Bog Turtle Distribution in Virginia: Assessing Proposed Methods for Finding New Localities and Examining Movement Between Wetlands

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ABSTRACT (ACADEMIC)

Freshwater turtles are among the most threatened groups of taxa globally, and the bog turtle, *Glyptemys muhlenbergii* is among the most imperiled in North America. In Virginia, USA, bog turtles are restricted to occupying Appalachian Mountain fens. Fens are naturally small and fragmented wetlands characterized by elevated water tables and an open canopy. Although there is a strong need to document and monitor populations of bog turtles, efforts to do so are often limited by the low detection of the species. The first objective of this thesis was to assess proposed methodologies for locating populations of turtles on the landscape. My first chapter assessed a previously-developed habitat distribution model for bog turtles using an occupancy modeling approach. I conducted 216 surveys of 49 discretely predicted patches of habitat, recording conditions such as weather, size of wetland and time of year, hypothesized to affect detection during each survey. In addition, I assessed factors including stream entrenchment, grazing presence and surrounding impervious surfaces for each surveyed patch to identify data sources that could improve future models or better assess sites. I found that sites with larger total wetland area had higher detection per survey, possibly due to larger sites having higher densities of turtles (among other explanations), and that sites with higher amounts of impervious surfaces within their drainage were less likely to be occupied.

In addition to the bog turtle, several plant species also occur in mountain fens. These species usually have a locally rare distribution or are disjuncts from a more northern latitude. Because of these traits, a high diversity of specialist plants may be indicative of a fen with a robust hydrology that has historically been less disturbed. Past site quality analyses have proposed using indicator diversity to assess sites, but no study has found if these species to tend to co-occur. My second chapter examines this hypothesis. I first chose a list of plant species that would most likely have habitat requirements similar to those of turtles. Then, at 12 sites, 6 with turtles and 6 without, I conducted a complete floral inventory. I first tested community-wide differences between the floral communities of these sites and found no difference, but when I narrowed my analysis to examining occurrence patterns of plant species determined *a priori* to be fen specialists and *Glyptemys muhlenbergii*, a pattern of co-occurrence was found. This lends support to the idea that indicator plants could be used as a tool to better evaluate sites that may have bog turtles.

My last chapter investigated movement of bog turtles in a landscape impacted by anthropogenic development. Movement of turtles between adjacent sites is critical to maintaining genetic diversity and maintaining metapopulation integrity. Despite this importance, records of long distances movements among wetlands are scarce in the literature, likely due to the lack of long-term studies for areas with multiple adjacent sites. In Virginia, mark recapture monitoring has been done intermittently in a cluster of sites for over 32 years. To determine the prevalence of movement among sites for bog turtles, I examined the dataset for all instances of turtles found at sites different from their last capture. I calculated the straight-line distance for each recorded movement. I also examined the sex of the turtle to test whether sex influences movement the

frequency and distance of movements. For a subset of movements, I calculated least-cost pathways to identify possible barriers to movement using a previously published resistance model. I found 21 instances where a turtle was caught at a different site than its last capture over 32 years of monitoring. Neither sex was more likely to move farther than the other. Although the study's observed rate of movement may appear low, it is likely an underestimate when detection and asymmetric sampling are taken into account. The least cost pathways analysis suggested that roads or driveways were likely crossed for a significant portion of movement events. Finally, to examine how movement may be affecting the current distribution of bog turtles, I described a method to test whether adjacency to known populations influences the probability of a new site being occupied by turtles. I prove the utility of the method by applying it to a map of bog turtle occurrences collected over this study and show that it can account for habitat differences and barriers to movement between sites as well. In spite of plausibility of the method, limitations in how occurrence data are currently collected prevent its immediate application.

Together, this thesis will help managers not only find and assess wetlands on the landscape, it will also provide information about the network of connected patches on the landscape. Knowing where bog turtles are and what wetlands or sub-populations are potentially connected will allowed for a more directed and informed regional management strategy.

Bog Turtles Distribution in Virginia: Assessing Proposed Methods for Finding New Localities and Examining Movement Between Wetlands

Joseph C Barron II

ABSTRACT (GENERAL AUDIENCE)

Freshwater turtles are facing population declines worldwide, and the bog turtle *Glyptemys muhlenbergii* is among the most imperiled in North America. Bog turtles occupy naturally small, specialized wetlands called Appalachian Mountain fens. The prevalence of fens on the landscape has declined over recent decades due to agricultural practices. Although there is a strong need to document and monitor bog turtle populations due to their threatened status, bog turtles are difficult to find due to their small size and ability to burrow completely into substrate. Thus, considerable effort must be expended to find populations and track their status. The first overall objective of this thesis was to assess methods for locating populations of bog turtles. My first chapter tests a habitat distribution model that uses publicly available landscape data such as topopgraphy and land cover to predict areas likely to contain turtles. To do this, I systematically surveyed 49 predicted sites multiple times each over 2 years. Simultaneously, I recorded variables such as the time of year, size of the wetland and the weather to determine whether any factor significantly explained the ability to find turtles on any given survey. In addition, I was able to record several variables relating to wetland quality and isolation that were not in the initial model. I found that larger wetlands were easier to search than smaller wetlands, possibly due to larger sites having more turtles, and that wetlands near more impermeable surfaces (such as roads and buildings) were less likely to have bog turtles.

As another potential method to find bog turtles and assess sites, we tested the use of ‘pristine indicator’ plants as a metric for potential wetlands. Mountain fens have specific attributes, such as high groundwater influence and exposure to a large amount of sunlight. Several species, including the bog turtle, are specialized to these factors and are rarely found in the surrounding landscape. Because a distinct community exists for mountain fens in this region, sites with a higher diversity of fen specialist plants may be indicative of a higher quality site which can support more specialists, including the bog turtle. My second chapter tests this hypothesis. I first chose a list of species that would most likely have habitat requirements similar to those of bog turtles. Then, at 12 sites I documented every plant species I encountered within the wetland. I compared the plant community as a whole between bog turtle-occupied and unoccupied sites and found no significant difference between the two. When I narrowed my analysis to focus on plants I previously identified as sharing habitat requirements with the bog turtle, I found a strong pattern of their co-occurrence with bog turtles. This lends support to the idea that these ‘pristine indicator’ plants could be used as a tool to better evaluate sites that may have bog turtles.

My last chapter investigates movement of bog turtles in a landscape impacted by human development. Movement of turtles between adjacent wetlands is critical to maintaining long term regional viability of the species, as it lets turtles colonize new sites and exchange genes. Despite the importance of these movements, records of turtles moving long distances between two wetlands is scarce in the literature, likely due to the lack of long-term studies for areas with multiple adjacent wetlands. One method of recording movements is by marking turtles with a unique ID and recording where it was encountered as wetlands are surveyed on the landscape. In

Virginia, this procedure has been conducted at multiple sites over 32 years. To understand the prevalence of movement between sites for this species, I examined this dataset and examined all instances of a turtle being found at a site different from its last capture. I recorded the straight-line distance moved for each recorded movement as well as the sex of the turtle, to test if either sex was more or less likely to undertake these movements. Then, for a subset of movements, I calculated least-cost pathways, a metric that accounts for landscape features and plots the easiest route for turtles to move. This way, I could evaluate the prevalence of barriers to movement, such as roads or development, on the landscape. I found 21 documented movements among sites over 32 years of monitoring. Neither sex was more likely to move further than the other. Compared to studies looking at other freshwater turtles, the observed rate of movement appeared low, but this was likely an underestimate due to the difficulty of capturing specific individuals. I also found evidence of significant barriers to movement in 13 out of 17 evaluated least-costs paths, usually roads or driveways. Finally, to examine how movement affects bog turtle distribution, I describe a methodology of testing if adjacency to known populations influences the probability of a new site being occupied by turtles. I demonstrate the plausibility of the method by applying it to a map of occurrences collected over this study and show that it can account for habitat differences and barriers to movement between sites as well. However, limitations in my sampling scheme limit conclusions from my dataset.

Together, these findings will help future managers find where turtles are and which sites may be connected. These results will help managers make more informed decisions for managing bog turtles at a statewide level.

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The fact that I'm here writing a thesis is the result of the support of so many other people throughout my life. My mom, dad and grandma actually just came down this month to cook me a dinner and provide encouragement. They've been encouraging me since the very beginning, putting up with noisy frogs I caught outside and buying me all the amphibian books I asked for. My friends from back home too deserve a shout out, always giving me outlets to throw memes at and providing endless banter through long nights. The graduate department at VT is among the most inclusive and friendly places you could hope to find yourself, and their perspectives and encouragement always drove me to do the best work possible. My roommate Vance Nepomuceno deserves an extra shoutout for picking me up after my car rolled into Claytor Lake. Finally, I want to thank my fellow Haas lab researchers, George Brooks, Houston Chandler, and

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Chapter 1: Use of occupancy modeling to estimate species detection and field test a habitat distribution model for *Glyptemys muhlenbergii*

Abstract

In many parts of their range, finding previously unknown populations remains a priority for the conservation of the federally threatened bog turtle, *Glyptemys muhlenbergii*. Bog turtles are known to have low detection rates, and adequately assessing potential sites requires considerable resources. To this end, habitat models have been constructed to better predict areas of occurrence for bog turtles, and field testing their efficacy can help refine them and confirm their utility for management. Potential sites are often surveyed by hand probing, and best practices for probing surveys have yet to be quantitatively tested for the significance. In this study, I field test a habitat model for *G. muhlenbergii* using an occupancy modeling approach. I, along with several other trained observers conducted 216 surveys of 49 discrete patches of highly predicted habitat standardized to 4 person-hours per hectare of mapped core habitat. For each site, I measured and calculated several additional factors not included in the habitat model, such as total wetland area, core wetland area, presence of grazing and an index of surrounding impervious surfaces. At each survey, I took data such as time of day, time of year and weather conditions to find their effect on detection. Out of 216 surveys, I had 21 detections of turtles at 9 sites. I found that surrounding impervious surfaces had a significant, negative relationship with turtle occupancy and that total wetland area positively had a significant, positive relationship with detection. The processes underlying these relationships require further study; however, this occupancy model highlights a potential limiting factor to turtle occurrence in addition to a quantitative detection estimate to better inform survey procedure.

Introduction

Previous conservation plans for *Glyptemys muhlenbergii* have listed identifying previously unknown populations and localities as critical objectives (see North Carolina Wildlife Resources Commission 2018 for an example). Towards this goal, several studies have constructed species distribution or habitat suitability models to help direct the search for new populations in the southern range of *G. muhlenbergii*. These models have identified that much of the potential habitat exists on private land (Feaga 2010, Stratmann et al. 2016, Howard and Chazel 2017). Surveying private land requires a considerable amount of landowner engagement, which has limited the number and extent of surveys to date. It is imperative however, that these models be field tested for their efficacy. A model that has proven to reliably predict habitat and populations can direct conservation effort more efficiently.

Searching for potential new localities in a structured way could not only reveal new populations – a worthy conservation goal in itself – but also reveal factors affecting the distribution of *G. muhlenbergii* at a finer scale than those previously investigated by species distribution and habitat suitability models. The models cited above have thus far used presence-only modeling techniques and robust survey data have not been used in determining differences between seemingly suitable wetlands. Comparing predicted sites with confirmed *G. muhlenbergii* presence to those without could reveal additional factors affecting their distribution. Finding comparable presences and absences is a difficult undertaking. Many freshwater wetlands are so small they are not documented in national wetland inventories (Leonard et al. 2012). By complementing landscape models with field surveying, the efficacy of these models can be tested while also investigating if additional factors not previously considered have an impact on the distribution of a rare, difficult to detect species.

Occupancy modeling (MacKenzie et al. 2002) examines each site independently and calculates the likelihood that a site is occupied, based on survey effort and the detection rate of the organism. Occupancy modeling can also use site specific variables to test if they correlate with presence. Ecologically speaking, this would imply that some sites are more likely to support a population than others. These site-specific variables can be remotely sensed or field measured. If predictive variables can be remotely sensed, then managers can assess the likelihood that a population is present, even if access is unobtainable. If field-measured variables have significant predictive power, then managers should be wary to make decisions on areas they cannot access. Through occupancy modeling and model-based inference, I will test a suite of factors to extract which ones are most likely to correlate with bog turtle presence at a site.

The output of habitat models used to find species is usually in a raster format, with square pixels attached to a relative score. Models predicting bog turtle presence use datasets which cover the entire study area, such as digital elevation or soil maps (Feaga 2010, Stratmann et al. 2016). Previously-mapped landscape-level data have been used successfully to identify new populations in at least one study (Stratmann et al. 2016), however many studies have found relationships between bog turtle presence and other factors, many of which would not be covered in these landscape level datasets (see Ernst and Lovich 2009 for an overview). The translation of a continuous raster into discrete, surveyable patches allows additional factors to be investigated through structured surveys in which effort can be allocated by the size of the habitat. Field surveys would allow for the assessment of potential factors that cannot be remotely sensed. For example, detailed mapping of predicted patch would present the size and structure of the wetland at a finer scale than the source raster. Possible degradation could be assessed from stream incision, which correlates with a lower water table (Schilling et al. 2004, Blann et al. 2009). In addition to field measurements, identification of discrete patches rather than the continuum of a raster allows for the incorporation of the immediate surrounding landscape to be assessed along with patch specific metrics. Predicted patches could be assessed for the predicted size and isolation relative to other patches, which are hypothesized to be important factors related to bog turtle presence (Myers and Gibbs 2013, Shoemaker and Gibbs 2013). Pressure from nearby development could also affect the presence of a population at a particular wetland. Increased impervious surface from development could affect a wetland's hydrology, making it less stable overtime (North Carolina Wildlife Resources Commission 2018). Additionally, housing and other development might generate food sources that subsidize predators of nests or adults, decreasing recruitment and survival at a site (Zappalorti et al. 2017, Knoerr et al. 2021). By testing a habitat model through occupancy modeling, more types of data can be assessed for their effects on turtle presence then investigated by points of occurrence on a raster.

A major limitation to studying the distribution of this species has been its perceived low detection, even where it is relatively abundant (Somers and Mansfield-Jones 2008, Stratmann et al. 2016). This is because turtles spend much of their time out of view in mud and under dense vegetation. While it is widely accepted that *G. muhlenbergii* is hard to detect, a quantitative estimate of detection probability in various habitat conditions is currently lacking in the literature. Occupancy modeling was developed to account for imperfect detection of a species by estimating the probability that one will find an individual during a single survey given that it is present (MacKenzie et al. 2002). Currently, the only published detection estimates for *G. muhlenbergii* are for trapping studies (Stratmann 2015). No detection estimate for probing is currently published, and estimates can only be derived from historical data of occupied sites, which are often difficult to compare due to variability in turtle density and surveyor skill and technique. While intense trapping at a site is a useful tool for evaluating one site thoroughly, it quickly becomes infeasible when many sites must be trapped concurrently. Visual encounter surveying combined with probing in the mud is more conducive to rapid assessments of many sites, and is still a standard surveying technique for the northern extent of the species range (U. S. Fish and Wildlife Service 2018). Without a quantitative measurement of detection probability however, land managers must make guesses on how much effort to spend on a site. An informed estimate of the survey effort needed for each potential site could help develop future guidelines used to identify locations with turtles and to limit the possibility of designating occupied sites as unoccupied. Additionally, knowing if season, time of day, or weather effects detection would allow surveyors to sample more effectively by targeting the best conditions under which to conduct surveys.

