). Over the past twenty years more has been learned about NSWO, but the bulk of this research has taken place in the northeastern US and Canada. Less is known about the Southern Appalachia population because of their small population size, which is estimated to be ~500 birds (Milling et al. 1997).

Northern Saw-whet Owls in the northern US and southern Canada are generally found in coniferous and hardwood forests, seeming to prefer densely vegetated areas with little openness (Ray 2014). But they have also been found in bogs, swamps, and disturbed areas in their northern and western ranges. The northern and western populations are considered habitat generalists, being found in many different forest types, although they do seem to prefer coniferous woods when available (Ray 2014). For the past 40 years, the consensus has been that the disjunct population of NSWO in Southern Appalachia is a habitat specialist only found in spruce-fir forests at elevations above 1,350 m, because this is where northern coniferous forests are found in Southern Appalachia (Milling et al. 1997). If NSWO in Southern Appalachia are habitat specialists that only breed in the spruce-fir forests, then it is cause for concern. Spruce-fir forests in this area are under threat due to a history of intense logging and the introduction of the balsam woolly adelgid (*Adelges piceae*), which has killed the majority of Fraser Firs (*Abies fraseri*) in this region (Milling et al. 1997). This habitat reduction and fragmentation along with climate change could pose serious threats to this small disjunct population (Milling et al. 1997).

In contrast to the idea that NSWO only breed in spruce-fir forests, the 1997 study conducted by the US Department of Agriculture (USDA), found that NSWO were not affected by the mass die-off of firs in Southern Appalachia (Milling et al. 1997). This suggests these owls are more adaptable than previously thought, otherwise their densities post-woolly adelgid infection would have dropped. The USDA study also did not record the presence of owls below 1,219 m, while a 2014 study found owls present near 1,066 m (Milling et al. 1997, McCormick 2014). McCormick (2014) also found NSWO present in true hardwood forests with no spruce or fir just as frequently as they found NSWO in spruce-fir forests. This suggests that elevation is more important than habitat type, and that NSWO inhabits various habitats above 1,066 m. This could mean that since 1997, NSWO in this region have expanded their range, or that they are more of a habitat generalist than previously thought (McCormick 2014). However, there are only two studies that have looked at NSWO habitat in Southern Appalachia in-depth (Milling et al. 1997, McCormick 2014). In order to efficiently implement conservation management strategies for NSWO in this region, biologists need to have a better understanding of their habitat requirements, including the best locations to place NSWO nest boxes. The presence of NSWO in the nest boxes has been documented 13 times by the Blue Ridge Bird Observatory (BRBO) since 2007. Effective conservation is important because research suggests that increased habitat fragmentation and destruction decreases prey abundance and NSWO reproductive success and increases their stress levels. This can lead to a decrease in population size over time (Hinam and Claire 2008).

Nest boxes have been used in conservation efforts for other cavity dwelling species in western North Carolina, like the Carolina Northern Flying Squirrel (CNFS; *Glaucomys sabrinus coloratus*). The CNFS is another disjunct population remnant from the last glacial maximum that prefers high elevation habitats that resemble forests in northeastern US and southern Canada (Odom et al. 2001, Ford et al. 2015). When placed in the appropriate habitat, nest boxes provide shelter for breeding and help maintain the species' population. Nest boxes are especially useful for NSWO in habitats where natural cavities are limited (Maine Natural History Observatory). Research has shown that nest boxes located near natural cavities are used less by NSWO than nest boxes in areas with few or no natural cavities (Elias and Stoleson 2021).

Nest boxes can also help managers create habitat prediction models. Carolina Northern Flying Squirrel (CNFS) nest boxes have been used to formulate presence/absence data that was then used to predict which habitats CNFS were likely to be found in based on different topographic and environmental factors (Ford et al. 2015). Predictive habitat and occupancy modeling are very useful tools in conservation management and would help streamline NSWO conservation efforts. Nest boxes may also be a way to estimate NSWO populations in Southern Appalachia. Northern Saw-whet Owls migrate south to winter but return north to breed and lay eggs (Marks and Doremus

2000). This means that any NSWO using nest boxes in this region are more than likely part of the disjunct population and are not just passing through. Nest boxes can also offer insight on the biological effects of conservation management tools. One study found that nest boxes placed in habitats preferred by the cavity-nesting Great Tit (*Parus major*) led to higher breeding densities, decreased food resources, and possibly increased predation. This decreased reproductive success and created an ecological trap (Mänd et al. 2005). For the Great Tit, it was more beneficial for nest boxes to be placed in non-preferred habitat because the population densities and resource competition were lower (Mänd et al. 2005). It is important to know whether this also applies to NSWO in this region and which habitat is best for reproductive success.

Northern Saw-whet Owls are known for inhabiting natural cavities in dead trees instead of building their own nests. They often take over other animals' nests, like abandoned woodpecker holes, when choosing breeding sites (Bent 1937). Our habitat survey assessed the habitat variables that affected the use of the nest boxes by the NSWO during the breeding season. Previous studies show that the presence and reproductive success of NSWO are correlated with the abundance of food availability, more specifically the Red-backed Vole (*Myodes gapperi*) (Bowman et al. 2010, Confer et al. 2014, Marks et al. 2015). Few studies, however, have focused on assessing the habitat type of NWSO nest boxes to predict ideal locations for the nest boxes. In our survey we analyzed if habitat type affects nest box use by NSWO. We predict that habitat type affects which next boxes NSWO use, and that they prefer nest boxes in densely vegetated areas with some spruce-fir component.

METHODS

Site Description

Our two study sites were located in the Blue Ridge physiographic province at elevations >1,550 m. We surveyed 13 NSWO nest boxes out of the 47 placed by the BRBO at Big Bald Mountain (BB) in Yancey County, NC and Unicoi County, TN, and Mile High (MH) in Haywood County, NC. Out of the 13 nest boxes, seven were surveyed at MH, near Maggie Valley in North Carolina (35.5108°N, 83.1788°W) and six were surveyed at BB, at the North Carolina-Tennessee border (35.9897°N, 82.4905°W) (Figs. 1 and 2). Both sites were located on forested mountains, though Big Bald Mountain has a patch of grassy bald bordering the forested area.

Plot Characteristics

The plot characteristics measured were elevation, aspect, and the distance to nearest evergreen tree, rhododendron, and edge. The 400 m^2 plot was marked with brightly colored flags - a flag was placed 10 m from the nest box in all four cardinal directions to form a square. We obtained the elevation from Gaia GPS. The aspect was also measured using a compass. To measure the distance to the nearest evergreen and rhododendron, we identified the closest ones in the 400 m^2 plot around the nest box and measured the distance with a measuring tape. We measured the distance to the closest edge habitat using ArcGIS Online map viewer. Because both of our sites were in national forests, the closest edge habitats were hiking trails or roads. We measured the distance from each nest box to the closest trail or road using the Measure tool and satellite imagery Basemap (Esri World Imagery).

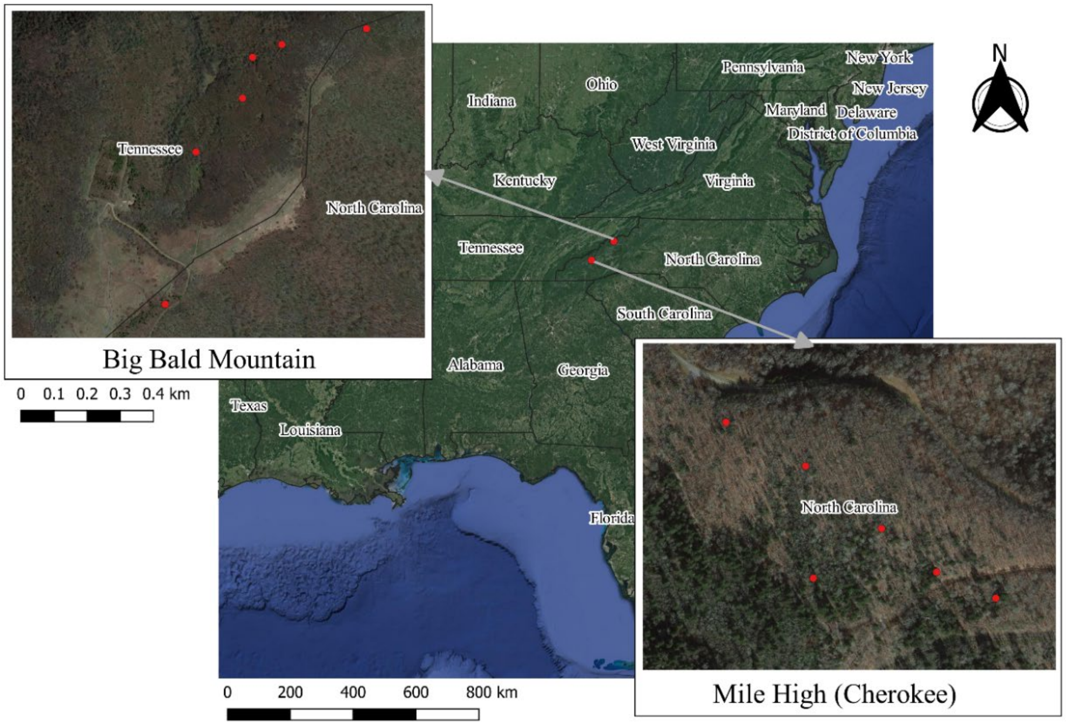

FIG. 1. Our nest boxes were located in western North Carolina at Mile High (MH) and Big Bald (BB), the latter which straddles the border of western North Carolina and eastern Tennessee

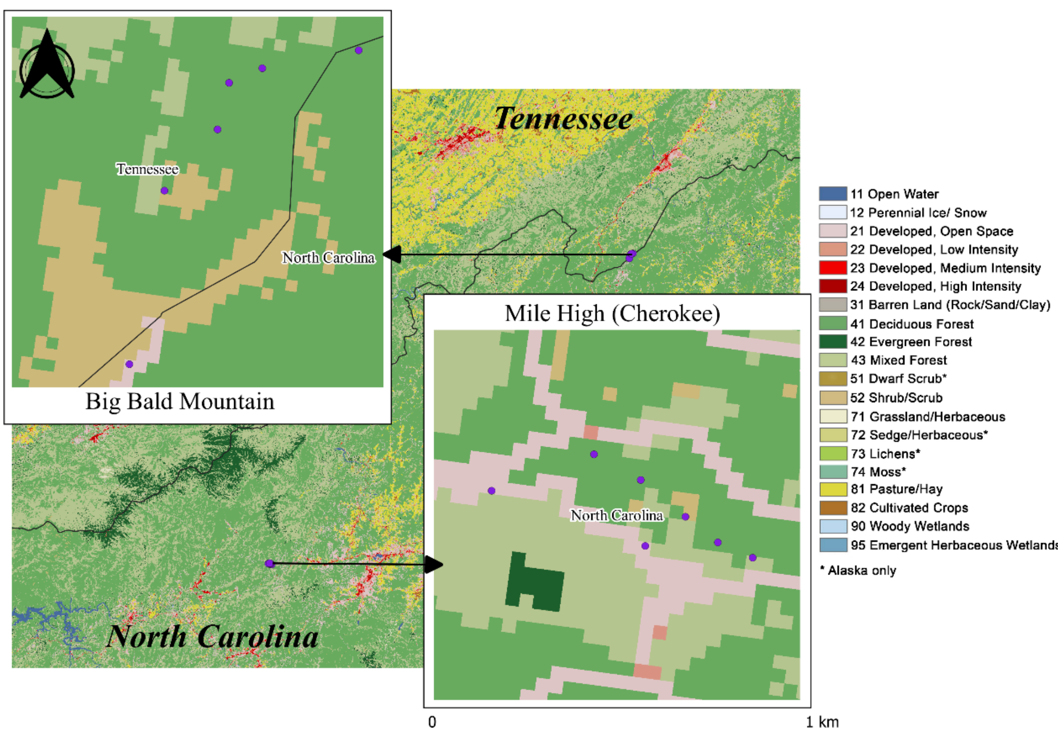

FIG. 2. All nest boxes were located in deciduous forests. The coarse resolution of the USGS 2019 National Land Cover Database shows some points occurring outside of deciduous forests.

Habitat Structure

To analyze habitat structure we measured basal area, canopy height and coverage, and the number of natural cavities in the 400 m^2 plot surrounding the nest box. To calculate basal area, we measured the DBH (diameter at breast height) of all trees ≥5 cm DBH; DBH was consistently measured at approximately 1.37 m above ground. If a tree had multiple stems that deviated near or above 1.37 m, we took the DBH of the entire trunk - not each individual stem. If the tree had multiple stems that deviated below breast height, we took the DBH of the circumference of all the stems. Because we did not take the DBH of each individual stem when deviation occurred below 1.37 m, there will be a slight overestimate in the basal area values. Fortunately, there were few plots that contained trees with this description. We also identified the tree species for each DBH measurement we took. We estimated canopy height by looking at the trees in the 400 m^2 plot and determining whether the majority were greater, less, or equal to ten meters in height. Canopy coverage was measured using a spherical densiometer. A densiometer measurement was taken at each cardinal point approximately two meters away from the nest box. Finally, we visually estimated the number of natural cavities we saw in the trees in the 400 m^2 plot. To estimate ground cover type, we took note of what was touching the pole (live woody, duff, leaf litter, etc.) when we placed it on the ground every two meters, for approximately 10 m in each cardinal direction.

Nest Box Characteristics

Northern Saw-whet Owl nest boxes were placed ≥3 m above the ground and built according to standard NSWO nest box dimension protocol - large enough for NSWO to enter but small enough to keep predators out (https://nestwatch.org). The habitat survey was designed to assess which habitat variables affect the use of the nest boxes by the NSWO during breeding season. At each nest box, we identified the tree species and recorded the box height and orientation. We used a 3.2-meter telescopic extension pole to measure the box height. The height was measured from the ground to the bottom of the box, adjusting the pole as needed. If the box height was <3.2 m, we adjusted the pole and then measured the height with a measuring tape. If the box height was >3.2 m, the pole was held touching the bottom of the nest box and a mark was made with washable chalk at the bottom of the pole, then the distance between the ground and the chalk mark was measured and added to the 3.2 m. We measured nest box orientation using a compass.

Mist Netting and Banding

We surveyed for NSWO on the night of September 13th, 2021. Before sunset we set up six mist nets on Big Bald Mountain, NC. Each mist net was 12 m long and made with 30 mm mesh. After sunset, at approximately 20:15, we played the audio lure. The audio lure consisted of different types of NSWO calls and was pointed north towards incoming migrating birds. We checked each mist net every thirty minutes over a two-hour span. Around 21:45, our third net check, we found a NSWO in mist net 14. A trained BRBO bird technician untangled the NSWO from the mist net and put it in a bag to prevent overstimulation. We then transported the owl to a table to safely take measurements and band it. The owl's leg was banded with an aluminum alloy band that had a unique tag number engraved in it. After banding the owl, we proceeded to measure its mass, wing length, tail length, culmen length, hallux length, and body fat, as well as determine its age and body and flight feather molt. We used a hanging scale to measure the mass of the bird to the nearest 0.1 g. We used a ruler to measure wing, tail, culmen, and hallux length to the nearest mm. To measure body fat and feather molt, the technician blew on the owl through a straw to examine feather characteristics. Sex was determined using a sex probability chart that took weight and wing chord length into account. We determined age by shining ultraviolet light on the inside of the owl's wing. Ultraviolet light was used to assess the presence of a feather protein that reflects a bright pink color. The ultraviolet light showed uniform bright pink across the underside of the wing - indicating the owl was likely a hatch year due to the abundance of the protein. While taking measurements, we kept a bag over the owl's head to keep it calm. When measurements were completed, we turned off all lights and took the bag off the owl's head to let its eyes adjust to the night for approximately five minutes. Then the owl was released from grasp and allowed to fly away once its eyes were adjusted.