Management agencies have implemented protocols to maximize detection based on perceived drivers of surface activity (U. S. Fish and Wildlife Service 2018). Through an occupancy modeling framework, I built several models to estimate detection and compare the relative support of these environmental factors as well as several others. In the above protocol for example, new sites must be assessed at least once in May or June, because these are thought to be when turtles are nesting and mating and therefore more likely to be on the surface. Although previous studies have shown that weekly movement rates of turtles do not significantly vary between April and August (Morrow et al. 2001), vegetation is lower and easier to search during the early active season and *G. muhlenbergii* counts have historically peaked in these months (Lovich et al. 1992). Bog turtle activity has also been tied to temperature (Ernst 1977). It was hypothesized that high temperatures in mid-summer may cause *G. muhlenbergii* to estivate underground (Bury 1979), but some authors hypothesize that this may be an abnormal behavior due to the deterioration of natural habitat (Bloomer 2004). By sampling throughout the year in different temperatures, seasonal and temperature effects on detection can be tested by an occupancy modeling framework. These behavioral trends may differ by time of day and weather conditions, so these factors were also assessed in my model.

Occupancy modeling has the potential to guide future surveys by providing a quantitative benchmark to finding *G. muhlenbergii* at new sites. It can help managers assess how many surveys are needed before one can be confident a site does not have a population of turtles, and what conditions might affect that number. The current standard is four surveys over a season (U. S. Fish and Wildlife 2016), however, in one instance a site was surveyed 6 times prior to a construction project – revealing no turtles until construction workers unearthed them (Somers 2000). It could be that surveyors have previously ruled out sites as not occupied, but more surveys are needed to obtain a high level of confidence. Some factors may confound management assessment as well. Grazing at a site has shown to apparently increase turtle density in at least one study (Tesauro and Ehrenfeld 2007), and grazing has been adopted as a management strategy in some areas. In the above study however, population estimates were not corrected for detection, which may affect turtle density estimates. As shown in the same study, grazing lowers the height of vegetation and lowers the prevalence of woody species, which may make turtles easier to detect. Occupancy modeling can help account for the effect of grazing on detection while evaluating whether grazed patches are more likely to be occupied by turtles.

This chapter will use an occupancy framework to investigate the current distribution of *G. muhlenbergii* in one county in southwest Virginia. Through this methodology I will determine detection rates for probing surveys on *G. muhlenbergii*, and the factors that influence this rate (Summarized in Table 1.1). Additionally, this study will build upon a local habitat model and determine if factors not well represented by landscape raster data can provide additional insight on where turtles are currently found.

Methods

Study Area and Sampling Design

This study was conducted in a single county in southwest Virginia, located within the Blue Ridge physiogeographic region near the border of North Carolina. For potential habitat to survey, I chose Feaga (2010) as my landscape model because this model was developed using data from this study area, and therefore most applicable to the region (Figure 1.1). This model exists in a raster format, with 10 x 10 m cells with an assigned relative value based on inverse wetness index, canopy cover, and proximity to a stream. To identify patches to survey, I selected all areas in the county where the pixel value was in the top 70th percentile of values in the model, an arbitrary benchmark that overlapped well with known distribution. Henceforth, the term 'patch' refers to a distinct group of pixels adjacent to each other in all 8 directions after all other pixels were nullified in the habitat raster. Since the start of the turtle marking program in Virginia in 1988, fewer than 40 sites contained marked turtles in my study area, with the majority of them falling within or near protected land. Feaga's model (2010), only accounting for the top 30% of the model, identified 1,216 patches of highly predicted habitat in my study area, many of which covered multiple privately owned parcels.

Because the number of predicted patches far surpassed what was possible to survey in the span of one or two years, I applied several filters on the model predicting suitable habitat to efficiently acquire landowner permission and increase breadth of spatial sampling. First, I overlaid a map of private land parcels in the county. Then, I filtered for parcels that have a residence on them. Having a residence on the property greatly increases the chances of being able to meet the owner in person. This, anecdotally, results in a much higher success rate when obtaining permission to survey as compared to requesting property access through the mail. I then stratified sampling by USGS Hydrologic Unit Code subwatershed (HUC12; U.S. Geological Survey and U.S. Department of Agriculture Natural Resources Conservation Service 2013) to spread the sampling over the entire county. My stratification procedure was as follows: first I took the proportion of my study area covered by each HUC12 drainage. I then allocated samples by the proportion of area covered by each of these 14 HUC12 units. I multiplied my target number of sites, determined logistically to be 40, by the proportion of area each HUC12 covers to get the target number of sites for each HUC12. Although this process was mostly followed, due to the unpredictable nature of access to private land, effort was asymmetrical (Table 1.2). Across all HUC12s, I surveyed 54 sites with habitat at least once (representing 51 patches, as 2 patches covered multiple landowners and were split). Any additional sites beyond the number targeted for that watershed were surveyed mostly due to convenience of access.

Field Surveys

I sampled sites between April and September of 2019, with additional sampling done between April and July of 2020. Sites were surveyed between 1 and 10 times over the entire study, with asymmetric numbers of samples largely due to difficulties in land owner contact. Originally, each site was to have at least 4 surveys, following the steps for optimal occupancy design outlined in Mackenzie and Royle (2005). To derive this target number, I used available historic sampling data from the area as benchmarks for occupancy and detection. In 2009, rapid assessments were done on 77 wetlands near the study area for a road project, and turtles were found at 14 of them (Feaga 2010). This number was used for an educated guess on occupancy rates. For detection, I used a data set from a historic study where 6 sites were surveyed multiple times for a mark recapture study in the study area. In that study, at least one turtle was detected in 79% of surveys (S. Carter, Virginia Department of Wildlife Resources, Unpublished Report), and this was my educated guess on detection rate. Although I recognize that well studied sites may have higher rates of detection due to familiarity with the site, the lack of mark recapture studies done in the area made this the best possible benchmark. In conjunction with my initial target set of 40 sites, the procedure in Mackenzie and Royle (2005) recommended surveying each site 4 times. As the sampling season went on, some sites became harder to access; therefore, effort was put into sites with confirmed occupancy to better estimate detection parameters as survey conditions changed. This challenge in obtaining repeated access resulted in 26 sites not being surveyed 4 times, but 42 sites were surveyed at least 3 times and the average surveys per site was 4.07.

Field Surveys and Measurements

To obtain habitat spatial data at a higher fidelity than raster data, sites were mapped using a Trimble GEO 7x GPS with decimeter accuracy or Trimble TDC-15. Site mapping was done using 3 separate delineations. First, mapping separated wetland habitat from the surrounding landscape, following established delineation definitions (Environmental Laboratory 1987). Within this wetland habitat, I attempted to visually assess the wetland stratum between ‘temporarily wet’ and the ‘usually wet’ or ‘always wet’ in Feaga et al. (2013), with ‘usually wet’ and ‘always wet’ combined into a single stratum henceforth referred to as ‘core wetland’. I made this distinction using two criteria. 1) core wetland would have enough moist substrate where a surveyor boot would sink at least 10 cm into the ground and 2) it was dominated by wetland obligate plants, such as *Scirpus expansis*. Within the mapped wetland, areas dominated by woody stemmed plants within the ‘core wetland’ were given an additional distinction – ‘woody wetland'. In cases where habitat was heterogeneous, such as scattered single stem woody plants or small pockets of moist soil, mapping only differentiated core wetland and woody wetland if it was at least 1m x 1m in continuous size. If no habitat could be found matching my definition of 'core wetland' the patch was ruled out for further surveys. At the conclusion of the sampling season, habitat stratum polygons were collated into a single map, removing overlapping polygons from mapping. If two polygons overlapped, the smaller feature was always given priority in the final map.

Because physically visiting a site allows for the measurement of parameters not captured in the habitat model, I field assessed two additional wetland parameters at each site. For sites adjacent to a stream, I measured the degree of stream incision within the wetland area by taking the height of the stream's low bank and the height at which it is bankfull – obtaining a ratio (Rosgen and Silvey 1996). If the site was not adjacent to any type of steam, this ratio was assumed to be one, but out of 53 sites, only three were not adjacent to a stream and one additional site had an inaccessible stream (also assumed to be one). I also made note if the site was actively grazed by livestock. Rotation of cattle throughout the study period interfered with my ability to objectively assess the intensity of grazing, so it was left as a binary attribute.

Concurrent with mapping, and at every subsequent visit after the initial visit to a site, trained surveyors and I searched the wetland for *G. muhlenbergii*. Those surveying were equipped with two ½'' dowel rods and a timer. At the onset of the survey, surveyors started their timers and attempted to locate *G. muhlenbergii* visually and through probing the soil. If at any time a surveyor stopped actively looking, they turned their timer off as to have a more accurate measurement of their time surveyed. Before the sites were mapped, I surveyed each site initially for the entire duration of mapping, with no standardization by wetland size. After wetlands were mapped, but before the maps were completely post processed, I scaled allotted survey time to 4 person hours of effort per hectare of core herbaceous wetland mapped at a site, an effort value that aligned with or was more intense than established benchmarks (U. S. Fish and Wildlife Service 2018). For logistic purposes, I also implemented a minimum actual survey time of one hour for the initial survey and 10 actual minutes for subsequent surveys (with a minimum person-time of 20 minute as my smallest team was two people). I set the minimum for the initial survey to allow for detailed mapping. For subsequent surveys, the purpose of this minimum was to reduce the bias of the inactive time spent walking between optimal habitats for sites with little core wetland area during the allotted time. In practice, median survey time was 23% more effort than what was allotted for surveys after removing surveys where a minimum time was enforced. This difference was likely due to basing effort goals on unprocessed maps, as polygons overlapped and therefore the sum area of herbaceous habitat was inflated.

In sites that have been repeatedly monitored, trained observers often know which areas of the site are best to search to maximize detection. To reproduce that knowledge, when surveying for *G. muhlenbergii* I stratified sampling effort within the wetland to include higher effort in areas where encounters were most likely to occur. While surveying, surveyors identified areas that previous telemetry studies have shown to be preferred habitat. In general, these areas closely aligned with soft soil close to open pockets of water, interspersed with tussock grasses or edged by alder shrubs (Carter et al. 1999). As density and abundance were not a part of this study, it was more important to survey areas with the highest likelihood of *G. muhlenbergii* to establish occupancy rather than sample the wetland entirely.

Finally, during each survey event, several conditions were reported. First I recorded ambient air temperature directly before the start of a survey in a spot away from direct sunlight.

Next, I noted sky and wind conditions using a quantitative scale, with 1 denoting both a clear sky and still air, increasing in level based on cloud cover and severity of precipitation and wind (Appendix A). For safety reasons, I did not survey in any conditions that involved lightning. Time of day and calendar date were also recorded, due to previous observed activity patterns with time and season (Arndt 1977, Lovich et al. 1992). Mean, minimum, and maximum value of all values measured in this study are presented in Table 1.3.

Additional Remotely Sensed Data

With discrete patches defined from the habitat model, I analyzed several additional factors both intrinsic to the predicted patch and from the surrounding landscape. First, I calculated the average score of each patch from the habitat model using the derived percentile to test if a higher specificity would lead to increased occupancy. Although I had previously filtered the landscape by percentile, a range between 70th and 100th percentile existed for this variable allowing a range of values to be evaluated. Using FRAGSTATS (McGarigal and Marks 1995), I also calculated each patch's area (in 10m^2 increments) and 'proximity index' to other predicted patches (Gustafson and Parker 1994). Patch area and proximity index were then be compared between patches to assess if larger and more connected patches were more likely to be occupied. Finally, to test for potential effects from surrounding development, I calculated an index of development pressure from the Multi-Resolution Land Characteristics Consortium (MRLC) NLCD impervious surfaces raster dataset (Jin et al. 2019). I chose impervious surfaces as they are likely to influence wetland hydrology (Chithra et al. 2015). Impervious surface was calculated within stream catchments mapped by the National Hydrographic Dataset (U.S. Geological Survey 2014). For each catchment that overlapped with a predicted patch I summed the values of pixels (a percent impervious score) of non-null values within any catchment the patch was within, then divided this sum by the total area of all catchments the patch was associated with. This gave a value of impervious surfaces scaled by area. These values are also summarized in Table 1.3.

Testing of Model Assumptions

Concurrent to sampling efforts in this study, two tests were conducted to test the underlying assumptions in several of the models to be tested. The first test was to determine if my assignment of habitat was accurate and robust throughout the year. To test this, I mapped 6 sites a second time in September of 2019. These sites were chosen based on a positive landowner relationship that would allow an extended mapping effort late into the season. After mapping, I conducted a one-sided paired t-test to determine if 'core wetland' area had changed significantly since the sites were previously mapped.

Additionally, to test the assumption that the vegetation density would be correlated with time of year and affected by grazing, I conducted an obstruction study during all 2020 surveys using a robel pole (methods explained in Stagliano 2016). Using maps of the wetland from the previous year, or satellite imagery in the case of several new sites added, I placed 20 random points in the 'core wetland' area of each site. Then, using a TRIMBLE TDC-150, I navigated to each point concurrent to every survey in 2020 to set up a single station at each point. My robel pole construction closely followed Toledo et al. (2008), with 40 alternating color bands. I then observed the pole through a peephole two meters away in each cardinal direction (each direction a specific reading), discarding a direction if it was inaccessible because of thick shrubs or steep elevation change. The number of stations set up during each survey was adaptable. I used a statistical calculator in field to calculate the coefficient of variance after 8 stations, and I added more stations using the formula $\frac{SD}{\hat{X}} * 8$ if necessary. The median of each station (up to 4 readings) was taken, then I calculated the mean of the station medians of each survey. I then constructed a suite of generalized linear mixed models using calendar day, grazing status (grazed or ungrazed) and an interaction term between them as predictor variables. Site was the random term in these mixed models. I compared all combinations of these factors, including an interaction term, using Bayesian Information Criterion (BIC) and examined the p-values of each factor in the final model for their significance.

Modeling

I used an occupancy modeling framework to derive estimates of detection and occupancy in my study area (MacKenzie et al. 2002). All analyses were done using R package *unmarked* (Fiske and Chandler 2011). First, for both occupancy and detection, I constructed 'null models' which only estimated an intercept parameter. The purpose of these null models was to serve as a target AICc for future models to determine the relevance of variables. Next, for both detection and occupancy I *a priori* chose a variable that I thought had the most support prior to the study. If I found it to have AICc support over the null, I kept this variable fixed in all additional models. This was done to compare additional variables to one thought to be most relevant and to reduce the number of models in my model set. For occupancy, I chose this to be the average model score of the patch from the habitat model used to select study wetlands as well as the predicted area of that patch, as this model directed my search most directly to finding new populations. For detection, I chose total wetland habitat at a site to be evaluated first. Due to the logistical difficulties of equal sampling throughout the season, I believed a more constant site variable would have the heterogeneity needed to model differences in detection at the site level. Additionally, larger sites fatigue surveyors and could provide more refugia for turtles to avoid capture. If no support was found, the *a priori* factors were removed from consideration. Once these variables were tested for support over the null model, I added in all additional variables defined above (Table 1). All variables affecting detection and occupancy were separate, with the exception of grazing, due to the evidence suggesting it might affect both occupancy and detection. Because grazing was used in both parameters, I conducted model selection on detection first, holding occupancy to a constant value. Under this procedure, any effect grazing may have on occupancy would be corrected by its effect on detection (provided I found support for it). Under best practice procedures for AIC model selection (Arnold 2010), I closely examined all models that fell within 2AIC of the top model. If a model omitted variables considered in the top model but included others, I considered it a valid alternative model. If a model included all terms in the top model with the addition of others, I did not consider it a valid alternative model and removed it from consideration.

Results

In 2019, I visited 54 predicted habitat patches in this study and 46 of them had at least $1m^2$ of core herbaceous habitat and were deemed appropriate for further surveys. One patch was broken up into 3 sites due to landowner access and one site was initially predicted but had its predicted value drop after updating the 2010 model with current landcover data, bringing the total to 49 surveyed sites. Between April and September of 2019, 184 surveys of the 49 sites were conducted covering 36.88 hectares of mapped habitat, although the majority (145 samples) of sampling took place between May and June. I had 9 sites with confirmed detections, 39 with no detections, and one site where only a plastron of an individual was recovered (and was considered unoccupied in this analysis). Additional surveys in 2020 took place between April and June, with 32 surveys of 9 sites, including 7 from the previous year (one was split into 2 sites due to landowner access) and 2 additional sites known to have turtles. All turtle captures in 2020 were at sites already known to have a population. Across both years, myself and trained surveyors recorded 393 hours of time actively probing over 216 surveys at 53 sites with 21 detections and 42 turtles found.

The goodness of fit test for dispersion parameter from the full model – the combination of all detection and occupancy variables measured — returned a p-value of 0.062 and a $\hat{c}$ of 2.17. Because the level of overdispersion was not significant to an alpha of 0.05, I did not use QAICc. Correlation between measured site variables was usually weak (r = <0.30), unless measurements derived from the same dataset (Appendix A). For example, area of core wetland habitat was greatly correlated with the total amount of wetland mapped (r =0.76). Because a larger wetland core inherently means larger total area mapped, I expected to find a correlation between these factors. I chose to include both as total habitat and core wetland habitat were modeled for detection and occupancy respectively. For survey variables, a slight correlation was seen with air temperature and start time of survey (r = 0.36), which is to be expected as temperatures were generally lower in the morning hours of the day (Appendix B).

I took the readings from 232 Robel stations set up over 2020 surveys to test the assumption that vegetation density was dependent on time of year and grazing pressure. Both grazing and time of year correlated with Robel pole obstruction (GLMM results presented in Figure 1.2 and Table 1.4). Grazed sites, on average, had 3.14 less bands obstructed by vegetation. As the sampling season continued through the summer, robel pole obstruction by vegetation density increased by an average of 1 band per week. No interaction term between the two was supported by BIC, and the modeled seasonal effect was left constant. In the final model, calendar day was found to be highly significant (p-value < 0.001) while grazing was significant only at an alpha value of 0.1 (p-value = 0.07). My second assumption dealt with habitat change over time. My second round of mapping at 6 sites documented significant change in wetland size through the course of the sampling season. On average, ‘core wetland’ (the sum of core herbaceous and woody wetland habitat) decreased by 77% over the sampling season ($t = -3.09$, p-value = 0.01), although without more samples I cannot comment on the rate of change or the effects weather had on these changes.

The first step of the occupancy modeling procedure was to estimate detection. The addition of the *a priori* chosen factor, total wetland habitat, lowered AICc by 5.83 points over the ‘null model’ that estimated detection only using capture histories and no additional variables. After I fixed total habitat into all subsequent models, addition of other all other factors did not lower AICc and were subsequently dropped from further consideration. The full table of models evaluated is shown in Appendix A. Because the addition of total habitat significantly improved model fit, in the subsequent evaluation of occupancy, total habitat was a constant factor affecting detection.

Unlike my detection models, the *a priori* chosen factors for occupancy did not improve model fit. The addition of the average score and size of the predicted patch from the habitat model was 3.1 AICc points higher than the null model. With no support that these variables improved model fit, they were removed from model consideration and no fixed effect was used in subsequent models. The top model after evaluating all other factors was one modeling a negative effect from surrounding impervious surfaces, with a AICc score 2.43 points lower than the null. Evaluating all models within 2AIC of this top model, it was found that all other top models included surrounding impervious surfaces in addition to other factors. In this case, following best practices discussed in Arnold (2010), the top model was chosen without the use of model averaging. The full table of all evaluated models for detection is shown in Appendix A.

In the final model set (Table 1.5), total habitat was included as a factor for detection and surrounding impervious surface was modeled for occupancy. In this final model, total habitat was found to have a positive effect on detection, increasing detection by 0.064 for every 1000m^2 of mapped wetland habitat. For occupancy, the impervious surface index modeled a negative effect, with occupancy decreasing by 0.0031 for every unit increase in the impervious surface index. 95% confidence intervals for effect size on the logit scale showed that total habitat did not cross 0 (0.00002 - 0.00011) but impervious surfaces did. Narrowing the confidence interval to 90% however did show a significant effect size between -2.4 and -0.19. Prediction plots showing the effect size through two standard deviations above and below the mean are presented in Figure 1.3.

Discussion

Out of 55 new patches I visited in this study, 47 of them had at least 1m^2 of core habitat according to my definition. representing a success rate of 86% for identifying suitable habitat. My definition of core habitat closely aligns with other managers (U. S. Fish and Wildlife Service 2018), although these guidelines do not make note of how large the core habitat needs as no minimum habitat size has been established thus far in the literature, making it difficult to set a reliable cut off. The smallest occupied site in this study had 612m^2 of core herbaceous habitat, although my study design cannot examine if this site sustains a population capable of long-term persistence. Using the 1m^2 cut off, my rate of finding suitable habitat was greater than Stratmann et al. (2016) in South Carolina and Georgia, who found suitable habitat at 11% of sites evaluated on the ground. As Stratmann et al. point out in their paper, their model was less restrictive than ideal, and states "It appears key missing data (e.g., better hydrologic and soil maps) do considerably limit the effectiveness of the SDM." The method in Feaga (2010)'s habitat model using inverse wetness index calculated from a slope layer appears to result in a 75% higher rate of success for identifying potential habitat. Additionally, while Stratmann et al (2016)'s model relies on the NWI mapping layer, which has been shown to be poor at finding small wetlands (Leonard et al. 2012), Feaga (2010) found no support for use of NWI, and was therefore unrestricted by it. No significant changes to the NWI dataset have been made since Feaga (2010) for the study area. Of the 47 patches with potential habitat that I visited in this study, 23 overlap with a mapped NWI emergent or forested wetland including 4 of the 9 newly identified populations (U.S. Fish and Wildlife Service 2020). This shows the value of hydrological modeling for *G. muhlenbergii* as many wetlands would have been overlooked if only NWI wetlands were considered.

Many factors previously thought to affect bog turtle detection were not selected in my top models. I emphasize that despite the lack of significance, my models do not refute their effect on detection. My study was complicated by several factors that prevented a study design that would allow for equal sampling across the range of relevant variables. Being that this study was done almost exclusively on private property, many sites had limited sampling due to lack of access. In addition, an academic schedule restricted much of my sampling so that a vast majority of my survey effort (160 out of 216) was done between May and July, with many sites not having a survey outside that range. The weather in 2019 was also remarkably homogenous, and weather conditions involving precipitation only occurred in 22 out of 216 surveys. Interestingly, I hypothesized that vegetation density would increase over the sampling season, and vegetation density would be lower in grazed sites. The results of the robel pole study supported both of these hypotheses, yet this did not appear to impact detection. There remains strong evidence both behaviorally and habitat wise that indicate seasonality could affect detection, but for this study the effect size was not large enough to be significant.

At an average site in this study, detection was approximately 20%, and the one factor in my study that influenced detection, total habitat at a site, did not follow the predicted direction. Greater total habitat at a site was associated with higher rates of detection. This effect was maintained even though effort expended at a site was biased towards small sites due to minimum survey times. The effect could be substantial, increasing the amount of effort needed to rule out a potential site considerably. For example, if I wanted to be 80% sure that I would detect turtles if they were present at a site, I would need to survey 14 times at a site $3363 m^2$ large versus 5 times at a site $11276 m^2$ large (representing one standard deviation above and below the mean site size, respectively) (Figure 1.4). The underlying cause of this relationship is worthy of future consideration, although I lack the data to fully examine it. Survey times were scaled to area of core herbaceous habitat, not total wetland habitat at a site - although a strong correlation existed between the two (0.76). One possible explanation could be that a wide basin of wetland was much easier to search than a smaller site. Many smaller sites were confined to wet ditches rather than a gradual shift from wetland ecotones, and these ditches were often deep and dominated by thick *Scirpus expansis* which was difficult to search. Larger wetland habitats might simply look better then smaller sites, motivating surveyors to look harder.

Perhaps most impactful, larger sites might also support a higher density of turtles, making encountering an individual more likely (McCarthy et al. 2013). This hypothesis could be tested by comparing time to first detection for a range of sites with different known densities, although getting site specific abundance estimates can prove challenging for the species (Holden 2021).

Although the drivers of detection still warrant further investigation, this model provides the first quantitative benchmark for the detection of turtles via hand probing.

Of all factors evaluated, the only factor affecting occupancy in this study was from impervious surfaces in the same catchment as a site. Impervious surfaces appear to have a strong, negative impact on bog turtle occupancy. The reasons for this impact could vary. For one, impervious surfaces may affect the recharge ability of wetlands (O'Driscoll et al. 2010), affecting the hydrological stability on which bog turtles rely (Feaga et al. 2013). Additionally, greater impervious surface around a wetland is probably related to increased road surface within a catchment, which may increase road mortality of individuals. During this study an individual was found struck by a car, although it is unknown how frequently this occurs. Another possible factor could be subsidized predators around human infrastructure, shown to be a strong source of egg mortality on bog turtle nests in North Carolina (Knoerr et al. 2021) or domesticated dogs, shown to predate on adult turtles (McCoy et al. 2020). My impervious surface index was simplistic and cannot investigate which of these sources was most significant, however the data used are readily available throughout the entirety of *G. muhlenbergii*'s range, allowing it to be applied to other study areas. This index could be added to future habitat predictive models by calculating the index for each catchment of interest and then rasterizing the catchments. Further investigation of the effects of urbanization on bog turtles could also find tools better able to predict occupied sites, but the index identified here provides an additional factor future models should consider.

Many other factors were hypothesized to affect occupancy, but upon evaluation did not have a significant effect. Despite hypothesizing them as the most important factors, neither average score of a predicted patch nor size of the patch had an impact on occupancy. This might indicate that my arbitrary cut off score, the top 70^{th} percentile of scores from the raster, may be specific enough to correctly identify extant bog turtle populations. Bank ratio of the associated stream also did not significantly impact occupancy. The single sample done at each site may not have been adequate to truly evaluate this parameter, although other factors, such as ditching, may have masked this effect on local hydrology. Proximity index, an index that measured distance to adjacent predicted patches and weighs them by the size of the adjacent patch was another non-significant factor. Although bog turtles are thought to disperse via stepping stones (Shoemaker and Gibbs 2013), this index may rely too heavily on patch area, shown to be non-significant as a factor for occupancy. Mapping the sites also did not result in a significant predictor for occupancy. Late season mapping showed that my initial mapping was not an accurate evaluation of areas with persistently moist substrate, and that managers should not use one visit to evaluate the extent of a site. A more labor intensive but likely more accurate measure would be to map multiple times over the year or install shallow wells to measure the water table consistently at a site to better map areas that remain consistently wet through the active season (Feaga et al. 2013). Finally, my model may appear to disagree with several papers suggesting that grazing is a viable management strategy for bog turtles, since grazing did not appear to influence occupancy at a site. I caution against this insight as my study had to limit grazing to a simple binary with density and duration of grazing unaccounted. A rigorous study, with measured stocking density with different seasonal stocking regimes would better investigate this role in managing bog turtle populations. Additionally, as shown by Stratmann et al. (2020), some factors can be beneficial to abundance while having no perceived effect on occupancy, and these processes may be decoupled.

Management Implications

My study evaluated and the efficacy of Feaga 's (2010) habitat model. Using this model, I was able to identify 9 new sites in southwest Virginia. Hydrological modeling appears to be a beneficial addition to predicting new populations of bog turtles and I encourage managers to incorporate it into future models. The sites examined in this study will provide a robust dataset to help the construction of updated models as well, as more data will help with parameter estimation of species distribution models.

This study also provides the first quantitative assessment of probing surveys for *Glyptemys muhlenbergii* and provides several benchmarks for future management plans. I provide a model, based off only delimiting the wetland at a site, to determine the number of 4 person-hours/hectare effort surveys to reach a defined threshold of certainty before efforts are allocated to other possible sites. Unfortunately, my data limits my ability to comment on any other possible factors that could influence detection, and I encourage further studies to be done investigating these trends. A study focusing on sampling known populations over more conditions will have more power to examine these possible effects.

Finally, my study found evidence that impervious surfaces surrounding a wetland negatively impact the probability of occupancy at a site. For my observed range of values (not accounting for sampling effort) occupancy was predicted to be between 0.01 and 0.71, indicating that there may be more populations unknown on private property in less developed areas. My method of estimating effect of impervious surfaces on each site was simple but uses data that are readily available throughout the range of the bog turtle. Because of this apparent effect, conservation of this species will likely require plans that not only incorporate protecting the wetland a population inhabits, but also mitigating the effects of surrounding development. Several studies have started to investigate the effects of development on bog turtle populations, such as increased nest predator density due to subsidization from development (Holden 2021). More research into the effects of surrounding development on bog turtle populations, such as road mortality or hydrological degradation will give managers the information the required to mitigate these effects. In addition, it may allow for the development of more nuanced metrics further refining habitat models to predict new populations.

Tables

Table 1.1. Factors evaluated for their effect on occupancy and detection of bog turtles in southwest Virginia, with explanations and their hypothesized effect. Only grazing was predicted to affect both detection and occupancy, and only one interaction term was hypothesized between detection variables, grazing and calendar day. These factors were weighed by AICc in a model occupancy framework to determine their relative support for predicting presence/absence of a turtle population within a given predicted wetland.

Variable	Explanation	Hypothesized Effect
Occupancy		
Area of Predicted Patch	Larger areas of predicted wetland are more likely to contain more suitable habitat (Feaga 2010)	Larger predicted areas from the model will be more likely to be occupied
Area of Core Wetland Habitat	Core habitat of *G. muhlenbergii* is characterized by soft, borrowable mud (Chase et al. 1989, Carter et al. 1999)	The more core habitat a wetland has, the more likely it is to be occupied
Surrounding Impervious Cover	The more surrounding development in an area, the more ground-water hydrology is disrupted (O'Driscoll et al. 2010) and other sources of anthropogenic mortality may become more prevalent	Wetlands with more surrounding impervious cover will be less likely to be occupied
Adjacent Stream Incision	The more incised the adjacent stream is, the lower the surrounding water table (Schilling et al. 2004, Blann et al. 2009)	If the adjacent stream is more incised, then the surrounding wetland will be less likely to be occupied
Average Model Value	Predicted wetlands with a higher average value will have a higher wetness index, lower forest cover, and closer proximity to a stream (Feaga 2010)	If the wetland has a lower average value, it is less likely to be occupied
Presence of Grazing	Grazing prevents succession of fen habitat into closed canopy forest, supporting turtle persistence (Tesauro and Ehrenfeld 2007)	Grazed sites will be more likely to be occupied
Detection		
Ambient Air Temperature	*G. muhlenbergii* may estivate during high temperatures (R. B. Bury 1979)	Detection will decrease as temperature increase
Sky Conditions	*G. muhlenbergii* might be less active during rain or cloud cover	Detection will decrease as sky code increases
Time of Survey	*G. muhlenbergii* could become more active during the day to bask (Arndt 1977).	Detections will higher later in the day
Calendar Day	*G. muhlenbergii* detections have historically peaked in mid to late spring (Lovich et al. 1992). Vegetation becomes harder to search as the growing season continues	Detection will decrease as the sampling season continues from April to September
Presence of Grazing	Grazing lowers the density and height of wetland vegetation (Tesauro and Ehrenfeld 2007)	Grazed sites will have higher probability of detection
Grazing x Calendar Day	Because both grazing and calendar day are predicted to significantly impact vegetation, seasonal trends will	Seasonal effects will have less effect size on grazed sites

	differ between grazed and ungrazed sites	
Total wetland Habitat	Larger wetlands will fatigue surveyors and provide more refugia for turtles to avoid capture	The larger the extent of wetland habitat at a site, the lower my detection will be.