FIG. 3. The NSWO we caught and banded September 13, 2021.We determined she was female based on weight and wing chord length. We determined she was a hatch year owl by examining proteins in her feathers.

Data Analysis

The data were processed using RStudio. We performed a Shapiro test for each variable to determine if we had parametric or non-parametric data which determined the type of analysis we used. We ran General Linear Models (GLM) for each variable to determine the p value and the statistical significance of each variable. Our alpha level for the p value was set to 0.05. We measured ground type and documented the tree species with ≥5 cm DBH at each plot for descriptive purposes but did not include them in the statistical analysis as the complexity of the data made it difficult to use categorically. However, it is worth noting that the nest boxes that were occupied at MH were at the two plots with the greatest number of spruce trees, and the occupied nest boxes at BB were at the three plots with the most beech trees. The tables for these habitat variables can be found in the appendix (see Appendix I and II). There were no natural cavities found at any of the plots, so we did not include these data in the results.

RESULTS

TABLE 1. Landscape characteristics of MH and BB in each of the 400 m^2 plots.

Nest Box ID	Elevation (m)	Aspect (degrees)	Nearest Evergreen Tree (m)	Nearest Rhododendron (m)	Nearest Edge (m)
MH-02	1,556	168 S	2.07	0.08	28.70
MH-04	1,588	176 S	2.70	7.10	30.70
MH-05	1,595	172 S	2.40	9.70	13.50
MH-09	1,551	230 SW	0.35	14.00	26.90
MH-11	1,623	180 S	4.70	NA	130.20
MH-13	1,600	190 S	1.90	NA	99.70
MH-14	1,620	246 SW	10.60	11.60	31.90
BB-07	1,552	335 NW	NA	11.80	20.60
BB-09	1,582	298 NW	NA	15.00	20.70
BB-11	1,583	305 NW	9.20	3.50	12.20
BB-13	1,603	288 W	NA	NA	5.09
BB-30	1,558	70 E	NA	NA	3.27
BB-Pole	1,598	180 S	9.20	5.80	18.30

There was more variation in aspect between plots at the BB site than at the MH site, with those at the MH site all facing south or southwest while those at BB had a mix of aspects (Table 1). The plots at MH all had an estimated canopy height >10 m but all the plots at BB had canopy heights of 10 m or less. Missing values for the nearest evergreen tree/rhododendron columns mean there were no evergreen trees or rhododendron visible from the plot. All the plots at MH had evergreen trees present while only two of the plots at BB did. Two plots from MH and BB did not have rhododendrons present. Two plots at MH, 11 and 13, had very high distance to nearest edge values and two plots at BB, 13 and 30, had relatively low distance to nearest edge values.

TABLE 2. Forest structure of each 400 m^2 plot at MH and BB.

Nest Box ID	Basal Area ($\boldsymbol{m^2}$)	Canopy Height (m)	Canopy Coverage (%)
MH-02	1.14	>10	92.4
MH-04	1.00	>10	98.4
MH-05	0.82	>10	98.7
MH-09	0.81	>10	95.8
MH-11	0.45	>10	90.1
MH-13	0.97	>10	89.9
MH-14	0.36	>10	82.8
BB-07	0.24	10	91.7
BB-09	0.58	<10	83.1
BB-11	0.26	<10	64.1
BB-13	0.66	<10	94.3
BB-30	0.64	<10	90.4
BB-Pole	2.80	<10	71.9

The most common tree species we documented were Red Spruce (*Picea rubens*), birch (*Betula*), American Beech (*Fagus grandifolia)*, oak (*Quercus*), and maple (*Acer*) (Appendix I). Canopy coverage ranged from 82.8–98.7% at MH and from 64.1–91.7% at BB (Table 2).

TABLE 3. Nest box characteristics at each plot at MH and BB.

Nest Box ID	Box Height (m)	Box Orientation (degrees)	Tree Species	DBH (cm)
MH-02	4.65	220 SW	Spruce	44.7
MH-04	3.60	30 NE	Spruce	42.3
MH-05	3.97	326 NW	Spruce	51.0
MH-09	3.50	60 NE	Spruce	62.3
MH-11	3.12	308 NW	Spruce	66.0
MH-13	3.28	300 NW	Spruce	24.3
MH-14	3.90	8 N	Spruce	36.2
BB-07	4.15	140 SE	Maple	71.0
BB-09	3.07	84 E	Birch	45.3
BB-11	3.10	49 NE	Pine	35.5
BB-13	4.50	353 N	Birch	48.5
BB-30	3.70	328 NW	Oak	64.3
BB-Pole	3.60	180 S	NA	NA

Nest box height at both sites did not vary much with the range being 3.12–4.65 m at MH and 3.07–4.50 m at BB (Table 3). All the trees that nest boxes were mounted on at MH were Red Spruce, while none of the trees at BB were Red Spruce. There is no tree species listed for BB-Pole because the nest box was mounted on a wooden pole.

TABLE 4. Min, max, mean, and confidence intervals for habitat variables of occupied and unoccupied nest boxes. (N. stands for nearest).

	Nest	Min	1st Q	Median	Mean	3rd Q	Max	sd	se	ci
Elevation	No	1551	1555	1578	1582	1605	1623	31.21	11.03	26.09
	Yes	1582	1583	1588	1590	1595	1603	8.82	3.94	10.94
Height	No	3.12	3.45	3.65	3.74	3.96	4.65	0.49	0.17	0.41
	Yes	3.07	3.10	3.60	3.65	3.97	4.50	0.61	0.27	0.75
DBH	No	5	33.23	53.50	46.73	64.73	71	23.52	8.31	19.66
	Yes	35.50	42.30	45.30	44.52	48.50	51	6.02	2.69	7.47
Basal area	No	0.24	0.43	0.73	0.93	1.01	2.80	0.82	0.29	0.68
	Yes	0.26	0.58	0.66	0.66	0.82	1	0.28	0.12	0.34
N. evergreen	No	0.35	2.03	6.95	8.60	12.95	20	7.88	2.79	6.59
	Yes	2.40	2.70	9.20	10.86	20	20	8.77	3.92	10.90
N. rhododendron	No	0.08	10.15	12.90	12.91	20	20	7.27	2.57	6.08
	Yes	3.50	7.10	9.70	11.06	15	20	6.52	2.92	8.10
N. edge	No	3.27	10.42	24.50	41.42	48.85	130.20	47.28	16.72	39.53
	Yes	13.50	20.60	20.70	22.08	26.9	28.7	6.02	2.69	7.47
Canopy Cover	No	71.92	88.11	90.25	90.25	91.88	95.84	7.50	2.65	6.27
	Yes	64.12	83.10	94.28	87.73	98.44	98.70	14.64	6.55	18.17

The difference in mean between occupied and unoccupied groups were greatest for basal area and distance to nearest edge (Table 4). The mean basal area was 0.66 m^2 for occupied nest boxes and 0.93 m^2 for unoccupied nest boxes. The mean distance to edge was 22 m for occupied nest boxes and 41 m for unoccupied nest boxes. There were large differences in confidence intervals between occupied and unoccupied nest boxes for elevation, DBH, basal area, distance to edge, and canopy cover. Large confidence intervals mean high variability in the data set and a less accurate estimate of mean. This makes sense if you look at the differences between the minimum and maximum values of occupied and unoccupied nest boxes for those variables.

TABLE 5. General linear model results show no significant difference between habitat variables occupied and unoccupied nest boxes.

Variable	Estimate	std. error	*p* value
Elevation	7.95	14.51	0.60
Height	-0.09	0.36	0.78
DBH	-2.21	10.89	0.84
Basal area	-0.34	0.47	0.49
N. evergreen	0.23	0.49	0.64
N. rhododendron	-1.85	4.00	0.65
N. edge	-0.63	0.65	0.35
Canopy Cover	-0.01	0.07	0.95

The results of our General Linear Model did not show any significant differences between the habitat variables we measured and nest occupancy (Table 5). The lowest *p* values generated by the general linear model were for distance to edge (p=0.49) and basal area (p=0.35).

DISCUSSION

Our results did not support our hypothesis that NSWO would favor habitats with greater basal area and spruce-fir components. There were no statistically significant differences between basal area in occupied and unoccupied nest boxes. And the three occupied nest boxes at BB had no Red Spruce or Fraser Fir in the plot. We expected to have the lowest *p* values for basal area and distance to neared edge based on the difference in means between occupied and unoccupied nest boxes. The noticeable difference in means was caused by outliers in the data. The distance to edge values for nest boxes 11 and 13 at MH were 99.70 m and 130.20 m. This skewed the mean distance to edge value for the unoccupied group. The mean distance to edge for the unoccupied nest boxes was 22 m without outliers, very close to the mean of the occupied nest boxes. The basal area value for the nest box pole at BB was 2.8 m^2, significantly larger than any of the other plots. This was expected because this plot had several trees with multiple stems deviating from below breast height. The method we used to measure DBH of these trees likely led to an overestimate in basal area. Without this outlier the mean basal area for the unoccupied nest boxes was 0.58 m^2.

Our sample size was too small to detect any statistical significance because of the high variability between sites. However, the fact that we did not find any statistical significance does not necessarily mean it does not exist. It may also be that our lack of statistical significance suggests NSWO are not habitat specialists at high elevations. This aligns with the results of the most recent NSWO research performed on this disjunct population (McCormick 2014). However, our study was not sufficient to definitively answer this question; because we did not have nest boxes at lower elevations in our sample, we cannot suggest they are habitat generalists.

If this study were to be replicated, the sample size would have to be much larger to account for high variability between sites. Ideally, all 47 nest boxes that BRBO mounted would be surveyed. Additionally, nest boxes at lower elevations should be mounted and monitored to see if NSWO can adapt to lower elevations if there are nesting cavities available. This larger sample size would account for variability and might produce statistically significant results. Another important addition to this study would be small mammal trapping at the nest box sites. Studies have shown reproductive success is linked to small prey abundance (Bowman et al. 2010, Drilling 2013, Confer et al. 2014, Marks et al. 2015). It is likely that prey abundance also affects nest box occupancy in Southern Appalachia. Moreover, with more resources, it would also be interesting to put GPS tracking devices on adult NSWO that are found in the BRBO nest boxes. Then we could track their movement to see if they migrate to lower elevations during the winter or if they migrate south. We would also be able to assess which elevation and habitat types they spend the majority of their time in. This would help with predictive habitat modeling.

The results of our study do align with the consensus that NSWO in northern US and southern Canada are habitat generalists (Ray 2014). However, because of our small sample size we cannot definitively suggest this. The study should be replicated to include the remaining 34 nest boxes at BB and MH and include new nest boxes at lower elevations. The larger sample size and the presence/absence of NSWO at lower elevations would reveal more about their habitat preference and adaptability. Additional habitat variables like prey availability should also be surveyed as it is directly tied to NSWO reproductive success and is important for effective conservation management.

ACKNOWLEDGMENTS

Thanks to Mark Hopey, the lead bander for the Blue Ridge Bird Observatory under Federal Master Banding permit #23821 and North Carolina Wildlife Commission Endangered Species permit #21-00284, for being our mentor. Thanks to our professors Jason Love and Dr. Rada Petric; without their help this would have not been possible. Also, thanks to the Eastern Band of Cherokee Indians Natural Resources Program for allowing us to work at Mile High and to the US Forest Service, Pisgah, and Cherokee National Forests for allowing us to work at Big Bald Mountain.

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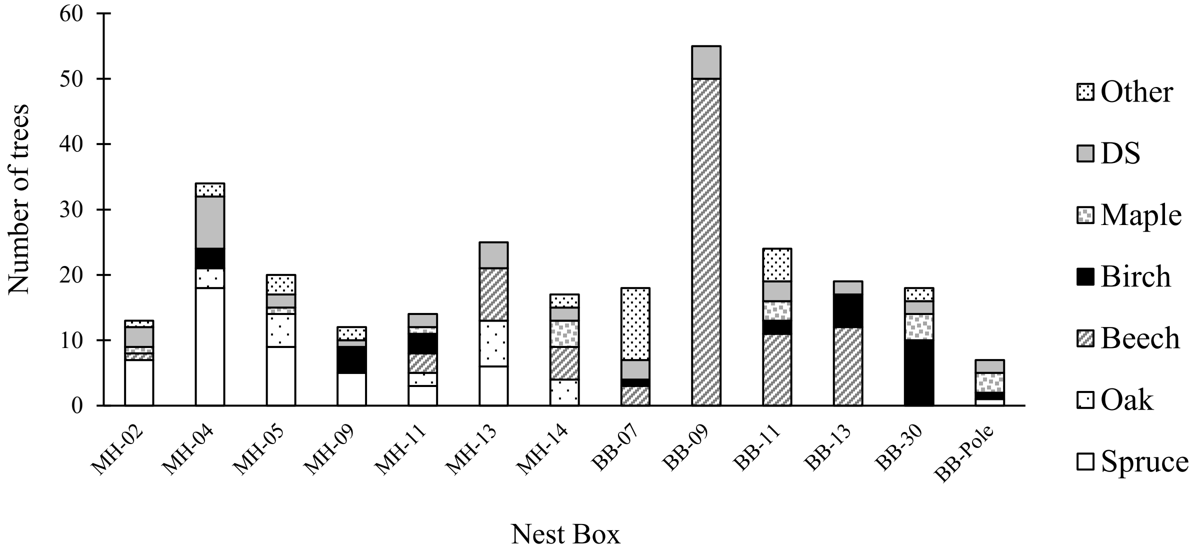
APPENDIX



APPENDIX I. The unequal distribution of tree species ≥5 cm DBH at each nest box plot.

APPENDIX II. Ground layer type visually observed at each nest box plot.

Nest Box ID	Ground Type
CSWO-02	Mainly duff, leaf litter, dead woody; some live herb and moss
CSWO-04	Mainly duff, leaf litter, and dead woody; some live woody and moss
CSWO-05	Mainly duff, leaf litter, dead woody; some live herb and moss
CSWO-09	Mainly duff, leaf litter, and live herb; some live woody and dead woody
CSWO-11	Mainly duff, leaf litter, and dead woody; some live herb touch and moss
CSWO-13	Mainly leaf litter, dead woody, and live herb; some duff and live woody
CSWO-14	Mainly leaf litter, dead woody, and live herb; some duff, moss, and live woody
BB-07	Mainly duff, leaf litter, and live herb; some dead woody, moss, and live woody
BB-09	Mainly leaf litter, dead woody, and live herb; some duff and live woody
BB-11	Mainly leaf litter, dead woody, and live herb; some duff, moss, and live woody
BB-13	Mainly leaf litter, live herb, and live woody; some duff, dead woody, and moss
BB-30	Mainly leaf litter, dead woody, and live herb
BB-Pole	Mainly duff, leaf litter, and live herb; some dead woody, moss, and live woody

BIG STORM, LITTLE PLASTICS: MICROPLASTIC CONCENTRATIONS AND DYNAMICS DURING A STORM EVENT AND ATMOSPHERIC DEPOSITION IN THE CHATTOOGA RIVER, NORTH CAROLINA

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Abstract. There is growing concern regarding the ubiquity of microplastics (<5 mm) in our environment potentially threatening the health of wildlife and humans; however, little research has been done to explore how microplastics behave in freshwater systems. In this study, we measured microplastic concentrations and loads at baseflow and during a storm event in the headwaters of the Chattooga River over a two-month period. We also measured the rate of atmospheric deposition of microplastics at three locations in the watershed. At two sites along the Chattooga River, we collected water samples throughout a storm event to assess how microplastic concentrations varied with discharge. We found that during the rising limb of the storm event, microplastic concentrations increased before decreasing at peak discharge. Consistent with the dynamics of other pollutants commonly found in river systems, our results suggest that microplastics from the surrounding terrain and buried in the benthic layer are re-suspended and transported during the initial flux of a storm event in a first flush phenomenon. We also found that atmospheric deposition of microplastics was greater during the storm event compared to non-storm conditions. These data indicate a potentially high concentration of microplastics polluting the Chattooga Watershed. Although this is a preliminary study, our results are important to furthering the current understanding of microplastic contamination and can hopefully be used to better inform future microplastic studies and management efforts.

Key words: atmospheric deposition; baseflow; Chattooga River; discharge; microplastics; stormflow; southern Appalachians; Wild and Scenic River; western North Carolina

INTRODUCTION

Plastics are durable, easy to produce, cheap, and disposable; thus, they have become a widespread pollutant in the environment (Jambeck et al. 2015). Only 9% of plastics ever produced have been recycled, with 79% ending up in landfills and 12% being incinerated (Geyer et al. 2017). If the status quo of production and waste management persists, an estimated 12,000 megatons of plastic waste will dominate landfills and the environment by 2050 (Geyer et al. 2017). As these plastics degrade, they form microplastics (MPs), which are defined as particles between 1 µm and 5 mm in length (Mani et al. 2015, Valsesia et al. 2021). Microplastics can be classified as fragments, foams, films, pellets, and fibers, and they can vary in coloration (Y. Zhang et al. 2020). Microplastics are categorized as either primary or secondary, with primary MPs originating in textiles, medicines, cosmetics, and feedstock, and secondary MPs derived from degradation of larger plastic debris (Cole et al. 2011, Boucher and Friot 2017). The processes by which these plastics deteriorate into secondary MPs include photodegradation, mechanical-physical weathering processes, chemical weathering processes (e.g., hydrolysis, oxidation), abrasion, and biodegradation (Klein et al. 2018).

Microplastics are transported to freshwater and marine environments from wastewater treatment plants, surface runoff, and discarded refuse (Gall and Thompson 2015). Specifically, wastewater treatment plants are a concentrated source of MPs entering waterways (Estahbanati and Fahrenfeld 2016, Magni et al. 2019). On average, wastewater treatment plants remove 88% of MPs, allowing the remaining 12% to accumulate in sewage sludge and effluents which are released into freshwater or agricultural fields (Iyare et al. 2020). Further, MPs are released into the earth's atmosphere, where they are transported and deposited in terrestrial and marine environments through both wet and dry deposition (Allen et al. 2019, Klein and Fischer 2019, Zhang et al. 2019, Roblin et al. 2020, Wright et al. 2020, Y. Zhang et al. 2020, Szewc et al. 2021).

Although only one published study has assessed the effect of storm events on MPs, rain and storm events have been found to be a source of MP contamination in freshwater systems (Hitchcock 2020). It is hypothesized that rain transports MPs to waterways in a similar fashion as runoff from nonpoint source pollutants (Wagner et al. 2019, Hitchcock 2020). Storms also increase river flows, which erode channel bed sediments from the benthic layer and re-suspend MPs in the water column (Horton and Dixon 2018). Moreover, heavy rainfall, and the resulting runoff, can overflow wastewater treatment systems, releasing unfiltered wastewater containing MPs into the environment (Fendall and Sewell 2009, Wagner et al. 2019). These combined effects result in increased MP concentrations in rivers during storm events (Wagner et al. 2019, Hitchcock 2020).

The ubiquity of MPs in the environment presents numerous health concerns for both ecosystems and humans. At the organismal level, MPs have been found to bioaccumulate with increasing trophic levels in aquatic environments (Eriksson and Burton 2003, Dawson et al. 2018). When exposed to MPs, aquatic biota may experience a range of toxic molecular and systemic physiological impacts, including alterations to immune, metabolic, feeding, growth, and reproductive functions, and changes to gene expression (Anbumani and Kakkar 2018, Franzellitti et al. 2019). The polymers and additives of which MPs are made, along with toxins sorbed on their surfaces, have the propensity to leach and desorb inside the bodies of humans and animals, leading to the aforementioned health effects (Li et al. 2018, Liao and Yang 2021).

The impacts of MPs on human health are still an emerging field, but routes of exposure include ingestion, inhalation, and dermal contact (Prata et al. 2020). Preliminary research has found that MPs have the potential to bioaccumulate in humans from an increased diet of seafood (Smith et al. 2018). Atmospheric MPs also present concerns, with several observational studies noting airway diseases, interstitial lung diseases, and cancer among people with elevated exposure to MPs (Prata 2018). Airborne MPs also pose a threat to our climate – in the first study of its kind, MPs were found to scatter light and produce a slight cooling effect. Yet, it is anticipated that with the increasing density of MPs in the boundary layer of the atmosphere, their effect could switch to generate a slight greenhouse effect due to infrared absorption (Revell et al. 2021).

We aim to contribute to the growing body of evidence of MP accumulation and contamination in North American freshwater systems, considering that less than 4% of MP research is focused on freshwaters (Lambert and Wagner 2018). While most MP studies focus on transport in marine environments, there are a limited number of studies conducted on river transport of MPs. Research of this nature is novel considering that no freshwater MP analysis in the southeastern US has been published and, to our knowledge, there is only one published study that has examined MP concentrations during storm events (Hitchcock 2020). Because it is likely that MPs vary with flow conditions, following the trend of other pollutants in waterways, it is important to investigate MP loads during periods of low, moderate, and high flow. However, studies on how MP concentrations vary with flow are currently lacking. Likewise, the significance of atmospheric deposition and wastewater treatment plants as sources of MPs are poorly researched fields. Considering storm events in calculations of MP concentrations is critical to our understanding of the maximum number of MPs to which aquatic ecosystems are exposed. Studying atmospheric deposition is important in understanding how much MP contamination in freshwater systems comes from the air. This research also highlights the importance of stormwater management in MP reduction efforts.

The primary goal of this study is to determine the abundance of MPs in the water of the upper Chattooga River in western North Carolina over a range of flow conditions, including storm events. The upper Chattooga River is located in Jackson County, North Carolina. The lower reaches of the river are designated as a National Wild and Scenic River, and portions of it pass through the Ellicott Rock Wilderness Area. This region is a biodiversity hotspot, making this research important to the prosperity of the unique wildlife and habitats in western North Carolina (Van Sickle 1999, Simon et al. 2005). We sampled the headwaters of the Chattooga River, downstream from a wastewater treatment plant.

In this study, we sampled two locations along the headwaters of the Chattooga River for MPs. One site was directly downstream from a wastewater treatment plant, and the second was a few kilometers downstream. We also sampled atmospheric deposition rates in throughfall and open field conditions. We hypothesized that there would be a difference in MP concentrations between the two sample sites along the Chattooga River. We expected the upstream reaches to possess higher baseflow MP concentrations than the downstream reaches due to the relative distance from the wastewater treatment plant. We also predicted that during major storm events, stormflow MP concentrations will be higher than baseflow MP concentrations due to increased sediment disturbance, atmospheric deposition, and nonpoint source runoff.

METHODS

Site Description

The upper Chattooga River watershed lies in the southern Blue Ridge physiographic province in Jackson County, North Carolina (Fig. 1). The study watershed is 19.50 km² and ranges in elevation from 1,489 m at the top of Whiteside Mountain to 859 m at our lower sample site known as Sliding Rock (SR). The river is a fourth-order stream. The watershed is primarily forested (81%), but also includes low-intensity development (14%), and medium to high-intensity development (0.02%) (MRLC National Land Cover Database 2019). The watershed of the Cashiers Creek (CC) site, which is a subbasin within SR watershed, has a total area of 3.28 km² and is predominantly covered with forest (43%), followed by developed open space (24%) (MRLC National Land Cover Database 2019). Cashiers Creek is a third-order stream. The small unincorporated town of Cashiers is served by the Trillium Wastewater Treatment Plant #3 which is close to CC. This wastewater treatment plant uses an aerobic treatment method, with chlorine disinfection and sodium sulfite dichlorination, that allows for the release of treated effluent into the upper reaches of the Chattooga River. During our sampling period, the mean discharge from the plant was 412 m^3/day (range = 250 - 1.01×10^3 m^3/day) (Tuckaseigee Water & Sewer Authority, Trillium Wastewater Treatment Plant, unpublished data).

The most downstream monitoring site was located at SR (35.07572 N, -83.10881 W) at an elevation of 859 m. The CC monitoring site (35.10310 N, -83.10718 W) sat at an elevation of 1,047 m. The riparian area of both SR and CC consisted of a deciduous forest and a dense canopy of Rosebay Rhododendron (*Rhododendron maximum*). The river bed at SR consisted of bedrock and sand, while at CC the substrate was primarily composed of alluvial sediments, including a mixture of sand, silt, and clay. A monitoring site was also installed at the Hall property (HP) (35.10864 N, -83.10916 W) at an elevation of 1,062 m, and was only used to measure the atmospheric deposition of MPs.

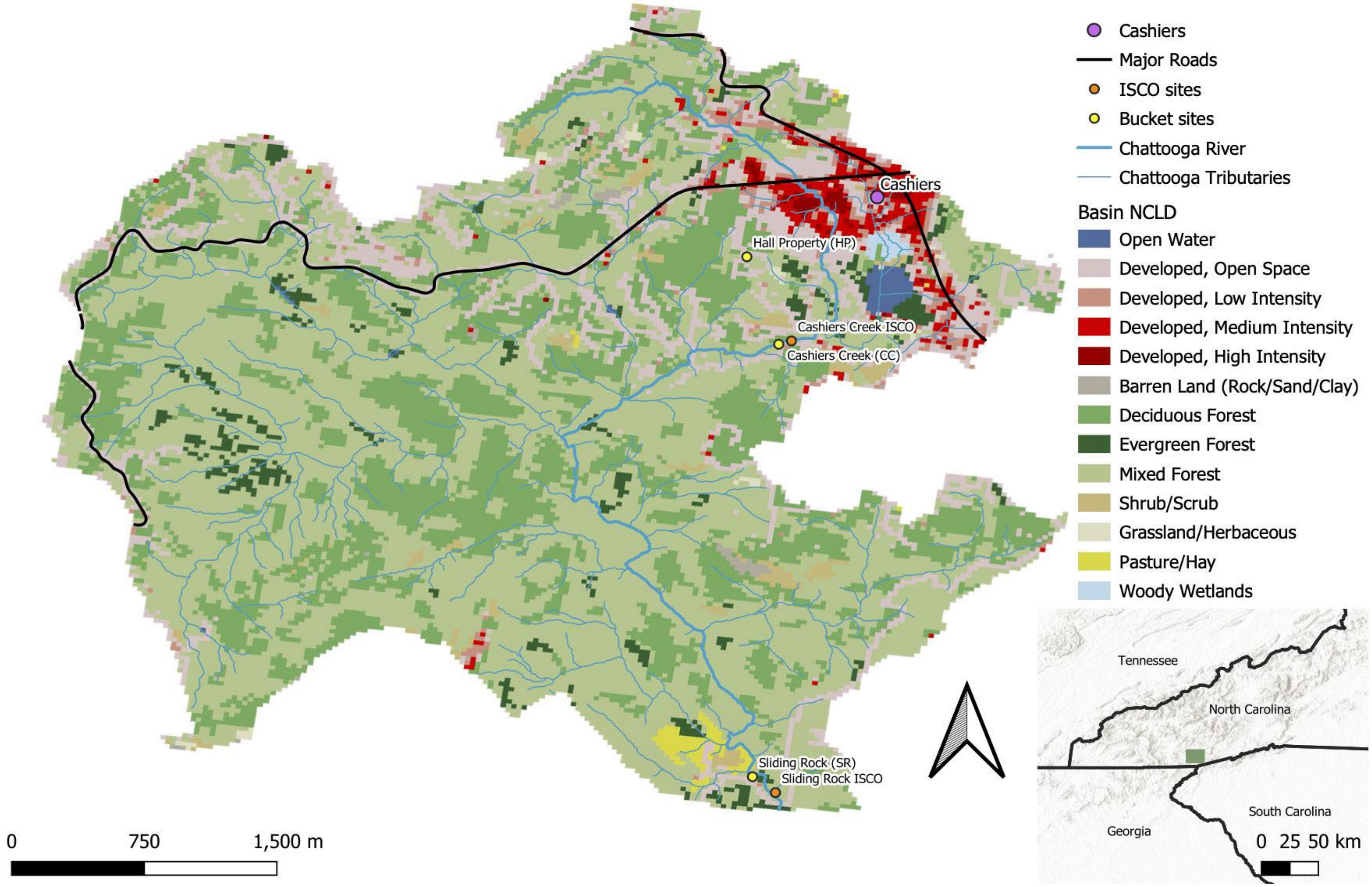

FIG. 1. Map of sample sites in the Chattooga watershed overlaid with National Land Cover Data. The watershed is primarily forested with scattered low-density housing developments. The unincorporated town of Cashiers sits in the northeastern part of the watershed. This map was created using QGIS software.

Water Sampling

Stream water samples were collected using Teledyne ISCO model 6712 full-size portable samplers (ISCO). We deployed two ISCOs along the Chattooga River. The first unit was deployed 190 m from the Trillium Wastewater Treatment Plant #3 in CC, and the second 4.8 km downstream from the wastewater treatment plant at SR. We placed the ISCOs adjacent to the river and above the floodplain. Water was collected from the river using a 7.26 m vinyl suction line that was connected to the ISCOs. Attached to the end of the suction lines was a strainer made of polypropylene (PP) (SR) or polyvinyl chloride (PVC) (CC). The PP strainer for the ISCO at CC broke and was subsequently replaced with a PVC pipe with drilled holes to mimic the original model. A pressure transducer was also connected to the ISCO, which measured instantaneous river depth every five minutes between August 2021-October 2021. At each site, a rebar was driven into the stream bed to serve as an anchor point for the strainers and pressure transducers. We attached the strainers and pressure transducers to the rebar with zip ties and stainless-steel hose clamps to keep them stabilized at a specific river depth as flow fluctuated. Both instruments were fully submerged in a shallow, steadily moving area of water with limited debris and rapids.

We loaded each ISCO with 24 350 mL glass bottles with polytetrafluoroethylene (PTFE) lined caps to collect samples when storm events occurred. We also used the ISCOs to collect weekly grab (river) and blank (filtered deionized water) samples. We manually programmed the ISCOs to collect 330 mL water samples during a predicted storm event; the sampling frequency was dependent on and unique to the length and severity of the storm. We aimed to collect water samples representative of the storm's entirety. Our selected storm event took place over the course of 6 days in October, starting at 04:00 on 6 October 2021 and ending at 00:00 on 11 October 2021, with sample collections every two and a half hours. This initial collection start time was selected based on the weather forecast for the storm event.

River Measurements

A discharge measurement of each sample site along the Chattooga River was collected to calibrate a rating curve modeled by Natural Resource Conservation Service's cross-section hydraulic analyzer (USDA Cross-Section Hydraulic Analyzer). The channel's cross-sectional dimensions and shape, along with slope, are required to model discharge with the hydraulic cross section analyzer. At SR, we deployed a Leica total station was set up perpendicular to river flow to measure horizontal and vertical distances at 16 locations along the river and its banks. Data were also collected to calculate the slope of the river. Cashiers Creek was surveyed using the tape and stadia rod method because the cross-sectional area was too vegetated to use a total station. The overall width of the river was measured, and depth measurements were taken at one-meter increments with a stadia rod. A hand-level was used to measure the slope. At both SR and CC, the river's cross-sectional width was divided into equal parts to measure discharge, and a wading rod was used to measure the depth of the river at the midpoint of each section. To generate discharge data, we measured average velocity at 0.6 times the depth using a Hach meter. The developed rating curve allowed the changes in water levels (as measured by the ISCO's pressure transducer) to be converted to discharge, which allowed for an assessment of the variations in MP concentrations with flow.