Table 1.2. Target number of sites per hydrologic unit code 12 (HUC12) watershed in my study area and the actual number of sites per HUC12 in 2019. I set a goal of 40 sites to visit, and calculated the goal number of sites by the relative area covered by each watershed in my study area. This goal was exceeded by 9 additional sites, however due to issues with landowner access 5 watershed units had less sites than the goal.

HUC12	Goal	Actual
A	2	4
B	2	2
C	3	2
D	4	9
E	6	9
F	6	8
G	6	5
H	3	2
I	3	5
J	3	2
K	0	1
L	2	0

Table 1.3. Summary statistics for variables that were measured in this study. Variables that were in the final model set are in **bold.** This data was used for an occupancy model predicting detection and presence/absence of G. muhlenbergii in southwest Virginia. Because some wetlands were spread over multiple landowners (and therefore made into separate sites), variables are denoted at the site, wetland, and survey level. For wetlands not split into multiple sites, wetland and site variables are interchangeable. An * indicates an outlier site that was surveyed before the habitat model was updated with current landcover data and was found not to be a discretely predicted patch in the updated model

	Variable	**Level**	**Mean**	**Min**	**Max**	**SD**
Detection	Calendar Day (1-365)	survey	164.45	109	264	30.22
	Grazing (0,1)	site	-	(22 sites without active grazing)	(31 sites with active grazing)	-
	Temperature (C)	survey	21.80	7.3	34.8	4.87
	Sky Code (1-7)	survey	2.5	1	6	1.32
	Start Time (Seconds from 12:00AM)	survey	43630.64	29700	60360	8244.74
	Total Habitat (m^2)	site	8489.10	97.76	41305.92	8660.95
Occupancy	Model Average (Percentile)	wetland	75.10	21*	81.97	8.40
	Area (m^2)	wetland	42.98	0*	222.4	49.57
	Proximity index	wetland	6.63	0.1025	77.40	12.72
	Soft Wetland (m^2)	site	2843.87	82.16	15240.32	3256.21
	Bank Ratio (Bank Height/Bankfull)	wetland	2.06	1	6.80	1.22
	Impervious Surface Index (Sum Percent/Area (km))	wetland	572.83	54.57	1649.40	414.61

Table 1.4. Model output of the generalized linear mixed model that examined the effects of calendar day and grazing on the average number of obstructed bands on a robel pole station. As the year progressed, more bands were obstructed by vegetation. Additionally, grazed sites on average had less obstruction, although the significance value was lower.

Fixed Effect	Estimate	SE	DF	t-value	p-value
Intercept	-9.81	1.85	222	-6.31	<0.0001
Presence of Grazing	-3.14	1.53	7	-2.05	0.0794
Calendar Day	0.14	0.009	222	14.53	<0.0001
Random Effect (N)	Variance	SD			
Site (9)	4.86	2.205			

Table 1.5. Table of the estimated betas for each parameter estimated in the bog turtle occupancy model for southwest Virginia on the logit scale with 95% confidence intervals denoted in parentheses. An impervious surface index, calculated by summing the percent value of pixels from the NLCD layer overlapping each site's catchment, was found to be significant for occupancy, while the total area of wetland habitat was found to be significant for detection.

	Varaible	**Unit**	**Beta**
State	Intercept (psi)		-0.69 (-1.98 - 0.061)
	Impervious Surface Index	Sum Percent/Area (km)	-0.0031 (-0.0063 - 0.000053)
Detection	Intercept (p)		-1.86 (-2.8 - -0.87)
	Total Wetland Habitat	(m^2)	0.000064 (0.00002 - 0.00011)

Figures

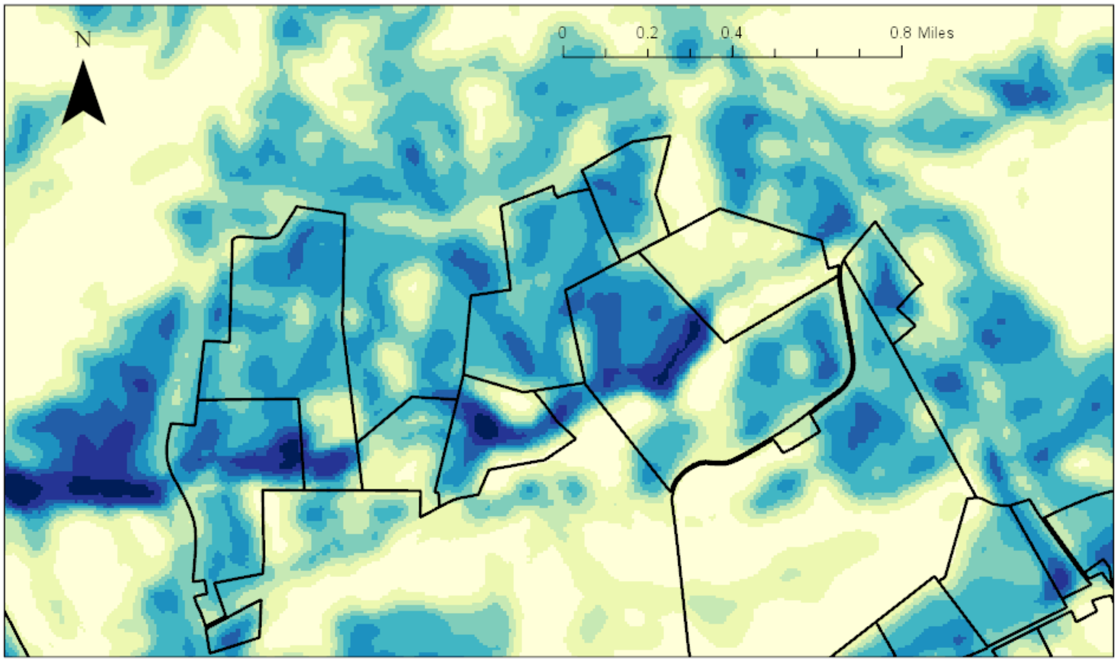

Figure 1.1. A small section of Feaga (2010)'s habitat model, with 10m x 10m pixels categorized by percentile of value output. The darker the pixel, the higher the value percentile of that pixel predicting habitat suitable for bog turtles in southwest Virginia. Pixels under the 70th percentile were then nullified and the remaining pixels were used as discrete patches for surveying. Dark lines represent land owner parcels that overlap with highly predicted habitat, showing the difficulty in obtaining access to an entire predicted area.

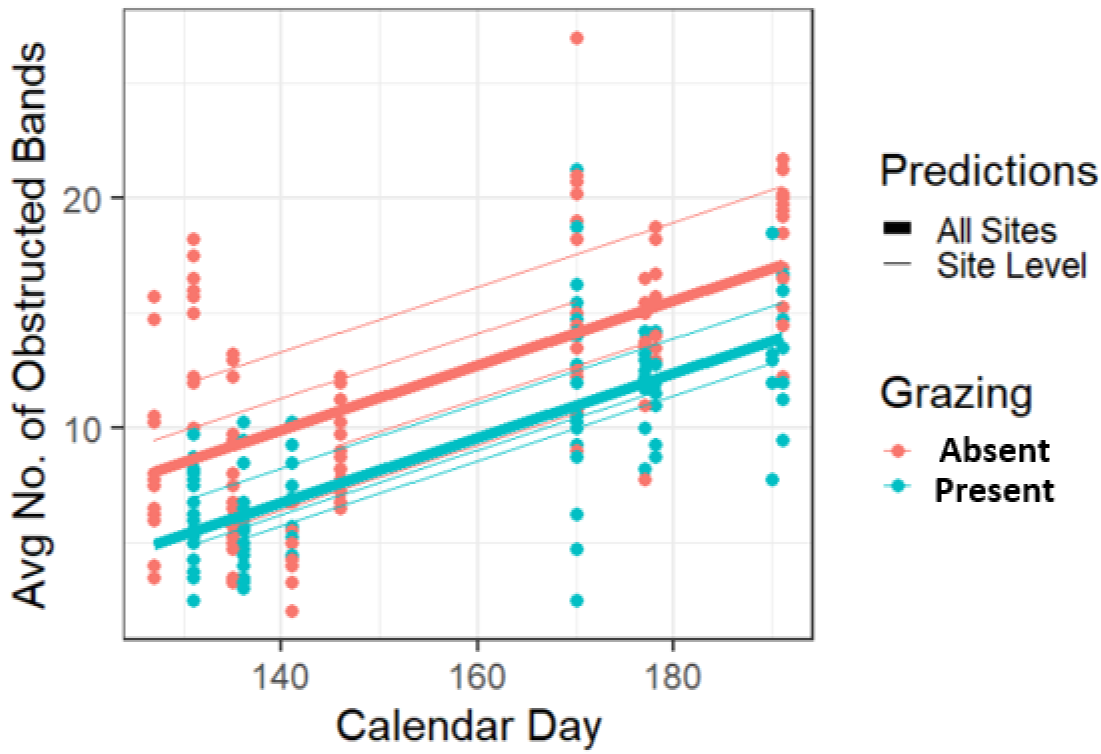

Figure 1.2. Output of the GLMM predicting Robel pole obstruction at 9 sites in southwest Virginia surveyed for bog turtles by calendar day and grazing. Data for this analysis were collected at all surveys conducted in 2020. On average, vegetation obstructed 1 more band of the Robel pole per week. Throughout the sampling season, grazed sites on average had 3.14 fewer obscured bands then sites without active grazing. No interaction term between the two factors was supported by BIC. In this model, calendar day was found to be significant to an α of 0.05 and grazing was found to be significant to an α of 0.1.

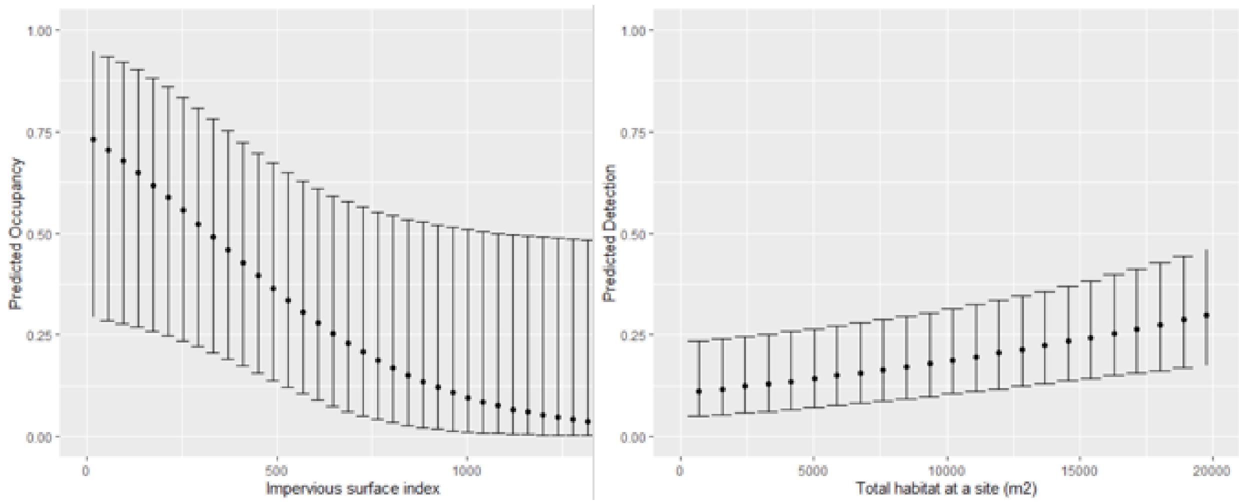

Figure 1.3. Predicted occupancy (left) and predicted detection (right) for bog turtles in southwest Virginia over the significant factors found in this study. An impervious surface index calculated by summing the percent impervious of each pixel within a wetland's catchment was found to negatively impact occupancy. For detection, the total area of wetland habitat at a site was found to be positively correlated with detection. The 95% confidence intervals are also shown, showing greater uncertainty of occupancy values relative to detection values. Both factors were plotted to two standard deviations above and below the mean value encountered in this study.

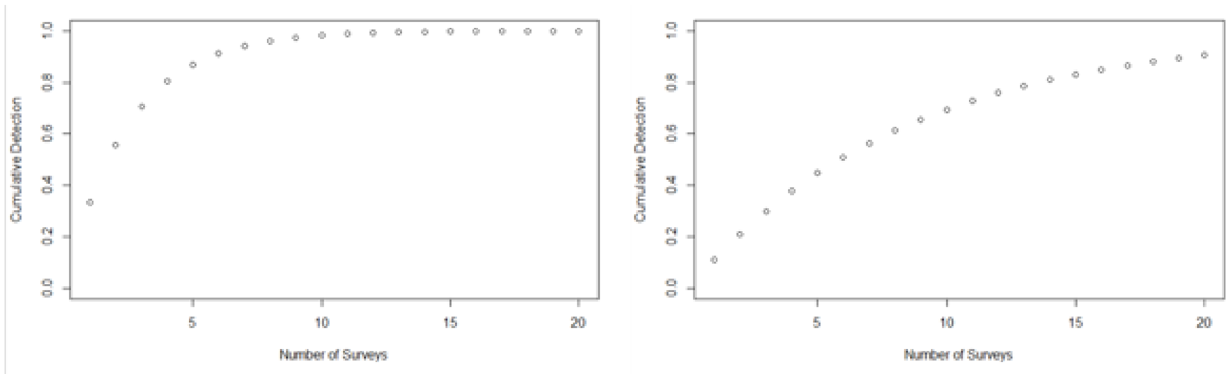

Figure 1.4. Cumulative detection probability for bog turtles over a number of surveys for two theoretical sites. As total wetland size was found to be positively correlated with detection, these two sites are equivalent save for their total wetland size. The panel to the left represents a site with a total wetland size that is one standard deviation above the mean wetland size in this study (11276m^2) and the panel on the right is a site that is one standard deviation below (3363m^2). It takes many more surveys to achieve a high cumulative detection for a smaller site than a larger site.

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Chapter 2: Co-occurrence of *Glyptemys muhlenbergii* with locally rare plant species: implications for future research and management

Abstract

Community composition can be a helpful tool for assessing quality at a site, especially when the species are rare and specialized to a certain set of conditions. Appalachian Mountain fens, the habitat of the bog turtle, *G. muhlenbergii*, are known to contain a large number of rare, often disjunct plant species specialized to open canopies and high water tables. It has been hypothesized that a high diversity of specialist plant species would indicate high quality habitat for bog turtles. The objectives of this study were to determine if turtle-occupied wetlands had distinctive communities, and separately, if pre-determined indicator plants were likely to co-occur with turtles. In this study, I first *a priori* determined a list of 30 'pristine indicator' species, building upon a previously published list and adding to it with expert opinion and a literature search. I then did a complete floral inventory at 12 sites, 6 with bog turtle presence and 6 without. After inventorying each site, I compared site wide species lists using a Jaccard index and tested for differences in turtle occupied sites using a PERMANOVA. Next, I narrowed my focus to *a priori* determined indicator species found at 2 or more sites and used a C-score test of co-occurrence to determine if these species were more likely to inhabit the same sites. I found high beta diversity at my sites, with 106 of 190 species found at only 1 or 2 sites. Because of this high beta diversity, my PERMANOVA test did not find significant differences in community composition between turtle occupied and unoccupied sites. From all surveys, 11 *a priori* pristine indicator species were found at more than one site. Despite the lack of overall difference between sites, the C-score analysis found that these 11 pristine indicators and *G. muhlenbergii* were likely to co-occur with each other on the landscape ($p\text{-value} < 0.001$). Using previously collected hydrology data at 3 of 12 sites, I find some evidence of a relationship between average depth to water table and 'pristine indicator' richness, but detailed studies are needed to explore the mechanisms that lead to the co-occurrence of rare species at the same sites. Despite this research need, this study has immediate application for the evaluation of sites for *G. muhlenbergii* presence using indicator species.

Introduction

Once thought to be a transitionary artifact of forest succession, montane grasslands are receiving attention as ongoing research suggests these habitats were once much more widespread and persistent on the landscape than previously thought (Weigl and Knowles 2014). One leading theory suggests a climate-herbivore hypothesis maintaining these habitats, where glacial disturbance opened areas of canopy to be colonized by grassland species that were maintained by grazing from megafauna for thousands of years (Weigl and Knowles 2014). One line of evidence suggesting this old origin of montane grasslands is the current distribution of relict or disjunct plant populations (Weakley and Schafale 1994). Given that smaller populations are more vulnerable to local extinction from stochastic processes (Hanski and Gaggiotti 2004, Lande et al. 2010), extant populations of these species suggest these habitats have been persistent and widespread enough to maintain them and in some cases speciate from their northern sister taxa.

Appalachian mountain fens are similar to other montane grasslands, in that they are open canopy habitats dominated by graminoid species (Weakley and Schafale 1994). Fen communities, in addition to an open canopy, require specific groundwater conditions to maintain the surface level water table on which many specialists rely (Bedford and Godwin 2003). This elevated water table creates a unique soil condition of low nutrient content and high volumetric water content (Bedford and Godwin 2003). Although individual sites can be small, often a hectare or less, they are found to support a rich diversity of species including many species found in northern latitudes where fens and bogs are more widespread (Weakley and Schafale 1994, Murdock 1999). Following the 'climate-herbivore' hypothesis above, a cooler climate would have prevented the establishment of canopy at many modern fens, perhaps aided by beavers (Wells et al. 2000, Sirois et al. 2014), populations of which have been increasing after a brief extirpation in the early 20th century (Jenkins and Busher 1979, Naiman et al. 1988). This open canopy was then maintained by grazing ungulates and the elevated water table at the site, preserving the community that colonized the area for long periods of time (Warren et al. 2004, Weigl and Knowles 2014).

Today, although many fens occur within active pasture where continual grazing by livestock may prevent succession, fen habitat is in decline due to hydrological disturbance (Moorhead and Rossell 1998, Bedford and Godwin 2003). On many properties, wetlands are ditched, lowering the water table and in turn lowering the persistence of fen specialist plants in favor of more generalist wetland species (Murdock 1999). Even as sites are restored through intentional management or as ditches become filled in with sediment, the increasing isolation of intact sites may lead to new sites not reaching their peak diversity for long periods of time due to lack of surrounding populations to colonize them (Nekola 1999). Such a ‘lag time’ where younger sites do not have all suitable species has been documented in many studies, and specifically on grasslands in other parts of the world (Lindborg and Eriksson 2004, Hájek et al. 2011).

In 1997, managers in southwest Virginia determined a list of ‘pristine indicator’ plant species for use in evaluating potential bog turtle sites, citing their indication of the “historical regime” of the wetland (Carter, Unpublished Report). Given the rarity of these species on the landscape, the driving hypothesis in this distinction was that sites that support these species are less impacted by disturbance relative to other sites, as any major disturbance would extirpate these species. *G. muhlenbergii* shares the habitat requirements of many of these species, requiring both the soft substrates caused by groundwater seepage to burrow and an open canopy to bask and incubate eggs. Additionally, bog turtles have a longer generation time relative to other vertebrates on the landscape, an attribute thought to lower colonization potential (Ebenhard 1991). Therefore, a high diversity of fen specialists at a site may not only indicate a site with suitable conditions but one that has maintained those conditions long enough to support a population of turtles.

This study investigates the use of pristine indicator species as a possible management tool for evaluating potential bog turtle sites. In addition, these rare plant communities may justify conservation for their own sake, so there is value in better documenting the distribution and occurrence of these species in southwest Virginia. I conducted a floristic inventory of 12 sites, 6 with confirmed turtle presence and 6 without capture, to determine if floristic composition of sites differs between them. I first examined the entire community using a multivariate approach, then I narrowed my focus to specialist fen plants. I predicted that turtle-occupied fens will not vary significantly from other possible sites, due to the large number of generalist species, but that specialist species will co-occur at the same sites as intact turtle populations, indicating specific conditions for groundwater specialists and a lower degree of past disturbance.

Methods

Study Area

This study was conducted in Floyd County, Virginia, after a county wide search for *G. muhlenbergii* was completed and 49 wetlands were mapped using a GPS with decimeter accuracy. From all the sites visited in that study, I selected a subset of sites based on certain criteria. First, sites had to have had a minimum of 600m^2 of herbaceous habitat that appeared to be perennially groundwater influenced, identified as areas where substrate was penetrable without the use of equipment at least 10 cm into the ground. This minimum area was established as a natural break in my data. Sites also had to have at least 0.5 ha of habitat dominated by hydrophilic herbaceous vegetation, regardless of soil condition. Finally, I only selected sites where grazing livestock did not appear to graze in the wetland a considerable amount. Although large herbivores were native to this system and likely maintain the open canopy, cropping of flowering structures through grazing affected my ability to reliably identify plants. From this subset of sites, I selected 3 sites of high observed diversity to better train observers on plant species identification and detection. The remaining 9 were randomly selected using a random number generator until a total of 6 sites with *G. muhlenbergii* presence and 6 without *G. muhlenbergii* presence were selected. Of these 12 sites, I purposely selected 3 from a previous subset of sites studied for their hydrology from Feaga (2010) to investigate patterns of hydrology and species richness.

Field Methods

Sampling took place in July 2019. This sampling period was chosen based on herbarium specimens previously collected from the area that indicated that July was peak bloom for many wetland species. Species that are actively blooming are both easier to detect and identify in the field. Plant species of interest were selected based on meeting both of the following criteria: (1) Species that require constant, flowing groundwater OR hydrophilic species that require a completely open canopy and (2) locally infrequent or rare. Although I chose some species *a priori* based on original bog turtle site quality assessments (Carter 2000), due to low occurrence of my initially selected species, the list was expanded using expert opinion in the field during initial surveys. Plant species identified, as well as local distribution, wetland status and how they were selected, are outlined in Table 2.1. Prior to sampling efforts, surveyors were trained on identification of wetland plant species using specimens from the Massey Herbarium at Virginia

Tech. A written test on plant identification was administered to qualify surveyors for the project using photos and herbarium specimens.

Sites were sampled in a “timed meander’ (Goff et al. 1982). Surveyors worked independently under a set window of time, scaled to at least 2.25 person hours per hectare of herbaceous habitat at the site. This specific effort value was determined using the most species-rich of the 12 sites, surveyed early in the study. At one specific site, a calculation error resulted in a site surveyed for only 1.25 person hours per ha per survey. Although this may have resulted in an under-representation of species at that site, the observation that no new species were recorded in the last 15 minutes suggests that I had already reached an asymptote in the species accumulation curve. Surveyors would identify plants when they were encountered in the field, and if permitting allowed, would collect a sample of the plant for confirmation of the ID at the Virginia Tech Massey Herbarium, where specimens were also stored. Some plants were identified to variety if this variety was notable and some plants were only identified to genera due to difficulties in identifying to species level. Varieties were included in the subsequent analyses, but genera were omitted, although they are reported in Appendix B. Due to the high diversity of plant species at these sites, collection of plants was limited to species of high interest. Where collection was not allowed, surveyors took notes and enough pictures for a confident ID of the specimen later in the lab.

Analysis

Two datasets were generated from my field data. One was a complete species list for each site and the other a subset of this list only including species *a priori* determined to be “pristine indicators” that were found at more than one site. I used a checkerboard analysis from Stone and Roberts (1990) to examine patterns of co-occurrence through C-scores for the second dataset.

This analysis was done using R package *EcoSimR* (Gotelli and Ellison 2013). This analysis has been shown to be robust to type 1 error, especially when the correct null hypothesis algorithm is used (Gotelli 2000). This analysis compares observed distribution to 1000 randomly generated null matrices. In my null matrices, total occurrence for each species was kept constant, but each site had an equal chance of being occupied by any plant species. A C-score is then calculated from each null, generating a normal distribution to which the observed C-score can be compared. A C-score far from the mean of the null distribution implies a pattern in species co-occurrence for the study community.

For the former dataset comparing entire floral communities between wetlands, a hierarchical cluster analysis using Jaccard dissimilarity measures was used to visualize differences between turtle occupied and unoccupied sites (Jaccard 1908). Presence/absence data from my plant species list was used to construct a species-by-site matrix. From this matrix, distance metrics were calculated and displayed using both a dendrogram and an ordination plot to show the relationship between sites (Quinn and Keough 2002). I chose the Jaccard to account for high expected beta diversity between sites (Walbridge 1994). In the Jaccard metric, shared zero values, meaning shared absences across sites, do not contribute to increased similarity between sites (Choi et al. 2009). Additionally, Jaccard metrics are unweighted, and I did not have abundance data to weight my species. To test for significant differences in plant communities in bog turtle occupied sites and unoccupied sites, a PERMANOVA test was conducted. A significant p-value from this test would give evidence that occupied sites differ in floral community structure from unoccupied sites (Anderson 2017).

Results

Over this study, surveyors detected and identified 190 species of plants (including different varieties but excluding genera-only identifications) across all sites. Alpha diversity of sites ranged from 51 species to 100 species, with a mean species diversity of 70.5. While some species were common across all sites, the majority of species were found either at just one site or several sites (Figure 2.1). My final list of 'pristine indicator' species (from earlier reports and publications and from in-field observations) totaled 30 species. Of these, 18 were found at least once and 11 were found multiple times, allowing their distributions to be compared across occupied and unoccupied sites.

The C-score analysis, taking all plant species of interest found at multiple sites as well as the bog turtle *G. muhlenbergii*, found that my observed distribution had higher co-occurrence than all 1000 simulations (analogous to a p-value of 0.001) (Figures 2.2 and 2.3), indicating high co-occurrence for this identified community. Another interesting finding was that pristine indicator species only found once (and therefore not used in this analysis) were all found at sites containing bog turtles.

An ordination analysis done on the Jaccard similarities of species composition between sites did not clearly define a pattern in species composition (Figures 2.4 and 2.5). All sites showed appeared equally dissimilar for one another and no clear grouping appeared between turtle occupied and absent sites. The NDMS analysis showed a stress value of 0.17, which is considered fair support that the ordination accurately reflects the differences in sites. Using turtle presence as a grouping variable, the PERMANOVA test for significant difference of species composition between occupied and unoccupied sites returned a p-value of 0.64, indicating I do not have enough evidence to show that occupied sites differ in species composition from unoccupied sites when the entire community is considered.

For 3 of my 12 sites, a previous hydrological study was done recording average depth to groundwater in 2007 through 2008 (Feaga et al. 2012). Two of these sites, SK and CG, have active bog turtle populations while EPC lacks one. Although I lack the sample size to adequately compare sites by their hydrology, it appeared that the site with the highest water table, SK, also had the highest number of indicator species, while CG, the lowest water table, had the least number of indicator species (Table 2.2). The total number of species appeared to be highest at EPC, with 23 more species then CG, which had the lowest number of species.

Discussion

Although the rare disjunct floral communities of montane grasslands and fens are well documented in the literature (Weakley and Schafale 1994, Murdock 1999), published species lists in the literature for fen sites are limited to public land (Walbridge 1994, Rossell and Wells 1999). Data from local private land surveys is focused on the species level, publishing locations and patterns of species occurrences rather than species lists for specific sites (see Wieboldt 1998). This study provides a landscape look into the community assemblage of plants among this habitat on private land, from which inference about distribution patterns can be examined. My sites, despite evidence of past alteration, still are inhabited by a diverse community of species. I found an average of 70.5 species per site, which is similar to surveys done in the West Virginia Plateau (Walbridge 1994), but less than a floristic study of sites in New York (Hajek 2014).

Analyzing differences between site communities, no clear distinction could be made between sites with bog turtles and those without. My ordination from the Jaccard dissimilarity scores identified some sites with relatively unique communities but many sites were similar to each other regardless of turtle presence. In mountain fens, beta diversity is often high, and species richness at a fen is influenced by many factors not covered in this study such as soil pH, microtopography and surrounding land use (Walbridge 1994, Hajek 2014). While I expected a high level of beta diversity, the number of species only appearing once in my study highlights the limitation of my analysis, as the Jaccard index does not examine shared absences for similarity. Complementing my site wide survey with transect methods to obtain estimates of density or abundance could better examine the drivers of diversity in fen wetlands (see Hajek 2014 as an example). A majority of the species I found in this study I only observed once. Although I did not examine every species' habitat requirements and local distribution, I hypothesize that many of these species are opportunistic colonizers of fens more common on the landscape, as my literature search would have uncovered them as possible indicator candidates. When I narrowed my investigation to plant species that were determined *a priori* to be rare fen habitat specialists however, a pattern of co-occurrence with bog turtles did emerge despite lack of difference in the community-wide scale.

My *a priori* determined indicator species share many important attributes, such as stringent groundwater and open canopy requirements and are thought to have a relatively small dispersal range compared to other more generalist wetland species (Hájek et al. 2011). My C-score analysis showed that these species were highly likely to cooccur on the landscape. This high co-occurrence suggests that sites with these species have favorable habitats to support a groundwater-dependent community, and their relative rarity on the landscape suggests that these are relict populations of previously wider distribution, rather than a recent colonization from an outside source. Although my subset of 3 sites with hydrological data support the claim that a higher water table supports a higher diversity of these species, I lack the spatial detail and sample size to disentangle the relative importance of habitat suitability from historical regime, as my habitat delineation approach was shown to exaggerate the amount of perennially groundwater-influenced habitat available at a site (Chapter 1), and it is likely that both play a role. In one study of fen plant dispersal, it was suggested that both dispersal vectors as well as optimal germination conditions are limiting to the distribution of these plants (Middleton et al. 2006). Still, the use of these species as indicators for groundwater-fed habitat favorable for *G. muhlenbergii* is promising.

Although not ubiquitous, most surveyed sites had some level of apparent hydrological alteration such as ditching or stream straightening. These actions, often to purposely dry wetlands, can lower the local water table and as a result make a wetland unsuitable for groundwater specialists (Moorhead and Rossell 1998). The effects of this alteration are not uniform. Some sites contract greatly, becoming an inundated ditch with little microtopography and habitat heterogeneity. Some sites slowly recover over time due to siltation of the ditches causing the water table to slowly rise and finally some sites only drain partially, allowing a marginal amount of perennially inundated groundwater habitat to endure (Tom Wieboldt, retired curator of Massey Herbarium, Virginia Tech, Personal Correspondence). This reliance on groundwater distinguishes fens from other montane grasslands which also likely benefit from the open canopy habitat that is maintained by livestock grazing. As more management action is taken to preserve the unique communities of montane grasslands, hydrology should be of special consideration for habitats identified as fens. While an open canopy wetland can support a wide diversity of species, groundwater specialists, often of highest conservation concern (Murdock 1999), need specific hydrological conditions to persist.

Examining the occurrence of a rare species with the community it occupies been done with other species, notably the Poweshiek skipperling in Michigan (Pogue et al. 2019). Many of the plants that were found to co-occur in their analysis directly related to the nesting behavior of the skipperling. Here, I do not make direct claims that my 'pristine indicator' plants directly contribute to the life history of bog turtle, rather I emphasize the sharing of a specialized niche space between these species. Notably, Pogue et al. (2019) also highlights the need for a more robust plant database for the state of Virginia. A database of the native status of encountered species, as well as a coefficient of conservation would be a helpful tool in further examining the patterns I found in this study. Conservation of these imperiled habitats will require multidisciplinary researchers and agencies sharing information to better examine the drivers of diversity for fens.