Atmospheric Deposition

We deployed purple plastic buckets within the Chattooga watershed to monitor the average daily atmospheric deposition of MPs. Three sites were chosen: an open riparian clearing at CC

(35.10310 N, -83.10718 W), under rhododendron thickets at SR (35.07572 N, -83.10881 W), and under a deciduous canopy at HP (35.10864 N, -83.10916W). The 5-gallon (18.93 L) buckets and lids were made of purple polypropylene plastic. The area of the buckets' opening was 660.5 cm^2. We intentionally utilized purple buckets to increase our ability to identify contamination from the buckets. We deployed buckets from 1 to 21 October 2021 and collected them weekly over this same period. We recorded the time at which each bucket was deployed and collected. The samples were analyzed to gather data on MP deposition per unit of surface area in relation to how much time the buckets spent in the field.

We transported the buckets to the sites with the lids on, and only removed them at the time of deployment to prevent contamination in transit. We designed and built debris sieves to sit atop the buckets and prevent leaves, sticks, and pine needles from entering (Fig. 2). The sieves were constructed of copper wire frames and 1 mm aluminum mesh screen. They sat 15 cm below the rim of the bucket. When we switched out buckets, we poured filtered deionized water (DI water) over the sieves to ensure that any MPs that may have been on top of the debris were rinsed into the bucket.

FIG. 2. The sieve used on the interior of atmospheric deposition buckets to catch debris while still allowing for the collection of rainwater.

Sample Filtering

To filter our water samples, we used a glass vacuum filtration system that consists of a 1-L Erlenmeyer flask with a glass stopper inside. On top of the glass stopper we placed a 47 mm diameter GE Healthcare and Life Sciences Whatman glass microfiber circular filter paper with a pore size of 5 μm, which was clamped beneath a glass beaker through which we poured the samples. When we were not pouring the samples, we covered the top of the beaker with a watch-glass to reduce potential contamination from outside particles. The Erlenmeyer flask was connected by a rubber hose to a vacuum system, through which water was pulled to expedite the filtration process. After the samples were filtered, we measured the water volume by pouring it into a graduated cylinder; we did not repeat this for the samples used to monitor atmospheric deposition because the volume was not necessary for those measurements. We then rinsed the top beaker with filtered DI water to ensure that all remaining sediments and MPs were on the filter paper and did not remain on the sides of the beaker. We then removed the filter paper with metal tweezers and placed the filter onto a petri dish and secured it with a lid. All parts of the filtration system were washed thoroughly with filtered DI water between each sample except the Erlenmeyer flask, as it was only used for wastewater. This process was replicated for all samples, including grab, storm, quality control, blank, and deposition samples.

Microplastic Identification

After filtration, each sample was examined under the microscope and analyzed for the presence of MPs. Microscope analysis took place at the Highlands Biological Station and Western Carolina University laboratories. At both Highlands Biological Station and Western Carolina, we used stereo microscopes to view the samples at magnification ranging from 10× to 50×. Each MP particle was classified according to its color and plastic type (fiber, fragment, foam, or film). Physical features were used to identify MPs, which were generally shiny, colorful, curved, and continuous. We determined that root material generally lacked luster, had visible cells, were either yellow or a dark black, had branching appendages, and were straight. For a subset of samples analyzed at Western Carolina University, a picture was taken of the particles to create a library to which we could later refer. We recorded the total number of MPs found per sample and subsequently calculated the concentration of MPs (MPs/L) and the load of MPs (MPs/s).

Quality Control

We employed multiple methods to control contamination in our samples. Purple cotton lab coats were worn to easily identify any MPs added to the samples during the analysis process. We wore these lab coats when we cleaned materials, deployed atmospheric deposition buckets, and filtered samples. Blank samples of filtered DI water were analyzed periodically to account for fragments of the polyvinyl tubing and plastic strainers. Further, we developed a six-step washing method to decontaminate sample bottles and deposition buckets:

1. Clean off the washing brush with filtered DI water.
2. Pour half a teaspoon of soap into the bottom of the bottle, fill it halfway with DI water, and use a brush to scrub out the inside (scrub at least 12 times). Dump out the water.
3. Fill the bottle to the brim with water and dump.
4. Fill the bottle to the brim with water and dump.
5. Rinse off cap, fill the bottle halfway with water, screw on cap, and shake. Dump water.
6. Rinse off cap, fill the bottle to brim with water and dump. Quickly screw on the cap.

The buckets were washed twice using this method. All DI water was filtered through a 5-micron stainless steel screen prior to use. Every twelfth glass sample bottle was filled with filtered DI water which was then filtered and examined under a microscope as quality control to ensure the efficacy of our washing remained consistent. We subtracted the mean number of MPs found in our control samples from our grab and storm samples to account for outside contamination, referred to herein as adjusted MP. We also collected DI water samples from four washed atmospheric deposition buckets we would be placing in the field and analyzed the sample for MP contamination. We then subtracted the mean number of MPs found in these bucket samples from the number of MPs found in the storm event bucket samples to further account for contamination.

RESULTS

Hydrology

The mean Chattooga River base flow discharge for Cashiers Creek (CC) was 0.07 cubic meters pers second (cms), and the mean discharge during the storm event was 0.28 cms (range = 0.08 - 4.21 cms). The mean base flow discharge for Sliding Rock (SR) was 0.95 cms, and the mean discharge during the storm event was 1.47 cms (range = 0.40 - 7.48 cms). The developed statistical model used to convert water levels to discharge was calibrated on the basis of only one field measurement. Thus, the presented discharge values possess a high degree of uncertainty.

Quality Control

Baseline MP contamination was calculated from the analysis of quality control and blank samples. Mean contamination from ISCO and DI water blanks was 3 MPs per 330 mL sample, with a range of 0 to 5 MPs. Another potential source of error was incorrectly determining if a particle was a MP, cotton fiber, or other organic material. We subtracted three MPs from all storm and grab samples in order to account for contamination in our samples. This became our adjusted MP. Atmospheric bucket MP measurements were adjusted for contamination as well. We had four control buckets over the span of three weeks with an average contamination of 4.5 MPs (range = 1 – 9 MPs).

Cashiers Creek Microplastics

We collected and analyzed four grab samples during baseflow conditions, and we collected 48 storm samples, 36 of which were analyzed for MPs. A programming error led to a gap in sample collections during the falling limb of the storm. During baseflow, MPs were primarily black (60.0%), purple (20.0%), and blue (13.3%) (Fig. 3); 86.7% of the baseflow MPs were fibers (Fig. 4). During the storm event, the most prominent colors were black (39.6%), purple (31.9%), and blue (17.6%) (Fig. 3); 81.7% of the storm MPs were fibers (Fig. 4).

Microplastic concentrations were highest before and after the main body of the flood (Fig. 5). Concentrations decreased as discharge levels reached their peak (4 cms), creating a clockwise loop (Fig. 6). As the river returned to base flow conditions, MP concentrations spiked two additional times (Fig. 5). The average stormflow MP concentration was 4.3×10^3 MPs/m^3 (range = 0.0 - 34.4×10^3 MPs/m^3) and the average baseflow concentration was 2.38×10^3 MPs/m^3 (n=4, range = 0 - 9.5×10^3 MPs/m^3). Load during peak discharge (3.7 cms) was 3.67×10^4 MPs/s. The Trillium Wastewater Treatment Plant #3 released an increased daily discharge that peaked on the same day as river discharge (Wastewater Treatment Plant peak discharge = 1.01×10^4 m^3/day on 7 October 2021) (Fig. 7).

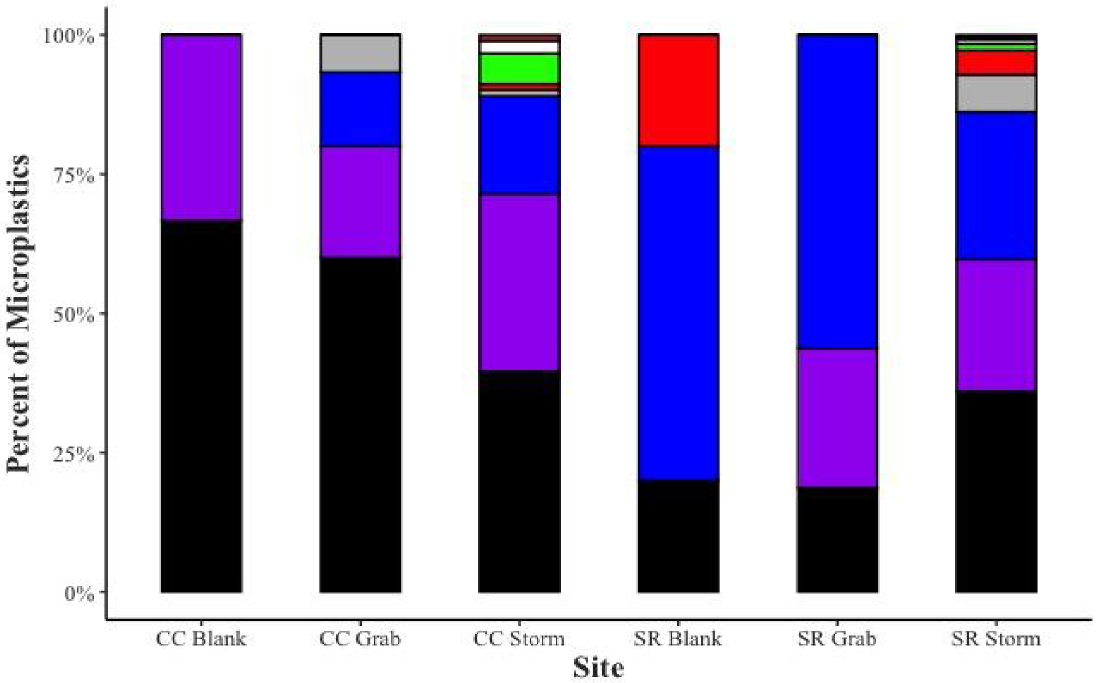

FIG. 3. The distribution of colors of the MPs found in blank, grab, and storm samples. The primary colors found were black, purple, and blue. From left to right, the weekly total counts of MPs were the following: 3, 15, 91, 5, 16, 253. A total of 109 MPs were identified at CC and 274 MPs were identified at SR.

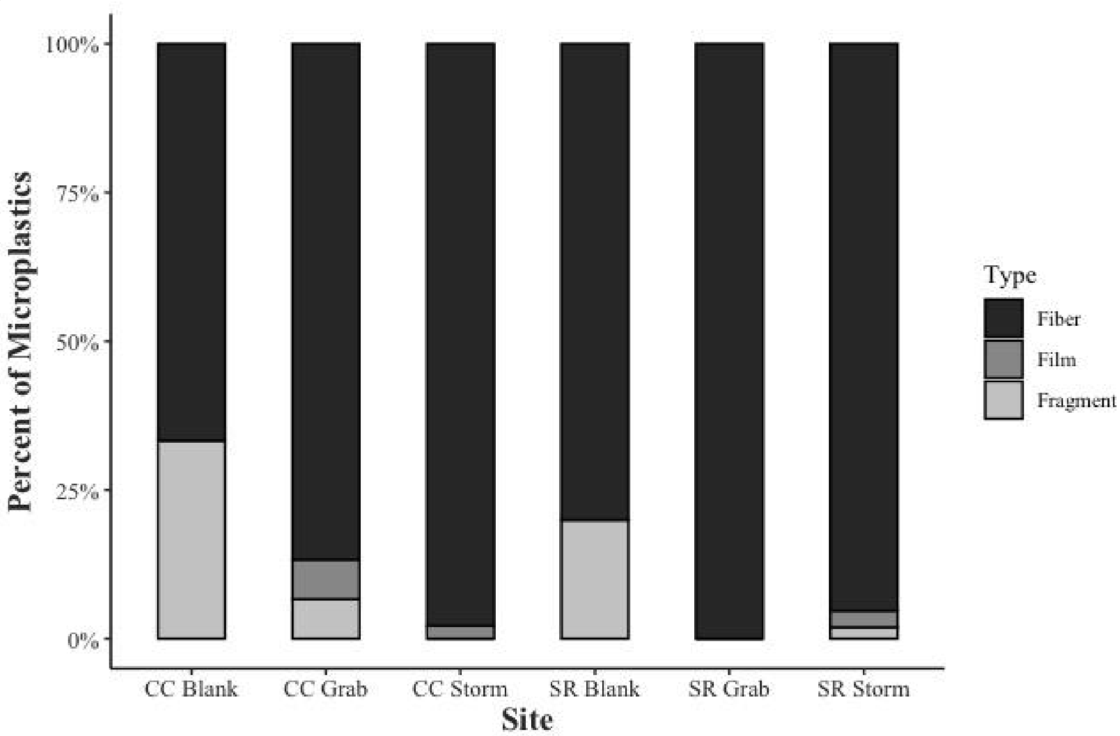

FIG. 4. Percentages of MP types from three collection methods at SR and CC. The majority of MPs were identified as fibers throughout all the samples. From left to right, the weekly total counts of MPs were the following: 3, 15, 91, 5, 16, 253. A total of 109 MPs were identified at CC and 274 MPs were identified at SR.

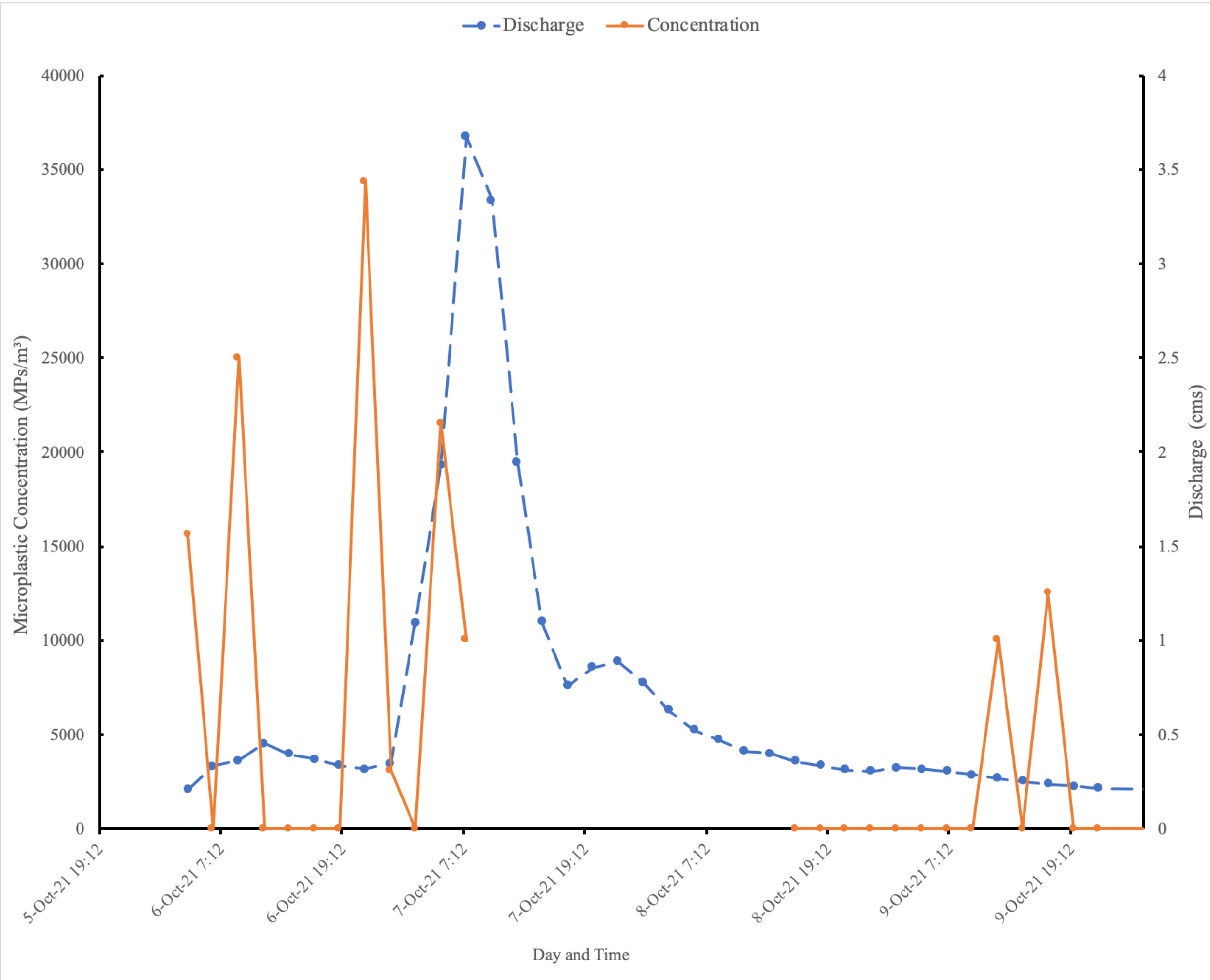

FIG. 5. Change in MP concentration at Cashiers Creek as discharge (cms) changed during the storm event. Generally, MP concentrations rose as discharge increased initially, then MP concentrations decreased when discharged peaked. MP sampling did not occur during the majority of the falling limb of the storm. Once CC returned to base flow level discharges, MP concentrations spiked.