Management Implications

The co-occurrence of bog turtles with other fen specialist plants has several broad implications for both conservation of turtles and of Appalachian fens. For turtles specifically, I supported a prior hypothesis by previous managers that 'pristine indicator' plant species may be a good metric for quality of an evaluated site. Because these species co-occur and share habitat requirements, finding more of these species at a site could indicate a turtle population is likely to be present, and that the site may be better able to sustain the groundwater needed for their long-term survival. I may also start to think of bog turtles as a candidate for an 'umbrella species.' If more sites are conserved for the purpose of sustaining a population of turtles, this management action is also likely to support populations of these rare plants, which may later be identified as conservation priorities. Conservation actions to improve fen quality may help bog turtles, plants, and other associated species such as the Mitchell's satyr (U.S. Fish and Wildlife Service 2019).

A long term study following hydrological restoration to increase turtle abundance is yet to be published, but studies show that restoration returns plant communities to those adapted for elevated groundwater (Patterson and Cooper 2007). Species specific responses will likely depend on the seed bank at the site however, given the low dispersal capacity of fen species (Rossell and Wells 1999, Haapalehto et al. 2011).

I recommend that managers surveying for bog turtles be educated on these plants and document their presence when found (Table 1). With the exception of several graminoid species, the species identified to co-occur with bog turtles in this study are relatively easy to identify. Many management agencies acknowledge the need for conservation of imperiled mountain fen habitat and cite both bog turtles and several of these plants as reasons (see The Nature Conservancy n.d.). Enacting conservation not only at the species level but the community level could allow for more resources and agencies to work together to preserve this rare ecosystem into the future.

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Tables

Table 2.1. Plants identified as rare habitat specialists in this study, with distribution in the Virginia mountains based on Virginia Botanical Associates (2019). Plant species were chosen based on the criteria that they (1) were specific either to groundwater or sunlight conditions similar to *G. muhlenbergii* and (2) were locally infrequent or rare in my study area. Many of these species represent disjunct populations of more widespread species, but some are endemic to montane fens. Wetland status from U.S. Army Corps of Engineers (2018). Not all of the species below were confirmed to occur in any of the 12 Appalachian fens that I surveyed in Floyd County, Virginia.

Scientific Name	Source	Local Distribution	Wetland Status
Arethusa bulbosa	Carter, Unpublished Report	Rare	OBL
Bartonia virginica	Carter, Unpublished Report	Infrequent	FACW
Calopogon tuberosus	Literature Review	Rare	FACW
Carex atlantica	Expert Opinion During Initial Surveys	Frequent to Common	FACW
Carex leptalea	Literature Review	Not documented	OBL
Chelone cuthbertii	Literature Review	Rare	OBL
Dalibarda repens	Carter, Unpublished Report	No records in VA	FAC
Dichanthelium lucidum	Expert Opinion During Initial Surveys	Rare	No data
Drosera rotundifolia	Carter, Unpublished Report	Infrequent	OBL
Dryopteris cristata	Literature Review	Infrequent	FACW
Epilobium leptophyllum	Carter, Unpublished Report	Rare	OBL
Eriophorum virginicum	Carter, Unpublished Report	Infrequent and Local	OBL
Glyceria striata	Expert Opinion During Initial Surveys	Common	OBL
Helonias bullata	Carter, Unpublished Report	Rare	OBL
Hypericum canadense	Carter, Unpublished Report	Infrequent	FACW

Juncus dudleyi	Expert Opinion During Initial Surveys	Frequent	FACW
Lilium grayi[1] *(inc. var. pseudograyi)*	Carter, Unpublished Report	Rare	FACU
Melanthium virginicum	Expert Opinion During Initial Surveys	Infrequent	FACW
Platanthera flava var. herbiola	Expert Opinion During Initial Surveys	Infrequent	FACW
Polygala cruciata	Expert Opinion During Initial Surveys	Disjunct and Isolated	FACW
Polygala sanguinea	Expert Opinion During Initial Surveys	Frequent	FAC
Rhexia virginica	Carter, Unpublished Report	Infrequent	OBL
Sarracenia jonesii	Carter, Unpublished Report	No Records in VA	OBL
Solidago patula	Carter, Unpublished Report	Infrequent to locally common	OBL
Thelypteris palustris	Expert Opinion During Initial Surveys	Frequent to locally common	FACW
Thelypteris simulata	Carter, Unpublished Report	Disjunct only to Giles Co.	FACW
Vaccinium macrocarpon	Carter, Unpublished Report	Rare and Scattered	OBL
Veronicastrum virginicum	Expert Opinion During Initial Surveys	Infrequent	FACU
Viola primulifolia	Expert Opinion During Initial Surveys	Infrequent	FAC
Xyris torta	Carter, Unpublished Report	Infrequent and local	OBL

Table 2.2. Comparison of three wetland sites with historic well data used in Feaga et al. (2013). The total number of species is recorded, as well as the number and species of a priori defined pristine indicators. Site SK had the highest watertable relative to the other two sites and also had the highest number of pristine indicator species, as well as a population of bog turtles. The highest number of species overall was site EPC, a site without turtles and with a lower water table than SK. Due to low sample size, no statistical tests were conducted.

Site	Well Average (cm)	Turtles Present	Number of plant species	Number of 'pristine indicator' Species	Pristine Species List
CG	-12.16	Y	59	4	Carex atlantica, Solidago patula, Xyris torta, Hypericum canadense
SK	-9.218	Y	75	6	Carex atlantica, Dryopteris Cristata, Polygala sanguinea, Solidago patula, Thelypteris palustris, Epilobium leptophyllum
EPC	-11.38	N	82	5	Carex atlantica, Carex leptalea, Dryopteris cristata, Solidago patula, Thelypteris palustris

Figures

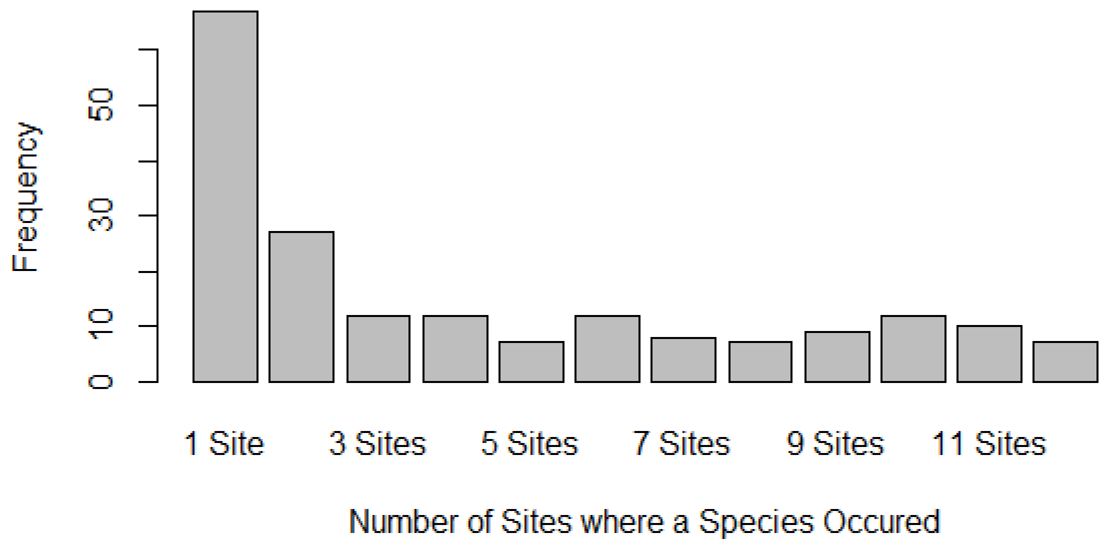

Figure 2.1. Barchart showing the number of site occurrences for every given plant species in Appalachian mountain fens in Floyd County, Virginia. Many species only occurred at one site, although a few were present at all or a majority of sites. Because of the large number of species only found on a minority of sites in this study, beta diversity was high between sites.

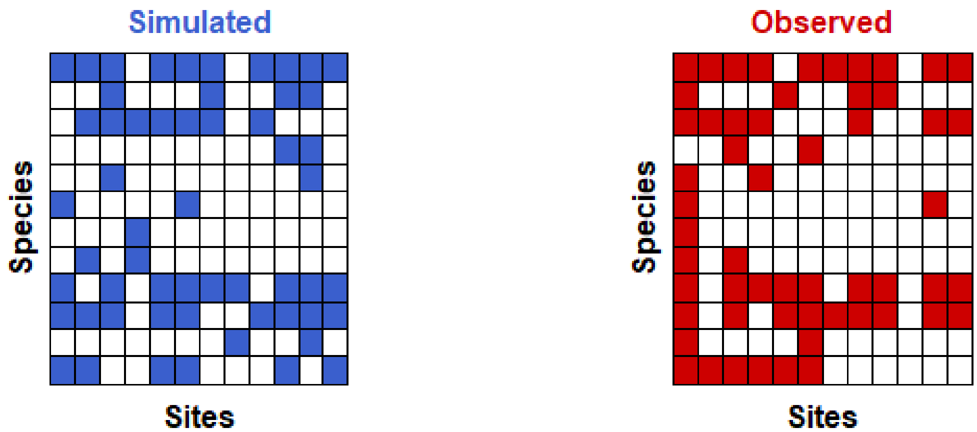

Figure 2.2. Matrices for 11 species of specialist plants and *G. muhlenbergii*. The simulated matrix represents an example matrix generated from the C-score analysis, where numbers of occurrence were kept constant for each species but each site had an equal probability of being occupied. An additional 999 matrices were generated in this manner and the corresponding C-score was calculated, generating a normal distribution. The observed matrix (right) was then compared to this distribution.

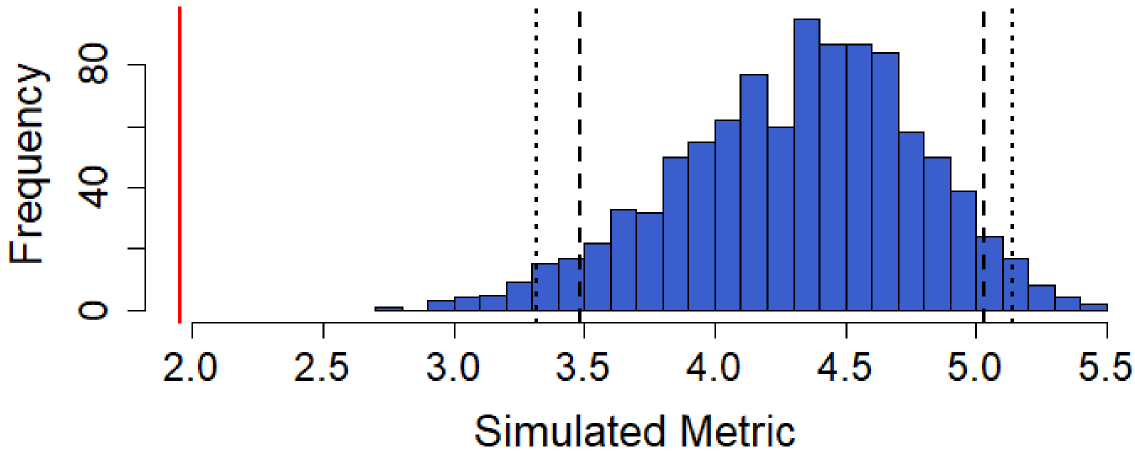

Figure 2.3. Histogram of calculated C-scores for 1000 simulated distributions of the 10 fen-specialist plants and bog turtle community data from southwest Virginia fens. In each of these simulations, numbers of occurrence were kept constant for each species but each site had an equal probability of being occupied. The lower the C score, the more the pattern simulated/observed favors species to overlap with eachother. The red line represents the C-score from the observed distribution, which was lower than all 1000 simulations. Dotted lines represent the 95% and 90% confidence interval from the average simulated C-score.

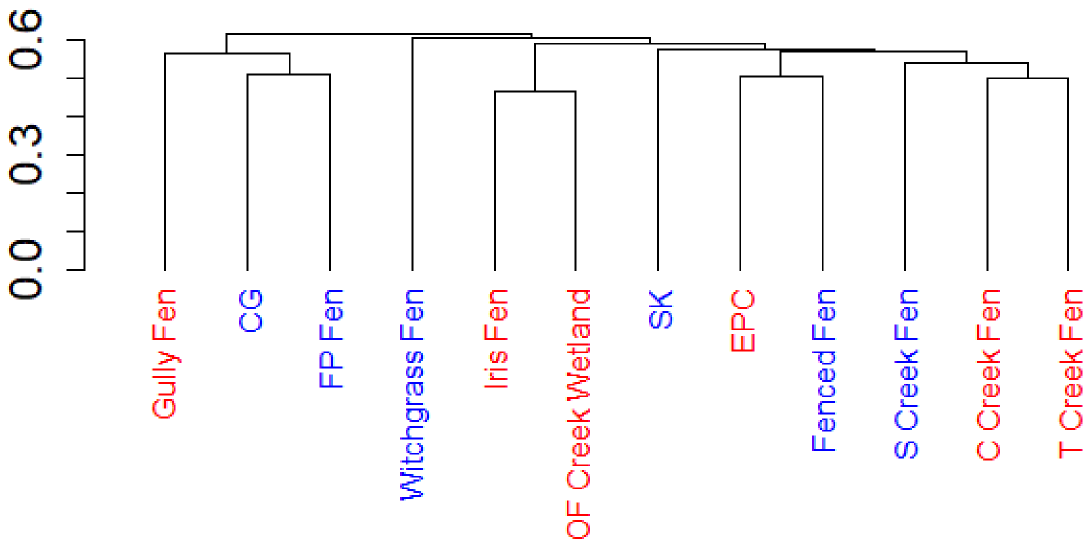

Figure 2.4. Dendrogram comparing similarity of species composition between 12 Appalachian fen sites in Floyd County, Virginia. Sites in blue represent sites with a known bog turtle population and sites in red are sites without a bog turtle detection. The highest dissimilarity between sites is approximately 0.6, with some sites clustered at a dissimilarity of 0.4.