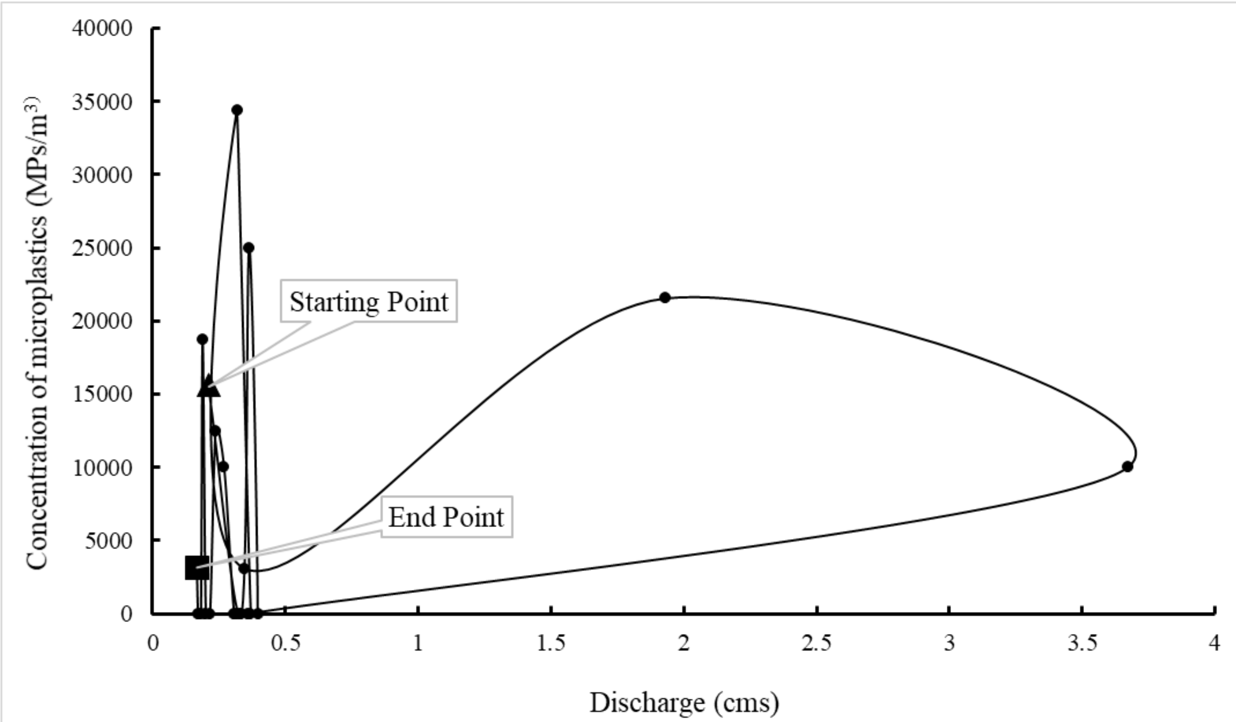

FIG. 6. Change in MP concentration as discharge changed throughout the span of the storm event at Cashiers Creek. The clockwise loop exhibited during the rising limb of the storm event may indicate the flushing of MPs into the river from nearby sources.

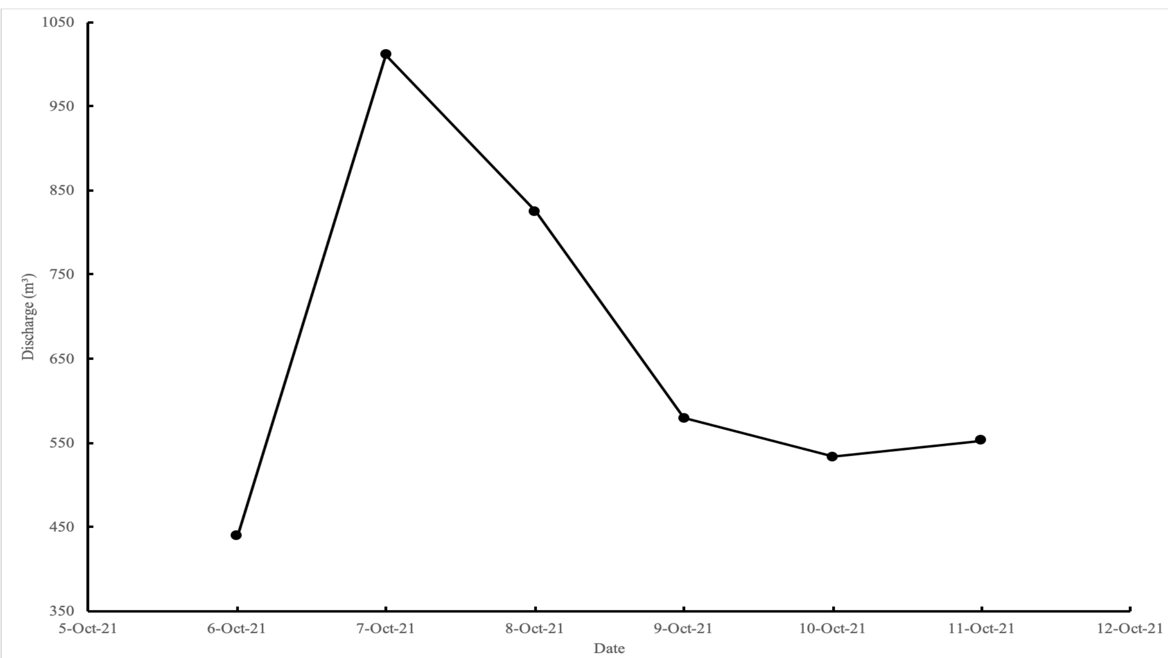

FIG. 7. Daily discharge of the Trillium Wastewater Treatment Plant #3 during the storm event. Discharge peaked on 7 October 2021.

Sliding Rock Microplastics

We collected and analyzed four grab samples during baseflow conditions, and we collected 48 storm samples, 34 of which were analyzed for MPs. A programming error led to a gap in sample collections during the falling limb of the storm. During baseflow, the majority of MPs found were blue (52.9%), purple (23.5%), and black (17.7%) (Fig. 3). Fibers represented 100% of the MPs identified (Fig. 4). During the storm event, the majority of MPs were black (36.0%), blue (26.5%), and purple (23.7%) (Fig. 3); 95.3% of the MPs were fibers (Fig. 4).

When discharge peaked during the storm event, MP concentrations decreased (Fig. 8). During the initial stage of the storm, MP concentrations increased in a counterclockwise loop (Fig. 9). In the subsequent rising limb of the storm, MP concentrations increased in a figure-eight hysteresis loop. The average stormflow MP concentration was 1.29×10^4 MPs/m^3 (range = 0.0 - 5.08×10^4 MPs/m^3) and the average baseflow concentrations from our grab samples was 0.0 MPs/m^3 (n=4). Load during peak discharge (7.4 cms) was 1.14×10^5 MPs/s.

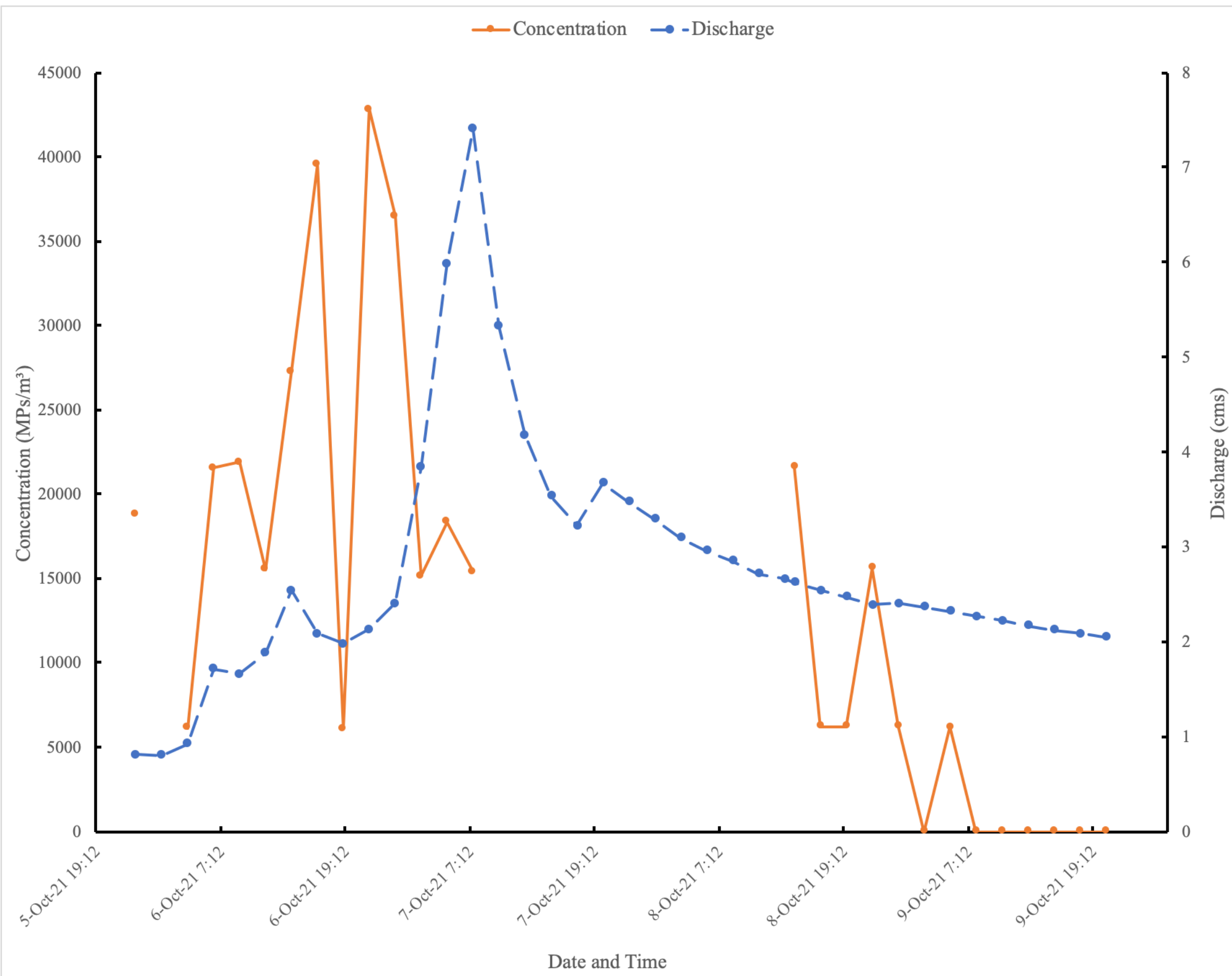

FIG. 8. Change in MP concentration at Sliding Rock as discharge (cms) changed during the storm event. As discharge rose initially MP concentrations varied greatly, then decreased when discharge peaked. MP sampling did not occur during the majority of the falling limb of the storm.

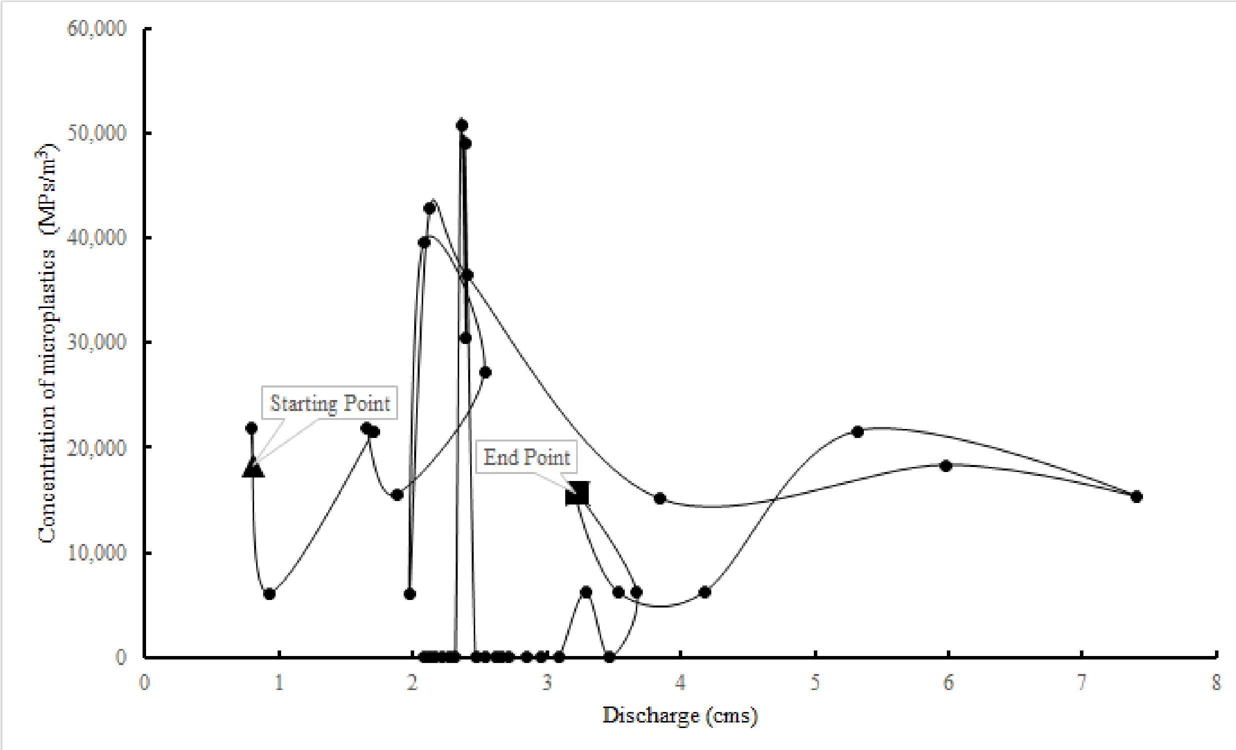

FIG. 9. Change in MP concentration as discharge changes throughout the span of the storm event at Sliding Rock. A counter-clockwise loop is shown initially indicating a delayed flush of MPs from the upper reaches of the river. A figure-eight loop is exhibited during the rising limb of the storm event indicating the flushing of MPs from nearby sources.

Atmospheric Deposition

The highest concentration of MPs at all three atmospheric deposition sites (SR, CC, and HP) occurred from 1 to 8 October, which was the same period of the storm event (Table 1). The colors of the collected MPs varied over the three-week bucket collection period at all three sites. During the storm event that occurred in week one, the colors of MPs at the SR atmospheric deposition site were 44.2% blue, 37.2% black, 8.1% purple, 7.0% red, 2.3% grey, and 1.2% green (Fig. 10). Week one atmospheric deposition results at the CC site were 50.0% blue, 41.7% black, and 8.3% purple (Fig. 11). Week one atmospheric deposition results at the HP site were 46.7% blue, 26.7% black, 20.0% purple, and 6.7% green (Fig. 12). During the storm event, 98.8% of MPs collected were fibers and 1.2% were fragments at the SR bucket collection site (Fig. 13). The MPs from CC and HP consisted entirely of fibers. We did not account for MP bucket contamination in the color and type charts.

TABLE 1. Amount of atmospheric deposition (MPs/m^2/day) at sites in the upper Chattooga River watershed, Jackson Co., NC between 1 and 21 October 2021. Sliding Rock and Hall Property sites were throughfall deposition. Deposition was highest at Cashiers Creek which was an open field site.

Location	Date Deployed	Date Collected	Total Time (days)	MPs (m^2/day)
Sliding Rock	1 Oct	8 Oct	7.0	175.7
	8 Oct	15 Oct	6.9	1.1
	15 Oct	21 Oct	6.1	23.6
Cashiers Creek	1 Oct	8 Oct	6.9	190.8
	8 Oct	15 Oct	6.9	12.0
	15 Oct	21 Oct	6.1	8.7
Hall Property	1 Oct.	8 Oct	7.1	162.0
	8 Oct	15 Oct	6.9	12.0
	15 Oct	21 Oct	6.2	18.4

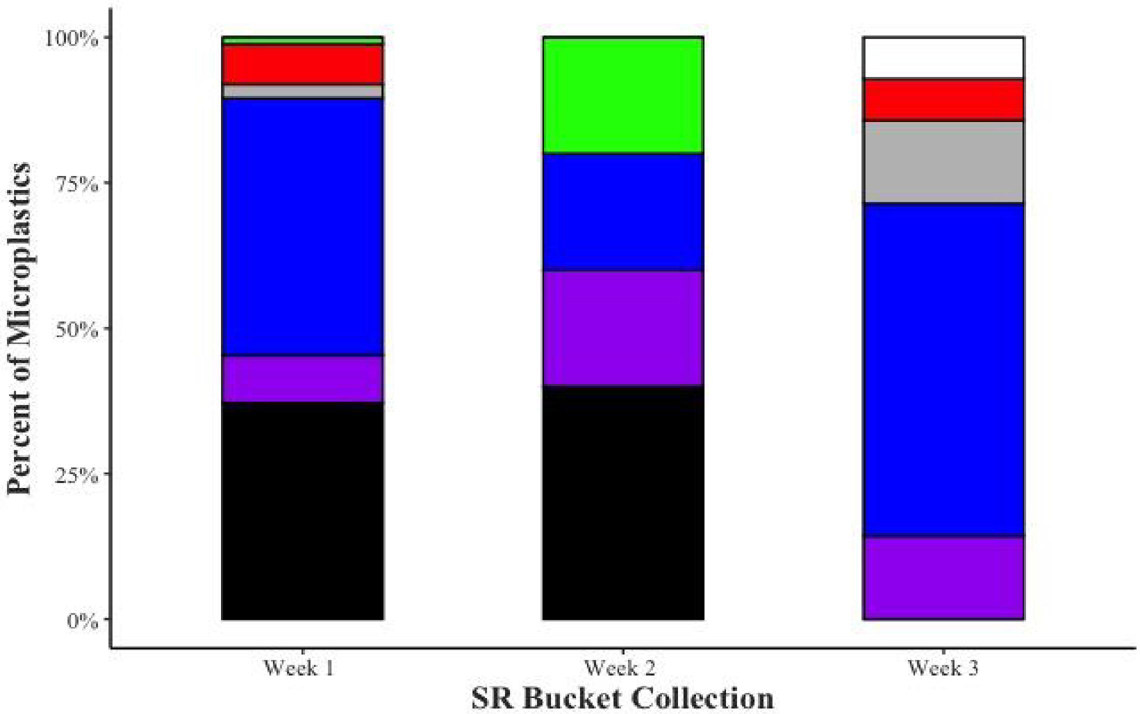

FIG. 10. Color distribution of the MPs found in the atmospheric deposition bucket at SR.

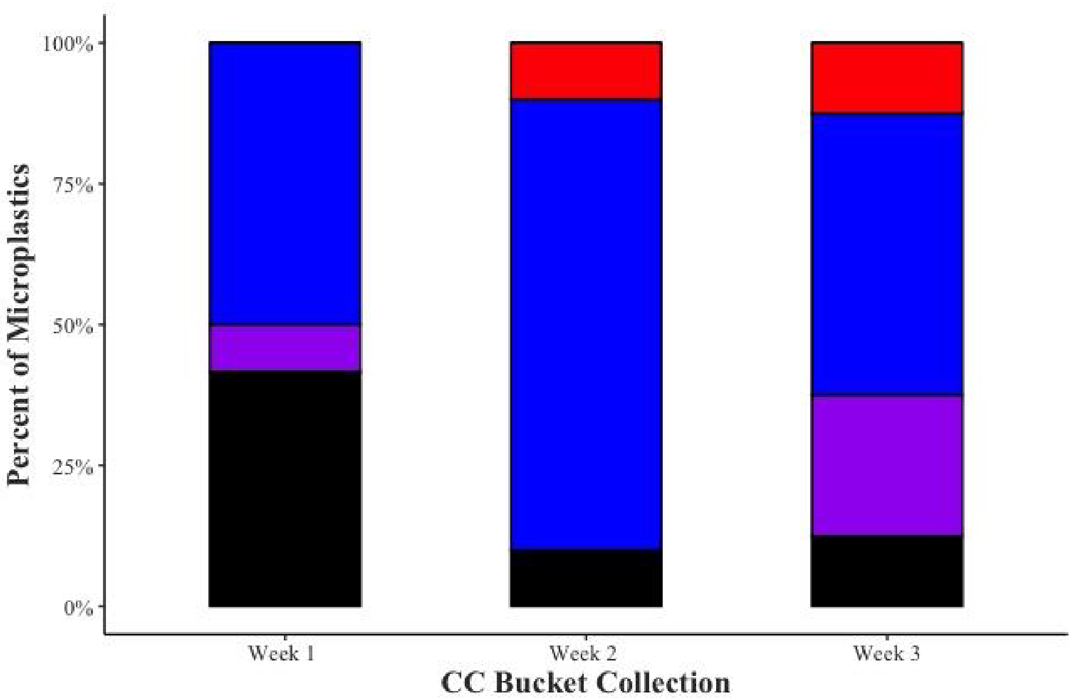

FIG. 11. Color distribution of the MPs found in the atmospheric deposition bucket at CC.

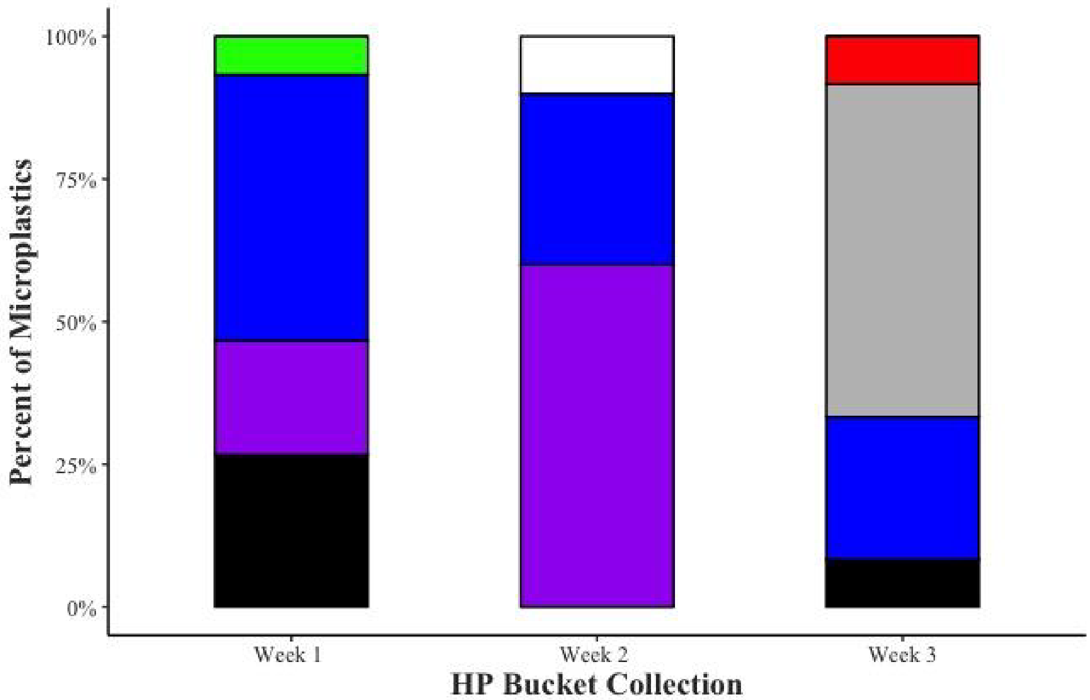

FIG. 12. Color distribution of the MPs found in the atmospheric deposition bucket at the Hall Property (HP) Site.

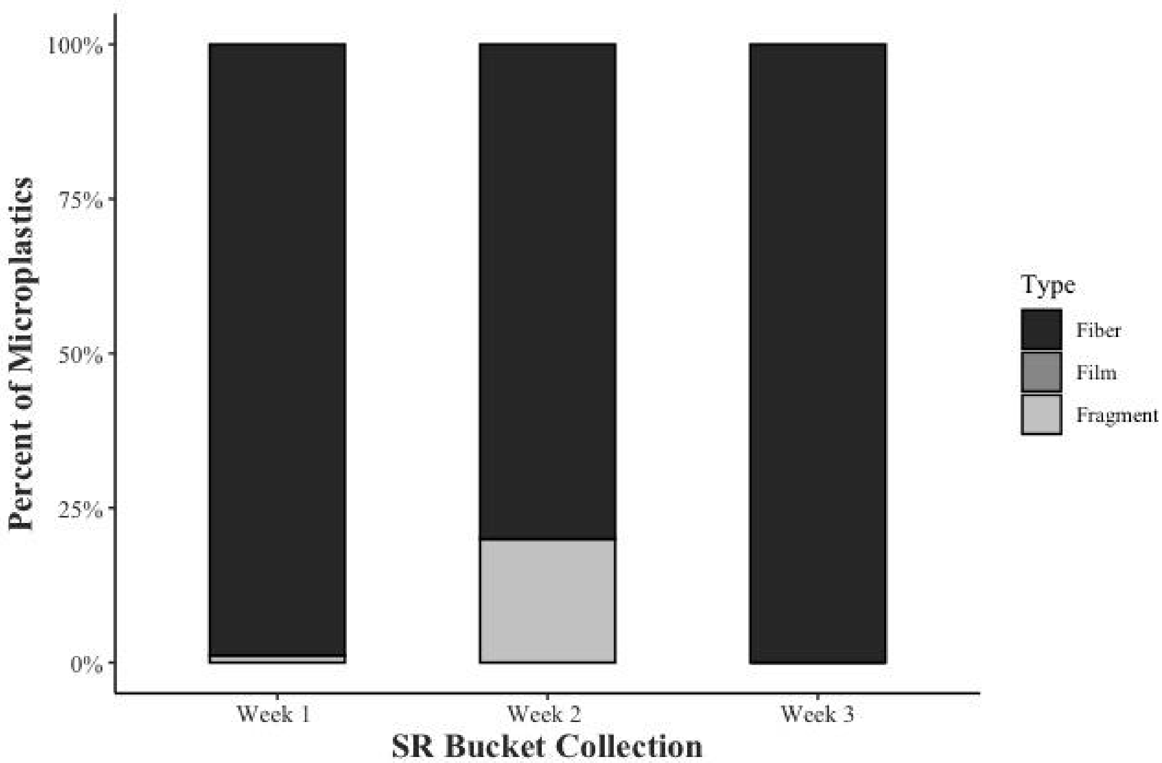

FIG. 13. Types of MPs found in the atmospheric deposition bucket at SR. The majority of MPs were fibers at SR.

Discussion

Abundance of Microplastics

Our results support the hypothesis that MP concentrations would be higher during stormflow when compared to baseflow conditions. Average MP concentrations at SR increased by 1.29×10^4 MPs/m^3 from baseflow to average stormflow conditions (range = 0.0 - 5.08×10^4 MPs/m^3). Average MP concentrations at CC increased by 1.90×10^3 MPs/m^3 from baseflow to stormflow conditions (range = 0.0 - 3.44×10^4 MPs/m^3). However, MP concentrations at CC during baseflow had several spikes in concentration. These spikes could be attributed to gaps in our data collection or releases from the WWTP.

Contrary to our prediction that MPs would increase as the storm event intensified, data from CC suggest a first flush phenomenon, where MP concentrations peaked while discharge levels were still rising. Once the storm reached peak discharge, there appeared to be a major decrease in MP concentration. This phenomenon is described by a clockwise hysteresis loop (Fig. 6) The maximum concentrations of MPs found during the initial stage of the storm potentially resulted from MPs aggregated in the surrounding environment washing into the stream as heavy rainfall began. Similarly, MPs settled in the stream's benthic layer during low flow conditions could have been resuspended during the initial rise in discharge. This same first flush phenomenon has been characterized by other pollutants - including nutrients, heavy metals, and organic compounds (Stenstrom and Kayhanian 2005). At SR, the data were not fully indicative of a first flush. Instead, a counterclockwise hysteresis loop at the beginning of the storm suggests that particles from upstream reached the monitoring site after the initial spike in discharge (Fig. 9). However, the second part of the flood exhibits a figure-eight loop which is somewhat indicative of a first flush phenomenon.

Table 2 demonstrates how our study compares to other MP freshwater river studies around the world. Our study's estimated maximum number of MPs found in one sample was 5.08×10^4 MPs/m^3. This is about 4 magnitudes higher than most of the other results presented in this table. However, this number represents a sample that was taken during the rising limb of the storm, whereas the values presented by the other studies were taken at baseflow. Also, other cited studies utilized nets as their sampling apparatus, while we used ISCO water samplers and vacuum filtration methods; these different methods may have a significant impact on the comparison of MPs from different studies as studies that use nets and that focus on baseflow may have significantly underestimated the number of MPs in the water. The average baseflow MP concentration is much lower than the maximum number of MPs found throughout our study, and it is likely the most comparable value to previous studies found in the table. However, our average baseflow from both streams still holds over 1.1 x 10^3 more MPs than the next-highest sample in comparable studies. Despite limitations during the quality control for this experiment that may have skewed our results to some extent, we can surmise that the Chattooga River still has an extremely significant concentration of MPs. These high concentrations can also be supported by unpublished data showing similar MP levels in the Tennessee River which is in the same region as the Chattooga River (Table 2).

TABLE 2. Comparison of average abundances of MPs from river studies around the world.

River	MPs/m^3	Sampling Method	Reference
Middle and Lower Yangtze River (China)	0.9	333 µm net	(Xiong et al. 2019)
Great Lakes tributaries (USA)	0.05-32	333 µm net	(Baldwin et al. 2016)
River Seine, urban area (France)	0.35	333 µm net	(Dris et al. 2015)
Various rivers (Switzerland)	7	300 µm net	(Faure et al. 2015)
River Danube (Australia)	0.32	500 µm net	(Lechner et al. 2014)
Nine different rivers (USA)	1.94-17.93	100 µm net	(McCormick et al. 2014)
Snake and Lower Columbia rivers (USA)	2.57	100 µm net	(Kapp and Yeatman 2018)
Tennessee River (USA)	16,000	Vacuum pump	(Fath 2018, unpublished data)
Chattooga River (USA)	$0\text{-}5.08 \times 10^4$	ISCO sampler and filtration	This study

Atmospheric deposition at all three sites showed elevated MP concentrations during the storm event compared to non-storm MP deposition levels (Table 1). Based on this difference, we infer that the increase in MP volume was brought about by the rainfall. Roblin et al. (2020) also found increased concentrations of MPs in wet deposition of ambient MPs. They suggest that rainfall washout and air mass movement are important predictors of MP deposition, which is consistent with our results.