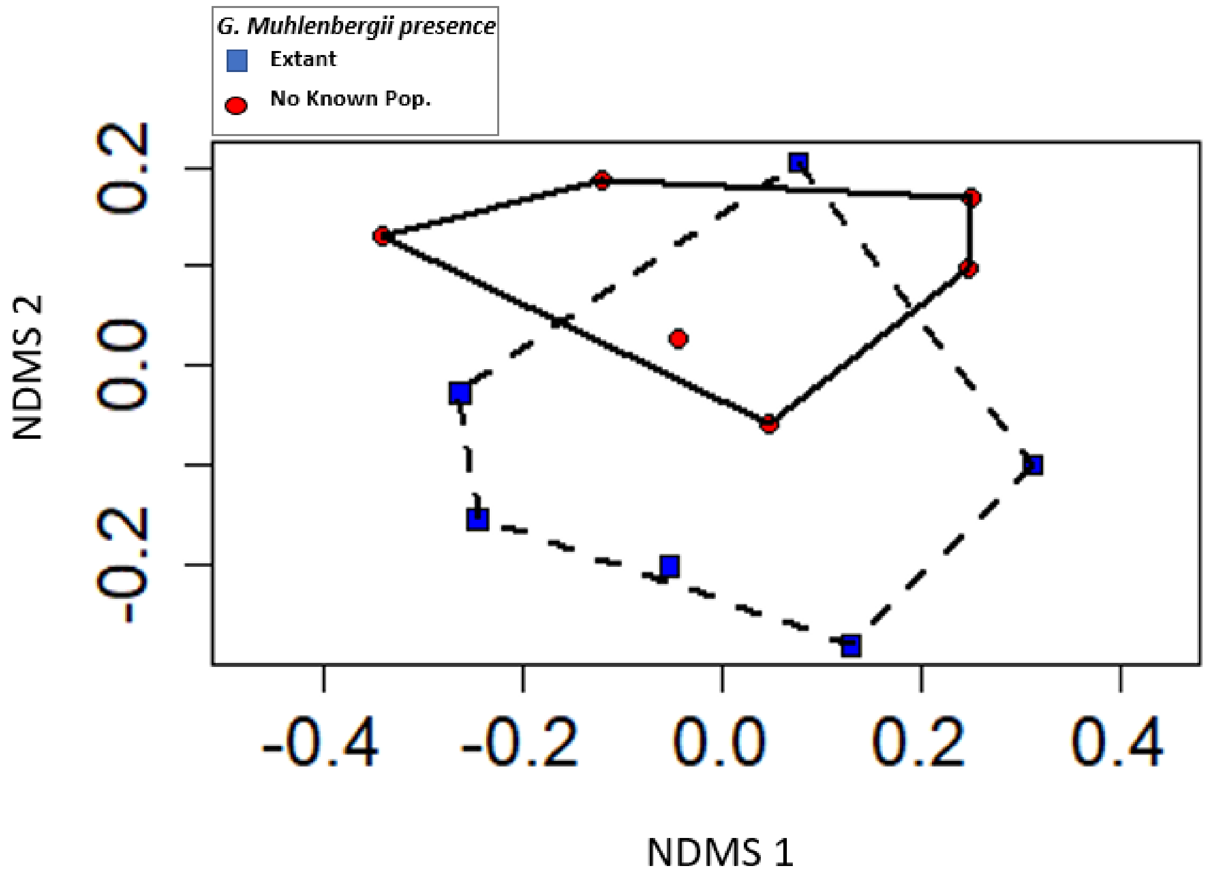

Figure 2.5. NDMS Ordination of plant communities in 12 Appalachian fen sites in Floyd County, Virginia, representing where defined groups are laid out in ordination space. Blue squares represent sites with an extant population of *G. muhlenbergii*, and red circles are sites with no known population. There two groups show much overlap, and a PERMANOVA analysis showed no significant different between sites.

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Chapter 3: Examining patterns of inter-wetland movement over 32 years of monitoring, and the evaluation of autocorrelation as a tool to assess connectivity between wetlands

Abstract

For long lived species, certain events in their life history may happen at a rate not adequately captured by short term studies. Although many radio telemetry studies exist examining the bog turtle (*Glyptemys muhlenbergii)*, long distance movements between discrete wetlands (here referred to as sites) are rarely documented. A long term study can potentially capture more of these events and provide insight into patterns and potential limiting factors. In this study, I examine 32 years of *G. muhlenbergii* mark recapture data, supplemented with my own trapping, from 1988 to 2020, and report on movements observed. Across this timespan, 21 marked turtles moved between 17 wetland sites, accounting for 6% of the turtle population marked at these sites. No significant difference was seen between sexes for average distance traveled in these events, but two long distance movements (6208 and 8999 m) exceed the longest movement in other states within the species range. To examine potential barriers to movement, I constructed least-cost pathways for 17 movements and recorded barriers crossed by these pathways. Of the 17 movements evaluated, 13 crossed either a rural unnamed road or a paved road with light traffic, indicating a prevalence of anthropogenic barriers to dispersal between wetlands. I also assess the utility of Moran's Eigenvector mapping, which only requires presence absence data, for establishing connectivity between wetland sites using data from a regional search for new populations. Although this approach has potential and can be used in conjunction with habitat and resistance models, current approaches for finding new sites do not provide a sampling scheme regular enough for this method to be applied and interpretable. A combination of continued monitoring, collection of genetic data from more sites, and a directed study examining autocorrelation will all be needed to better understand connectivity and geneflow between wetland sites for this species.

Introduction

Management for a species requires knowledge of its ecology, from its habitat preferences to reproductive strategy. Each of these life history traits informs consideration for management plans tailored to conserve the species into the future. Distribution and abundance are often used as goals for successful management of a species, but broadening the scale to a regional management approach requires knowledge of the species' ability to move between wetlands and the network populations establish on the landscape (Allen and Singh 2016). Among life history traits, movement range and dispersal capacity remain data deficient for many species. This is especially true for reptiles for which there are fewer studies on movement relative to other major taxa (Bowne and Bowers 2004). For many freshwater turtles, the wetlands to which they are tied are naturally patchily-distributed on the landscape, and movement between them must occur for colonization and genetic exchange to occur following classical metapopulation dynamics (Hanski 1998). The rate of exchange of individuals between discrete patches however, is a continuum, and not all movements between patches fit within the classic metapopulation model. For example, if resources are close enough that individuals can find and exploit them during routine foraging behavior, individuals may move fluidly between patches in search of resources, forming one 'patchy' population (Bowne and Bowers 2004, Baguette and Van Dyck 2007). By contrast, if resources were spread far apart on the landscape, movement between patches may only occur under special behavior (Van Dyck and Baguette 2005). For *Glyptemys muhlenbergii*, a freshwater turtle specialized to mountain fens in Virginia, lack of movement data among wetlands has limited the ability to define a population, understand habitat use, and to study potential drivers of long distance movement. By collecting data on known movement events, patterns can be assessed. A better understanding of movements would allow managers to both differentiate populations as well as plan a regional management strategy targeted at maintaining population viability at multiple sites.

G. muhlenbergii has been studied using radio transmitters, useful to track daily movements and estimate home ranges (e.g. Carter et al. 2000, Pittman and Dorcas 2009, Feaga 2010). While these studies examine spatial use over relatively short time scales and distances, documentation of long, straightline movements have been infrequent, usually a single individual during the study. A possible explanation for the lack of these records is that bog turtles are a long lived species, and even if an individual was likely to make one of these movements over its lifetime, a short term study may not capture it. Low detection also complicated obtaining movement data, as considerable effort is needed to find individuals that have moved to other sites (Holden 2021). Genetic analysis may circumvent these limitations, although other issues present. One study was able to identify 9 first generation migrants and estimate effective dispersal rates of 0.33 individuals per year for a cluster of sites (Shoemaker and Gibbs 2013*b*). This rate, however, reflects effective dispersal (successful breeding at a new site) rather than all movement rates. If turtles use multiple wetlands over the course of their lives, but do not breed at those sites, this genetic data might underestimate site exchange of individuals for other resources, such as hibernacula, which would imply a much more interconnected set of patches then genetic data would divulge. Additionally, in a species with a long generation time like *G. muhlenbergii*, genetic data can lag behind changes to the landscape that might affect current connectivity and exchange (Epps and Keyghobadi 2015). Although time intensive, complementing genetic data with mark recapture data has the potential to record movements before their genetic markers are seen in the population, and data can be collected during monitoring programs already in place to study population demographics.

Lack of inter-wetland exchange data has thus far limited the use of movement models for evaluating connectivity in populations of bog turtles. Travis et al. (2018) constructed a model of resistance to bog turtle movement in New York, but their model, based on best professional opinion, was only evaluated using 11 incidental captures in their study area. Refining future models that can better identify barriers to movement between wetland will require more data. Specifically, information on movement capacity of the species, what barriers they can cross, and the frequency of movement as it relates to distance are important areas of research that can greatly improve the regional management plans focused on maintaining geneflow.

Virginia is thought to have less site isolation then other parts of the range (Buhlmann et al. 1997), and this is supported by the lone genetic study on bog turtles in Virginia which found that all Virginia sites were closely related (Dresser et al. 2018). Based on telemetry data of movement events, several sites in Virginia exist close enough to possibly exchange individuals and individuals been marked at these locations for over 30 years. This provides a long term data set to possibly capture movement events that may only occur on a scale of multiple years. Patterns of movements may also help explain drivers of migration as well as the resource grain that individuals perceive on the landscape. For example, turtles have been recorded using hibernacula 314 meters apart in successive winters (Feaga 2010), given that the average linear range is 93 m for male and 173 m for females, this may be an example of non-dispersal behavior driving longer distance movements (Feaga 2010). External factors may also drive long distance movements, as unfavorable conditions can drive turtles to be more active on the surface (Feaga 2010). In the bog turtle’s southern range, some sites have undergone habitat alteration through stream incision (after deforestation and livestock grazing), removal of beaver dams, or ditching and draining wetlands for agricultural or other purposes (Buhlmann et al. 1997, Moorhead and

Rossell 1998). In my study area specifically, hydrological alteration by beavers has caused the suitable area of one site to decline considerably, and one site was significantly ditched. The populations at these sites appear to be extirpated (Holden 2021), although no examination of the capture records has been conducted to examine if individuals from these sites moved to adjacent undisturbed sites.

Probability to move to another site may also be a factor of a turtle's sex. Previous radio telemetry studies have found that daily movement does not differ by sex, but homerange may differ depending on which estimator was used (Carter et al. 1999, Feaga 2010). If long distance movement was a special behavior separate from daily movement, a bias towards one sex might be uncovered. Females can move considerable distances from their hibernacula to nesting grounds at a single site (Whitlock 2002). If no suitable nesting habitat is available at a site, a female turtle may choose to find another site with suitable habitat and undergo a long distance movement. While male turtles have no need to seek nesting habitat, male turtles may undergo large movements to find possible mates (Ernst and Lovich 2009).

Because of the effort required to collect movement and genetic data, other metrics, requiring fewer captures, should be considered for their utility on bog turtles. Another possible metric for assessing connectivity without individual movement data or genetic data is the use of autocorrelation in presence/absence data (Brooks et al. 2019). Bog turtles have life histories that are conducive to spatial patterning, which makes this approach appear promising. They are a long-lived, low fecundity species, and colonization events may occur on the scale of multiple years due to their long lifespan, aspects shown to contribute to spatial autocorrelation (Bahn et al. 2008, Shurin et al. 2009). If this tool was proven to be effective, resources for locating new populations could focus on areas close to known populations and connectivity between sites could be better inferred without movement data. This effect has been hypothesized before, and making note of proximity to other known sites is a factor in at least one site quality analysis (Carter, Unpublished Report). A Moran's eigenvector mapping (MEM) approach, which extracts spatial eigenvectors from a coordinate plane is a promising approach due to its ability to take into account habitat variables at a site and resistance in the matrix between sites (Borcard and Legendre 2002, Dray et al. 2006). This approach has the most power to detect spatial patterning when sampling is regular, with equal space between points and no gaps in sampling (Borcard and Legendre 2002). Issues arise however, when applying this method at the scale and irregularity that managers search for new populations (Brind'Amour et al. 2018). With irregular sampling, spatially explicit factors, although still valid, become hard to interpret as processes that occur in different spatial scales cannot be disentangled (Borcard and Legendre 2002). In addition, irregular sampling lowers the power of models to detect spatial patterns, especially at a fine scale (Brind'Amour et al. 2018). In 2019, I sampled 49 new sites repeatedly in 12 HUC12 drainages (Figure 3.1; U.S. Geological Survey and U.S. Department of Agriculture Natural Resources Conservation Service 2013). This 49 was out of 1216 possible sites predicted from a habitat model, and sites were intentionally sampled to be spread throughout the county (Chapter 1). Because habitat is fragmented for this species and access to privately-owned land often precludes nuanced sampling schemes, this distribution of sampled sites is highly irregular. By performing an MEM analysis on this dataset, I will assess the utility of this approach on data as currently collected for the species.

In this chapter I examine movement of *G. muhlenbergii* between sites and their pattern of distribution in southwest Virginia. I will first use a long-term data set from 32 years of monitoring to examine patterns of movement between a group of wetlands. In this study, a site was defined as a discrete wetland patch (or sometimes cluster of small patches separated from each other by less than 100 m of upland habitat). Through this, I will examine patterns both in the individuals that make such movements, but also patterns in the distance (and where possible the matrix) between suitable habitat that bog turtles are likely to cross. In addition, I will apply a Moran's eigenvector mapping approach to recently collected presence absence data, specifically, to assess its efficacy and investigate if populations appear asymmetrically distributed in the study area. This chapter will help managers better make inference on the connectivity between sites, the allocation of resources to known site clusters and the allocation of resources to finding new sites in the future. Finally, I will examine the distribution of a long-lived habitat specialist in a heavily altered environment, and discuss if natural movement, important to colonizing new sites and maintaining genetic diversity, is still occurring.

Methods

Obtaining Data on Movement Events

For this study, I define 'site' as any wetland or closely related set of wetland patches disconnected from each other by less than 100m. Often, wetlands were separated by parcel boundaries and discrete landscape changes such as a road or forest edge. I obtained historical data for this study from the Virginia Department of Wildlife Resources database of bog turtles marked in Virginia with turtle records dating back to 1988. Additional trapping was also undertaken at 4 sites adjacent to sites with large populations of marked turtles to capture more possible migrants. Significant effort was put in the database to verify capture record integrity, as mis-identification of turtles (typically because over the years, multiple turtles were marked with the same notch codes, but also because shell injury or wear obscured marks) was found to be an issue in many capture histories during initial investigation. This resulted in data that appeared as a movement, but rather was a double-marked or mis-identified individual. For this, master IDs were assigned to each capture history that could be verified. Sudden changes in sex, evidence of carapace damage and implausible changes in morphometric data were flags that warranted a capture history to be broken up into multiple master IDs. For my analysis, only movements between sites where both captures had the same master ID were examined.

Once movements between sites were confirmed, I collated captures by site and binned captures per site by year into 3 categories. 1) turtles unmarked at time of capture, 2) turtles previously marked in that site 3) turtles previously marked at another site. Due to the low number of captures at a site in any given year, detailed demographic modeling to estimate immigration parameters between sites is infeasible. Instead, I compared the number of recaptures per year of turtles previously caught at the same site to turtles previously caught at another site to visualize the prevalence of immigrants at each site. In this examination of the data, I only counted one capture per individual per site per year, to remove biases repeated capturing of the same individual, although a check was made to verify no turtle underwent a back and forth movement between two sites. In addition to the location of each capture, sex was recorded to identify possible demographic patterns in movement.

Once movements had been collected and their prevalence visualized, for captures that occurred within the one county for which I obtained detailed landcover data, least-cost pathways between each site were constructed using R packages *leastcostpath* and *gdistance* (van Etten 2017, Lewis 2020). For the resistance layer used to construct the path, Travis et al. (2018)'s model was reconstructed for part of my study area. Due to differences in available data, some changes were made in the construction of the resistance raster. Data for land use, including water surface, pasture land, cropland, upland, impervious surfaces and developed land was obtained from Virginia Geographic Information Network's landcover layer. This layer was based on 2011-2014 orthophotography and had a resolution of 1m (Virginia Department of Conservation and Recreation 2016). Examining this layer, it was discovered that while it overlapped NWI data closely in non-developed areas, it prioritized several development types over NWI classification, leading to under mapping of NWI wetlands on active pasture and developed land. Thus, I also brought in National Wetland Index shapefiles (U.S. Fish and Wildlife Service 2020). As most landcover types and non core wetland types were given the same weight of 1, I assigned NWI shapefiles as a weight of 1.1, so that all mapped wetlands in the area were of their original extent. All road data was obtained from Virginia Department of Transportation, except for private or unmarked roads, which were obtained from U.S. Bureau of the Census (2020) 'all lines data.' Roads were classified and buffered according to methods outlined in Travis et al. (2018). No railroads are present in my study area. All hydrography data, with the exception of open water areas which were mapped by the landcover layer, were obtained from (U.S. Geological Survey 2001) and streams were classified and buffered according to the methods outlined. The NHD dataset also contained digital elevation maps for the study area, from which the slope layer was calculated.