Table 3 demonstrates how our study compares to other MP atmospheric deposition studies around the world. Our range of MP concentrations (10.9-305.3 $MPs/m^2/day$) fits into the broad range of previously collected data. The sampling methods are varied, which influences the data and makes standardization difficult. One previous study differentiated data obtained from throughfall sites, which helped orient our sites, as two of our sites collected throughfall and one was in an open field (Klein and Fischer 2019). The throughfall data collected by Klein and Fischer did not differ greatly from our open field data. Conversely, we found higher MP concentrations in the open field compared to throughfall sites. We postulate that more MPs were collected in the open field because there was no canopy which prevented MPs from depositing in our collection buckets.

TABLE 3. Comparison of average abundances of MPs from atmospheric deposition between multiple studies.

Location	Range of MP Concentration (MPs/m^2/day)	Sampling Method	Comments	Reference
French Pyrenees	300- ~460	Collectors		(Allen et al. 2019)
Hamburg, Germany: Urban	136.5-260.6	Bulk precipitation samplers	Two throughfall sites	(Klein et al. 2019)
Hamburg, Germany: Rural	331.4-512.0	Bulk precipitation samplers	Two throughfall sites	(Klein et al. 2019)
Coastal Ireland	9-15	Precipitation chemistry monitoring stations	Only microfibers were collected	(Roblin et al. 2020)
Central London	575-1008	Aluminum rain gauge with a 0.03 m^2 orifice- 50 m above ground		(Wright et al. 2020)
Gulf of Gdansk, Gdynia, Poland	0-30	Steel barrel, steel funnel (0.33 m^2), and 20L glass jar with aluminum top		(Szewc et al. 2021)
Chattooga Watershed, North Carolina	10.9-305.3	18.93 L purple plastic buckets with debris sieves and an opening of 660.5 cm^2	Two throughfall sites and one open field site	This study

Potential Sources of Microplastics

Urban development in the southern Appalachians has been increasing over the past few decades and is projected to continue to rise at an even greater rate (Terando et al. 2014). While public roads have not increased significantly, our study area has experienced a 361% increase in private road development and expansion between 1954 and 2009 (Kirk et al. 2012). Urbanization and impervious surfaces minimize the capacity for rainwater to infiltrate into soils, leading to increased runoff (Mejía and Moglen 2010) that directly influences runoff pollutant concentrations and loads (Hatt et al. 2004). Storm events represent periods of increased inputs of nonpoint source pollution which may increase mobilization and contamination of MPs into waterways (Hitchcock 2020). We speculate that the increase in MP concentrations during the storm event seen during this study could be attributed to the initial influx of MPs flushed into the rivers by runoff, and the impact of the first flush phenomenon exhibited at CC could be due to the urbanization of the surrounding town of Cashiers. Moreover, previous studies have found that sediment in the channel becomes resuspended from increased river discharge, where MPs become remobilized and are found in higher concentrations (Gellis 2013, Wagner et al. 2019). With increased urbanization, more MPs could become settled in the benthic layer, leading to greater concentrations of MPs when upwelled by elevated discharge. Atmospheric deposition of MPs could be another source of MP contamination in the Chattooga River. Increased MP deposition from during rainfall events could contribute to the first flush phenomenon.

There was a noticeable difference in MPs concentration between SR and CC during stormflow. Sliding Rock's maximum MP concentration was 1.64×10^4 MPs/m^3 higher than CC's maximum MPs concentration. These concentration differences may possibly be attributed to the downstream location of SR from CC, along with SR being a higher order stream. Because SR is a higher order stream, there are several sources with the potential to increase its MPs concentration. Other studies have shown that MPs concentrations may increase significantly downstream due to an increase in nonpoint sources of pollution (Simmerman and Coleman Wasik 2020, Gerolin et al. 2020). We used the North Carolina Division of Water Resources Map Locator to determine if there were any other private wastewater treatment plants or plots with non-discharge permits in our study watershed (NC-DEQ 2021). Non-discharge permits are needed to use sewage sludge application on agricultural fields, which has been linked to high concentrations of MPs in agricultural soils (Corradini et al. 2019). However, we found no other wastewater treatment plants or any non-discharge permits in our watershed, suggesting that the higher concentration of MPs at SR might be coming from a more localized source. Potential sources could be septic tanks or gray water systems used by local landowners with older homes. Research has shown that just a single landowner can have a disproportionately large impact on stream quality (Jackson et al. 2021). There are several low-medium density developments upstream from SR that could have contributed to increased MP concentration.

Our second hypothesis, which stated that there would be a noticeable difference in MP concentrations between SR and CC due to their varying proximities to the wastewater treatment plant, was not supported by our findings. During baseflow, there were no major discrepancies in MP concentrations between the two sites. The average MPs concentration at CC was 7.33×10^3 MPs/m^3 and 1.66×10^4 MPs/m^3 at SR. This suggests that the Trillium Wastewater Treatment Plant #3 effectively removed MPs from their effluent and did not greatly contribute to the high concentrations of MPs found in the Chattooga River. Our findings are inconsistent with results from a study conducted by Uddin et al. (2020), which showed that while conventional wastewater plants with primary and secondary treatment removes the majority of MPs from wastewater, conservative estimates that treated effluent still adds approximately 1.47×10^{15} MPs into aquatic environments annually. Advanced final-stage wastewater treatment technologies were found to remove 99.9% of MPs, and these technologies, such as disc filters, rapid sand filters, dissolved air flotation, and membrane bioreactors, are needed to remove particularly small MPs from effluents (Talvitie et al. 2017). The Trillium Wastewater Treatment Plant #3 does not employ any of these advanced treatment technologies (Bryson 2021).

Fibers constituted the most numerous types of MP contamination at both SR and CC, similar to what Zheng et al. (2019) found in Jiaozhou Bay, China. Fibers are the primary type of MP contamination for the Chattooga River, indicating that the ultimate source may be the WWTP or atmospheric deposition or both. Multicolored synthetic textiles, along with tires, contribute to two thirds of global releases of primary MPs in aquatic systems (Boucher and Friot 2017). We also found that blue, purple, and black MPs constituted the majority of MPs found in atmospheric deposition, storm, and grab samples. Zhao et al. (2015) explained that the variety of MP colors is attributable to the enhancement of plastic usage in daily consumer activities. Zheng et al. (2019) likewise found an abundance of blue and black fibers in their study. Blue MPs are commonly found in fishing nets (Yin et al. 2020), and black MPs are from carbon black which is widely used in vehicular parts (Wagner et al. 2018). In a study by Ferreria et al. (2018), purple was the second most abundant MP in a tropical estuary, which they attribute to the weathering process of blue MPs to purple. This could explain the high percentages of purple MPs found in our study. However, it is also possible that our quality control methods of wearing purple cotton lab coats contributed to the high purple MP count.

Limitations and Future Directions

One limitation of the present study is that only one storm event was captured during the research period. In the future, capturing multiple storm events is necessary to confirm our findings, account for variation, and improve the accuracy of our results. Similarly, we must take more grab samples to gain a broader understanding of MP concentrations during baseflow conditions. Because of the ubiquity of MPs in the environment, our quality control methods were not guaranteed to be completely effective. Contamination could have still occurred throughout the cleaning, sample collection, filtration, and scoping processes. A secondary, chemically based analysis of filtered samples is necessary to either confirm our MP findings or to re-identify perceived MPs as organic debris. This will be done using microscopy or fluorescence. Because research of this nature has never been conducted, our methodology in collection and analysis of our data had to be developed and adapted throughout the project. We caution that this is a preliminary analysis, and we hope to confirm that the MPs identified during our microscopy are indeed MPs, and not roots, sediments, or organic fibers that resemble MPs.

Many studies have been performed on the harms macroplastic pollution has on aquatic environments, but little research has been done to understand the effect of MPs. Aggregation of MPs in aquatic systems can cause extensive negative impacts on local biota and ecosystems. A study done by McNeish et al. (2018) showed that 85% of the fish in their study had MPs in their digestive tissue, and they assert that MP abundance in fish could vary across a gradient of aquatic habitats. This study also found that MPs bioaccumulate in fish, indicating that species higher-up on the food chain contained increased levels of MPs. In addition, the aggregation of MPs could have extensive negative impacts not just on the environment, but on the human body as well. Microplastics can enter the body through many means, including inhalation, ingestion via water, plant matter, or animal consumption, and many potentially adverse health effects can accompany MP accumulation in the body (Karbalaei et al. 2018). Microplastic exposure can cause development of respiratory irritation, dyspnea, cancer, asthma, and cardiovascular disease (Karbalaei et al. 2018).

Our study introduces novel methods for collecting MPs and is the first of its kind to focus on MP concentrations in southern Appalachian watersheds. Our atmospheric deposition collection method utilizing a debris sieve has never been deployed in any other study, and an ISCO water sampler has never before been used for sample collection in a MP study. Microplastic pollution is a relatively novel issue facing humanity and our natural environment, and all efforts to study, understand, and eradicate them are valuable. We encourage future studies to reference, utilize, or improve upon our data collection and analysis methods to expand our knowledge of MPs. Further research could investigate how many MPs come from nonpoint source pollution by analyzing sheet flow data. A study focused primarily on atmospheric deposition of MPs could center around deposition bucket data and dive deeper into how deposition is affected by different weather conditions. Looking forward, we hope that researchers can use our data to continue to study the Chattooga River and the effects of MPs on southern Appalachian aquatic systems. We encourage wastewater treatment plants to consider incorporating final-stage treatment technologies that have the capability to filter MPs of all sizes out of effluents.

ACKNOWLEDGMENTS

We would like to thank Jason Love and Dr. Jerry Miller for their constant support and guidance throughout this semester. In addition, we would like to extend our appreciation to the Highlands Biological Station and Western Carolina University for allowing us access to their facilities, materials, and funds for this project. Thank you to the Nantahala National Forest and District Ranger Troy Waskey for permission to conduct research on US Forest Service land. Thank you to Ms. Sherry White and the Hall family for kindly allowing us to conduct our research on their properties. We hope that the results of this study shed light onto the serious issue of plastic waste and MP contamination in our environment.

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Finishing up field work at Cashier Creek monitoring site. (l-r): Vy Pham, Chloe Hall, Noa Meiri, and Rachel Maunus.

“All Terrain” Includes the Forbidden

Rachel found chicken of the woods on a tree
Behind the gym
And when we slipped back up
The red clay path from the river, potholes
Maintained by ATVs,
I picked up a smushed red bull can.
And when the van pulled away, the heap
Of trash in the middle of the cul-de-sac
Smiled at me in the blue
Five-o’clock light.
(And I smiled back.)
Felt like thinking about it
Really hard but still
Not being able to levitate.

They did an experiment where they
Took all the leaves and twigs out of
A stream and replaced them
With PVC pipes.
Did the water bugs notice?
Does it matter?
When I was nine my Mom taught
Me how to sign my name in
Cursive but I still slammed her hand
In the car door on accident anyway.

They say “cotton kills” but sometimes
I don’t even feel the rain
Through my T-shirt. I bet
The water bug does.

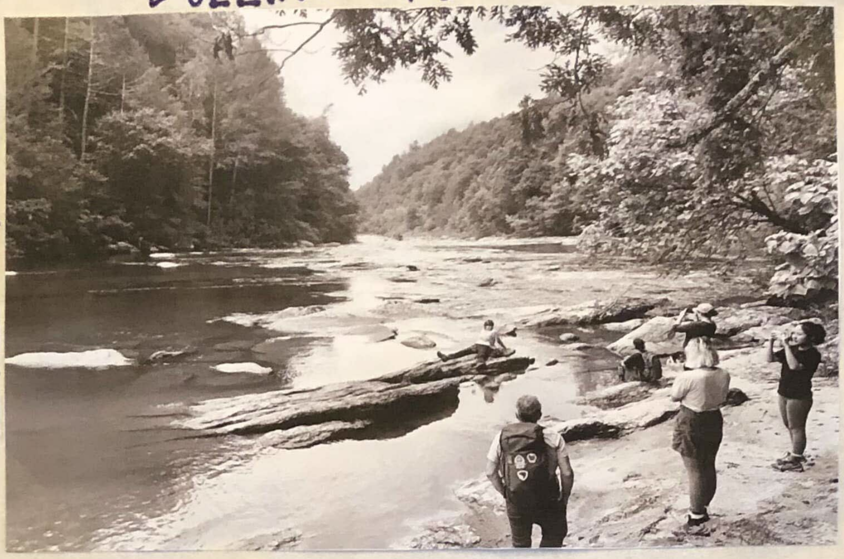
If only half of the cows are lying down,
Is it still going to rain?
I don’t really know
Anything. But I
Do know how it feels to be somewhere
And be certain I’ll never see it again
By steep trek or degradation.

Maybe my bones will glow in one
Thousand years, maybe the water bug
Will see it all.

- Scout Allen

DUELING Riverd[illegible]

by Chloe Hall
IE 2021

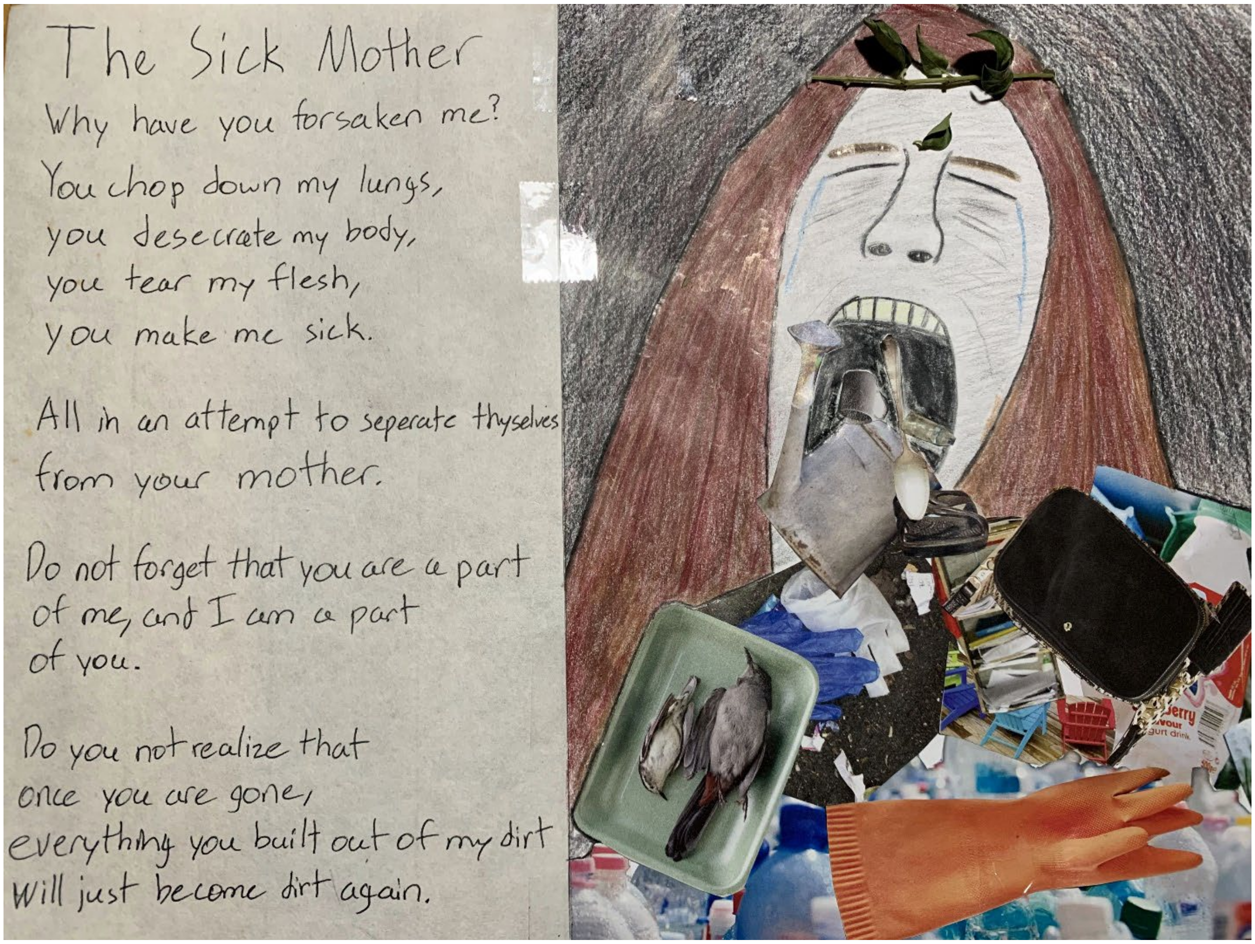


The wild & scenic
Stands TALL and
PROUD,
pounding it's chest
in rebellion –
with ROARING
rapids
A scene from
time gone by.
Tempting
Taunting
Terrifying
...

The gated and
guarded,
holds steadfast
against
the will of nature.

Man's own personal
asset, behind
bars
Convicted by the
Department of
the Anthropocene
...

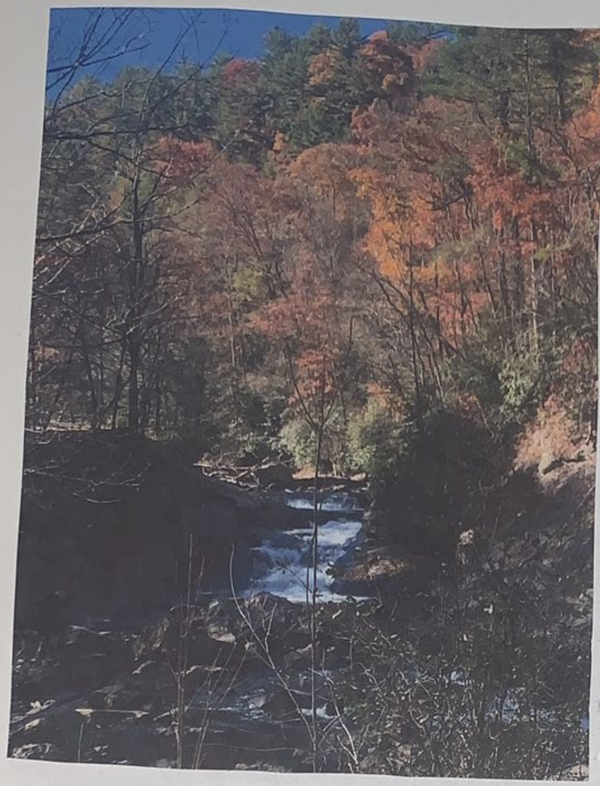
We often talk about the duality of man, but what about the duality of nature? The Chattooga River is a living, breathing, roaring oxymoron of what should be and what is. When I was first told we would be journeying to the Chattooga, I envisioned something similar to the mighty Wild & Scenic pictured above. Yet the gated off, amenity version was my first impression of this river. Seems as though the McMansion safe havens for the influent and affluent have come to include wild and scenic rivers as a community water feature. While these property owners may be able to fence off their lands, their gates cannot stop the flow of the river as old as time itself. I have hope that one day, the gates will open, the signs will fall, and the Wild & Scenic will reclaim what once was.


The Sick Mother
Why have you forsaken me?
You chop down my lungs,
you desecrate my body,
you tear my flesh,
you make me sick.
All in an attempt to seperate thyselves
from your mother.
Do not forget that you are a part
of me, and I am a part
of you.
Do you not realize that
once you are gone,
everything you built out of my dirt
will just become dirt again.

- Alex Hubbs

Grace Kinder

LIFE SPAN



It flows by
within a second
Down
Down
Down

Past the rocks and the
brush and the low
hanging branch thats
reaching towards the
water.

I've always wondered about
the lifespan of a water droplet,
Beginning with the formation
of H and O,
Always clumping up with
an incomprehensible number
of other drops, all with the
same path

I like to imagine they have their own lives
some end up on those trees and rocks, eventually soaked
up and rained down somewhere else while others make it
to broader homes

They've each lived many lives and seen the changing of
the leaves and the evolving of their homes, directed by
a long concrete slab as people emerge to take snapshots
of them flowing by

How odd, they must think, to take a picture of me.

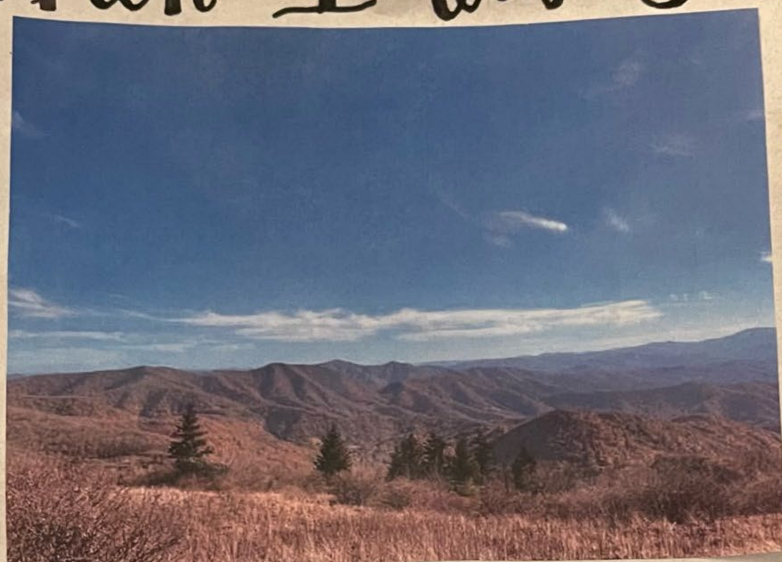
An Unbeknownst Poet's American Sonnet

Why does everyone else take those thalwegs,
so consumed with their fastest current, why won't
people see the island I'm on? When will someone shipwreck on
my desolate shores? I promise those that join will be happier
with this new life, we can listen to the lullabies sung by
water crashing on the bank. Unlike those that only see
from their boats of new, my island helps see in ways of old.

We know the turtles and birds used to rule this land,
but now they are a distant memory. Now all we know
are constant floods and dreams to "gaze at the stars."
All I can do is "smile through the pain" of my missed
connections. I continue to see people go through
the same rapids that run through my mind. Maybe
one day I'll have more than rocks for company.

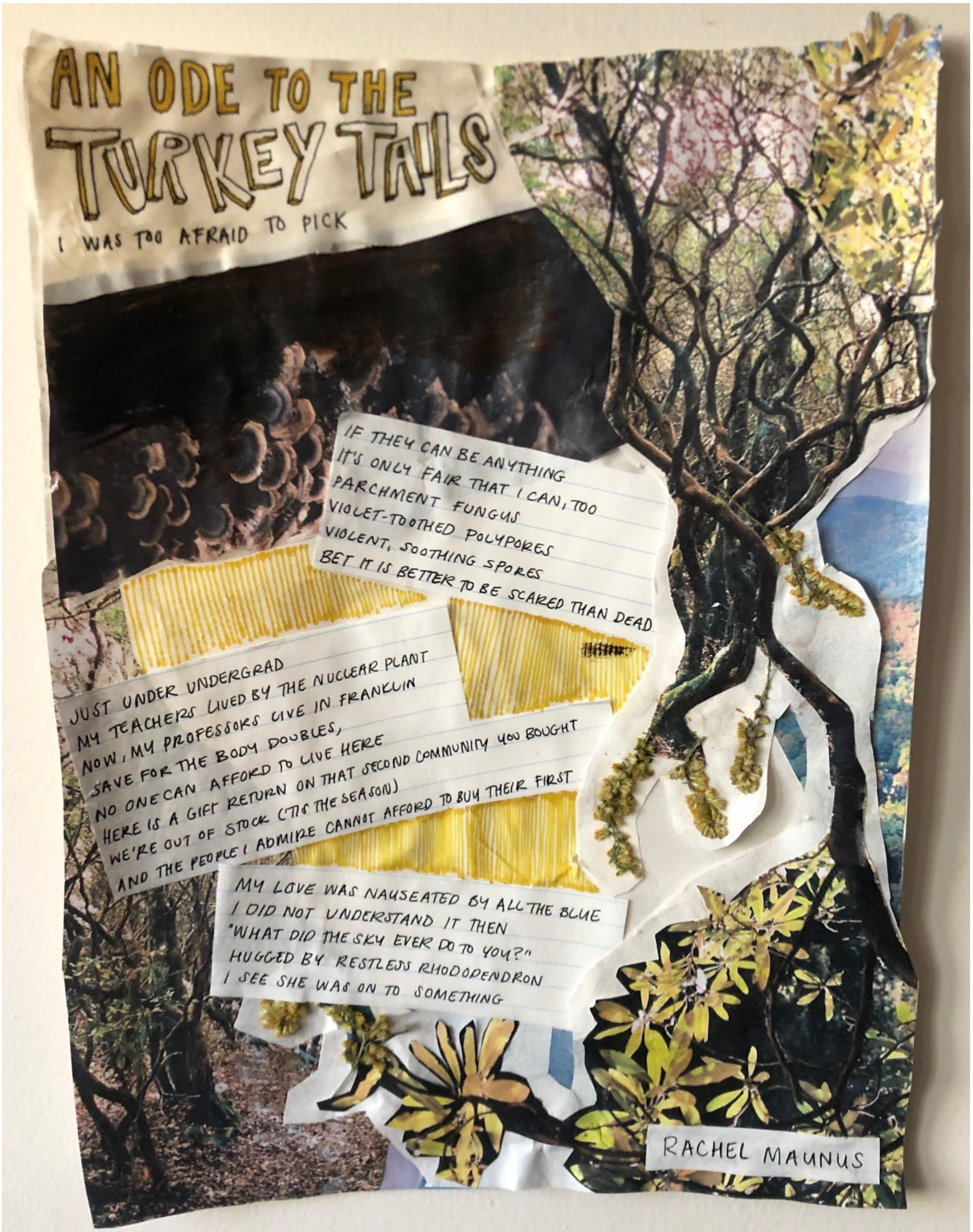
- Eva Kinney

When I was Small



I frequently ponder what it would be like to be small. I would wear flower petal dresses, ride damselflies and salamanders, and live in a tiny red mushroom. The closest I've gotten to feeling small was at the top of a bald, peering out at the vast majestic mountain ranges. I was dwarfed in comparison to the vista. At that moment, the shrinking powers of the Blue Ridge Mountains reached my ego. I realized how inconsequential I was in the grand scheme of things, How these mountains towered long before I existed and will look over these lands long after I'm gone. Maybe if everyone felt small once, we would be a little bit happier in this tumultuous age.

- Rachel Lopez


AN ODE TO THE TURKEY TAILS
I WAS TOO AFRAID TO PICK
IF THEY CAN BE ANYTHING
IT'S ONLY FAIR THAT I CAN, TOO
PARCHMENT FUNGUS
VIOLET-TOOTHED POLYPORES
VIOLENT, SOOTHING SPORES
BET IT IS BETTER TO BE SCARED THAN DEAD
JUST UNDER UNDERGRAD
MY TEACHERS LIVED BY THE NUCLEAR PLANT
NOW, MY PROFESSORS LIVE IN FRANKLIN
SAVE FOR THE BODY DOUBLES,
NO ONE CAN AFFORD TO LIVE HERE
HERE IS A GIFT RETURN ON THAT SECOND COMMUNITY YOU BOUGHT
WE'RE OUT OF STOCK ('TIS THE SEASON)
AND THE PEOPLE I ADMIRE CANNOT AFFORD TO BUY THEIR FIRST
MY LOVE WAS NAUSEATED BY ALL THE BLUE
I DID NOT UNDERSTAND IT THEN
"WHAT DID THE SKY EVER DO TO YOU?"
HUGGED BY RESTLESS RHODODENDRON
I SEE SHE WAS ON TO SOMETHING
RACHEL MAUNUS

In primordial waters
rivulets quench and temper a hardening blade
which slices a cell in two.
One becomes you and me, the other, a tree.
The river runs and sprints, creations
in ode to overwhelming abundance.
Cell walls and cellar walls can’t shield us
from ancient familial roots.

My fingers hover over their echo in the river which birthed me and the trees
These epiphanies percolating through my senses and synapses
infiltrating through my consciousness
meandering like droplets through the water table
Waves of adrenaline gathering steam
caressed by the moon’s gentle tug, every bit of me the river.

- Noa Meiri

Floating Down a Dream

Water is memory and it takes me back in time.

“Get in, jump in!” I dig my toes into the sand bank and bend my knees

Brother waits in the water before me with a grin and a glow

The initial slap of cold water turns into a comforting rush, engulfs my body.

Paddle splashes splish me and I’m jerked back to reality as cold water smacks my face.

Things fall into the water from the trees above

Sounds transport me back to the time of skinned knees and stubbed toes.

I stand on the edge of the splintery wooden dock

Look down at the opaque depth below

I land in the water and suddenly I’m floating in my kayak again slowly down this dream -
I mean stream!

Sure feels like a dream, a trip, but I’m sober.

Waves blur into each other

Dancing like freshly plucked guitar strings.

Nostalgia takes over as river ripples continue to rush, splash, and plop.

- Hannah Obenaus

One in a Life Time

Three months, four weeks fluttered by
so fast. Don’t even have time to peek at the past.
A period of growth, self-conscious and challenge
Abandoned my mother’s arms to embrace in bigger balance.
“Highlands, what is that place?” A place is roofed by the color greens,
and is wrapped by the green colored fear. But Highlands,
Became a place for reality escapers – Submerged in nature,
Rambling, and meandering up and down like a true mountaineer.
Jumping in Busted the Butts Fall in icy warm
Bathing behind Dry Falls during heavy storm
And fainting on top of Devil’s Courthouse after steep stable.
There are some moments in life that are unforgettable.
This was the moment that I would not want –
To trade with anything else.

- Vy Pham

Meandering Life

Dew on grass
Flight of my toes
My wet shoes and socks
Canvas shoes do not suit this environment
My meander recorded temporarily
Snail trail in my wake
Am I the brush and earth the canvas?
We all must be
Interwoven in the scarf of humanity
Paths cross, begin, end
And the stroke of one's life
May be thin or wide
But all make a mark
All leave a trail to follow, for a time
But follow the trail of another
And you will make no stroke of your own
But at least you kept your shoes dry.

\- Gus Winiker

Stitching before extinction

While I heal, while I heal within
how can I find a cure for Her
with so little to offer and so much that can be taken away
I am not needed here
there are no expectations
I observed her 400-year-old tulip poplar
growing and growing without anyone's aid
I could see her mending the injuries suffered
and at the same time the ghost of the humans that were there before
the humans that tried to destroy her
can She even be destroyed?
destroyed before we disappear?
what is left for us may be extinction
extinction or evolution

- Daniela Zarate-Arias

Many feet over Abrams Creek, Cades Cove, Great Smoky Mountains National Park. (l-r): Gus Winiker, Rachel Maunus, Rachel Lopez, Chloe Hall, Noa Meiri, Alex Hubbs, Daniela Zarate, Scout Allen, Hannah Obenaus, and Vy Pham.

Whitesides Mountain