Besides the slight correction to NWI wetlands outlined above, the only other significant change in my methodology was defining core wetland habitats. Because I only have detailed mapping data for a subset of sites in the study area, my best approximation for core habitat was the habitat suitability model from Feaga et al (2010). I used my predefined cut off of top 30% of model values to define core turtle habitat (Chapter 1). Because this layer was at a coarser resolution than the landcover layer (10m) it had a tendency to overlap with streams and road features. Therefore, I assigned it a weight value of 4, which was enough to prioritize it over land use types but removed any core habitat clearly on a stream or road. All other weights from the original model of 4 or higher weight were increased by one to reflect this change in order. After all features were accounted for, they were rasterized according to their corresponding resistance and weight at a resolution of 10m. A 10m resolution was chosen due as it was the coarsest grain size used in constructing the raster.

Least-cost pathways were then compared to straight line distances for average difference in distance. In addition, by examining the overlap between least-cost paths and features on the resistance raster, prevalence of barriers to dispersal were assessed. I defined a barrier to dispersal as any habitat crossed with a resistance value higher than 200 on the resistance raster, which usually corresponded with roads or developed areas.

Analysis of Autocorrelation using an MEM Approach

I examined data from a county wide search for new populations for patterns of spatial clustering in newly found turtle localities. In this search, I surveyed 49 discrete patches of wetland, with effort stratified by HUC12 watershed unit (U.S. Geological Survey and U.S. Department of Agriculture Natural Resources Conservation Service 2013) to allocate effort more evenly across my study area, as drainage unit has been proposed a possible limit to bog turtle range (Buhlmann et al. 1997). Historical presence data in my study area was omitted due to past spatial sampling bias. I examined the data from the 49 patches using a Moran's eigenvector mapping approach which uses the latitude and longitude of site centroids as a covariate in a spatial model examining presence and absence points (Dray et al. 2006, Bauman et al. 2018). The output of this approach are spatially explicit eigenvectors that account for autocorrelation among presences and absences. The interpretation of this approach is influenced heavily by the scale at which sites were sampled. Therefore, to understand if the sampling array could plausibly explain direct connectivity between sites, I constructed a neighborhood matrix between sites by iteratively finding the distance (by 1km) to which all sampled sites were connected to at least one neighbor. Once this distance was found, the mean neighbor distance was calculated and compared to known movement ranges of bog turtles.

Using presence absence data from my 49 sites, three sets of 6 models were constructed using the procedure outlined in Bauman et al. (2018) to derive spatial eigenvectors for my data set (Table 3.1). Because of the exploratory nature of this study, 3 different neighborhood matrixes: Gabriel graph, Delaunay triangulation, and minimum spanning tree were compared. In addition, two weighting functions were compared: (a) a linear decline in weight over distance and (b) a concave-down function where weight declines more sharply as distance increases using the formula $1 - (\frac{d}{d_{max}})^{0.5}$ where *d* is the distance evaluated and d_{max} is the maximum distance evaluated. Each model set therefore had 6 models. The 3 sets of models were constructed to correspond to different sources of both the spatial data and the response data being modeled. In the first data set, distance is derived from coordinates (WGS 1983), and distances are Euclidean. The response variable is presence or absence of turtles at a location. In the second model set, distance is again Euclidean, but instead of raw presence absence, an index of surrounding impervious surface cover, deemed significant through previous occupancy models, was used to build a logistic model on presence absence (Chapter 1), the response variable in the spatial model set is the residuals of each site from this logistic model rather than presence/absence. Finally, in my last model set, ecological distance, derived from the calculated least-cost pathway between each site from my resistance layer, was used as the explanatory variable and the residuals from the logistic model were used as the response. Because the R package *adespatial* does not accept distance matrixes as a valid input, my symmetric distance matrix was converted to a coordinate system using multidimensional scaling.

The package *adespatial* calculates all spatially explicit eigenvectors from the coordinates and response variable and then determines the significance of spatial patterning using adj-R^2 and a corresponding p-value, corrected for the number of eigenvectors evaluated (Bauman et al. 2018). If no significant eigenvectors can be derived, the output of the model is null and no spatial eigenvectors are extracted.

Results

Recorded Movement Events Between Sites

Between 1988 and 2020, I recorded 17 sites in which at least one individual was involved in an inter-site movement. Within these sites 21 turtles were recaptured at a site different than the one at which they were previously seen, accounting for 2.7% of total recaptures and 6.4% of turtles in my study period (739 recaptures, 313 individual turtles). I did uncover slightly more female movements than males with 11 females compared to 7 males (and 3 juveniles) however due to low sample size no significance test was applied. Comparing distances covered, neither sex moved further on average than the other (Figure 3.2; t-value 0.16, p-value 0.87). In addition to these inter-wetland movements, a search of the database uncovered 4 additional turtles found incidentally outside of a wetland (e.g. on a road, in someone's yard). Their straight line distance from last known capture was examined, but no least-cost path was constructed for their movement.

Between 2019 and 2020, at the 4 sites where I trapped, I had 1425 'trap-days' (number of traps x number of days they were deployed). In total, I caught nine turtles in traps – six without any previous markings, two previously marked at the sites at which they were captured, and one turtle that was previously caught 1500 meters away from its last capture (included in my dataset).

Euclidean distances for all movements (including both from site to site and incidental captures) are summarized in Table 3.2 and Figure 3.3. The mean distance traveled between where the turtle was captured and the site it was last seen in was 1414 meters. Outside of Virginia, the maximum recorded distance a turtle moved from its original site of capture was ca. 4700 meters (Travis 2018). Two recorded movements in this study exceed that: One male turtle found 6208 meters from its original capture site 6 years since it was last captured (previously reported in Feaga (2010) and Fleming et al. (2011)), and a female turtle found 8999 meters from its original capture, 14 years after it was last seen.

For 17 recorded movements, a least-cost pathway was calculated using Travis (2018)'s landscape resistance model (Mapped in Figure 3.4). These paths followed predicted core habitat and intermittent stream riparian corridors when available, but the majority of the distance traveled was upland pastures and forest area (Figure 3.5). On average, least-cost distance was 35% longer than straight Euclidean distance between sites, with a standard deviation of 9%. Roads were present in a majority of least-cost paths, with paved roads of traffic <1000 cars/day crossed in 11 individual movements, and unnamed private roads in 3 individual movements (Table 3.2).

In sites with recorded movement, the proportion of residents to immigrants was variable (Figure 3.6). In site ST, a centrally located site with 123 recaptures over the study period, only 1 turtle has been recorded migrating into the site. In contrast, site AG, a site monitored infrequently, had 4 immigrants out of 13 recaptures. This is despite site AG being across a trafficked road from the other sites and having less available habitat than site ST (Barron, Unpublished Data). The proportion of turtles captured at a new site and those that stayed in the same site was variable year to year, but remained low throughout the monitoring program after a significant number of turtles were marked (Figure 3.7).

Analysis of spatial autocorrelation across the study area

Sites examining spatial patterning of site occupancy were spread around the county by drainage, with 40 sites without bog turtle presence and 9 with. A minimum distance of 10km was needed to connect all sites to at least one neighbor, and using that cut off to assign neighbors, average Euclidean distance between sites was 5507m, further than all recorded movements reported here except for the two highest. Distances between sites followed a uniform distribution between 0 and 10 km with a slight bias towards longer distances (Figure 3.8). No model set, using either raw occurrences, residuals from a logistic model incorporating habitat information, nor correcting distances by Travis et al. (2018)'s resistance layer could successfully extract a significant eigenvector (Table 3.1). Interpreting this lack of significance is hindered by the uneven sampling and low prevalence of the species.

Discussion

Over the past 30 years, several studies have attached radio transmitters to bog turtles in order to better understand turtle movements, home ranges and habitat preferences (Eckler et al. 1990, Carter et al. 1999, Morrow et al. 2001, Pittman and Dorcas 2009, Feaga 2010). Long distance movements leaving a wetland have been observed with these studies, however the short term nature of these studies limits their window to assess how often they occur or estimate a plausible maximum range for the movement ability of this species over its long life span. Transmitter range may also limit detection of long distance movements. The major advantage of this study was its long time scale and large potential population of marked turtles to observe at other sites. Long term monitoring was able to show evidence of inter-wetland movements between 17 sites over a 32-year period and uncovered 21 distinct movement events where turtles moved from one site to another. Two movements recorded surpassed the current published longest straight line movement of the species. Barriers were also found to be prevalent in the matrix these movements went through.

Over the 32-year period during which these sites have been intermittently monitored, 21 confirmed exchanges of individuals have occurred, with 6.4% of all known turtles in the study system involved in a successful movement between sites. This sample is likely an underestimate of what is occurring for several reasons. Most of these sites were sampled in only 1-10 years out of the 32-year period, so any turtles who moved and returned or moved and died could have been missed, and even during a year when a site was intensively sampled likely only a small proportion of turtles present at the site were captured as individual detection estimates of individual bog turtles are low (see Holden 2021 as an example in this study area). At a per-year rate, this corresponds to an average of 0.625 movements per year (not accounting for more marked individuals as the monitoring program has continued), almost twice as large as reported in another site cluster using genetic data (Shoemaker and Gibbs 2013*b*). A per year movement rate might not be as biologically relevant to the species given its perceived long generation time however. No current estimate exists of generation time in the literature, but it is known that turtles mature at around 8 years of age, and have low clutch sizes throughout a long life span (Myhrvold et al. 2015). An educated guess might put the generation time of *G. muhlenbergii* at around 12-15 years. If this was the case, then my observed rate of individual exchange was 7.86-9.86 individuals per generation, with 2.13-2.66 generations occurring over my study. Although the potential for genetic exchange through these movements is promising, a more in-depth genetic study of this site cluster could evaluate if this level of movement is maintaining adequate genetic diversity to preserve the species into the future.

The longest distances recorded in this study were 2 turtles moving 9000 and 6200 meters, surpassing the longest distances recorded in the literature summarized by Travis et al. (2018). Although I cannot make inference on the rate at which these turtles moved due to the significant time between captures, previous radio telemetry studies can provide some insights. Feaga (2010) tracked a turtle moving 736 meters over 8 days, Pittman and Dorcas (2009) found a turtle moving 556 meters over the course of several days, and Carter et al. (2000) tracked a turtle moving 375 meters in 24 hours. In another study, turtles were recorded moving as far as 750 m in one season (Eckler et al. 1990). Typical movement behavior seen in this species shows that turtles will usually stay within 30 m of a recent location day to day (Carter et al. 2000). This high rate of movement relative to day to day movements points to these being specific dispersal behaviors (Van Dyck and Baguette 2005). This dispersal behavior may drive the majority of movements as almost all discrete areas of resources for this species are further than the average linear home range recorded (Feaga 2010).

Although these movements show that turtles can move a considerable amount over a short period of time, stepping stone wetlands are often hypothesized as the vector by which turtles can make long distance movements (Shoemaker and Gibbs 2013*b*). Capturing this behavior is challenging however and there is only one reported instance of a turtle temporarily moving into a wetland thought to be unoccupied (Feaga 2010). In the long distance movements reported, turtles were not found for several years, giving them opportunity to possibly make these movements over multiple seasons, potentially utilizing multiple wetlands.

Although some changes had to be made to the direct modeling procedure, owing to the lack of high fidelity mapping data and lack of mapped bog turtle wetlands, I was able to construct Travis et al. (2018)'s resistance layer for Virginia. Interestingly, many of the high resistance value layers, such as highways, railroads and open water features were either non-existent or did not seem to impact any mapped cost pathways. The highest resistance in close proximity to the sites evaluated was either low traffic roads or high slopes. One important limitation of this implementation of the data was the need to lower the resolution from 4 m in their paper to 10 m in my study area, and increasing grain size has been shown introduce significant error in simulation studies (Simpkins et al. 2017). Although I cannot comment on how this may have impacted the pathways, it is encouraging that the resistance layer can be transferred to new locations without sacrificing the number of resistance categories evaluated.

Examination of calculated least-cost pathways revealed two key insights. First, the path of least resistance between sites added on average, 35% more distance onto the straight line distance. This provides a metric to compare isolation by landscape resistance in the study area to others using Travis et al. (2018)'s model. In addition, the least-cost path calculated crossed a resistance value of 200 or greater in 13 out of 17 evaluated paths indicating high prevalence of anthropogenic barriers to dispersal in my study area. Two of the turtles found incidentally over the monitoring program were found crossing the road, with one struck by a car. An additional turtle was found struck by a car but was not included in my analysis due to ambiguity with its markings. Another turtle was described by Carter et al. (2000) crossing a road 2700 m away from its previous capture location, and then moving 375 m through a pine plantation the following day, before the transmitter signal was lost. Before long term monitoring was established, Buhlmann et al. (1997) found 11 turtles adjacent to a road. Despite these barriers, turtles do seem to attempt road crossings at a considerable rate relative to their lifespan, but with only a few incidental records of turtles struck by cars it is unknown how successful turtles are crossing the road and to what degree road morality is affecting local demography. I recommend that a future study be conducted to better examine the effects of roads on local populations of turtles. Corridor designs, such as the one proposed in Kaye et al. (2005) should be considered if road mortality is found to have significant impact.

To fully understand how well these populations are demographically and genetically linked, I propose two additional studies to build upon the findings presented here. First, the genetic structure should be examined more closely to investigate the structure between sites. Dresser et al. (2018) conducted a microsatellite study using 55 tissue samples from Virginia sites, but this only provided enough data to compare four sites. Additional genetic data would help to investigate if the movements reported here are allowing for adequate genetic exchange (maximizing heterozygosity while allowing for differentiation and local adaptations) between sites and could also compare the observed rate of exchange to the effective rate of exchange, as not all migrants may produce offspring (Mills and Allendorf 1996). Due to their low population size and long life span, bog turtles may represent a unique case for migrant rates needed for metapopulation stability, although this is beyond the scope of this thesis (Shoemaker et al. 2013*a*). More genetic data would allow for a second study that evaluates landscape resistance using new methodologies such as the R package *resistanceGA* (Peterman 2018), which have been shown to outperform models based on professional opinion.

A study on *Chelodina longicollis* in eastern Australia presents a similar study for comparing the movement rates of bog turtles to other turtle species. In that study, 33% of marked individuals were recorded moving between wetlands, compared to my 6% (Roe et al. 2009). The difference in apparent movement rates of these two species highlights the differences in habitat and life history between the two turtles. In the natural environment, *C. longicollis* inhabits both temporary and permanent wetlands, and the study found that relative rates of movement were much higher between temporary and permanent wetlands whereas movement between permanent wetlands was around 11%. This rate is still higher than my observed rate, although the low detection of bog turtles may make the rate reported here artificially low. The wetlands that *G. muhlenbergii* inhabit could be classified as 'temporary' over decades but not on a seasonal scale, rather several decade long trends such as beaver impoundment and tree establishment contribute to some fens being ephemeral on the landscape (Buhlmann et al. 1997, Murdock 1999, Sirois et al. 2014) although it is important to mention that recent research suggests that some fens have persisted since the Pleistocene (Weigl and Knowles 2014). Over my study period, wetland RM underwent a natural transition to an unsuitable environment due to beaver impoundment, and as a result, a number of turtles were later found in adjacent wetlands. *G. muhlenbergii* may not choose to permanently leave their wetland without a strong environmental stimulus or resource gradient, similar to the seasonal drying seen in Roe et al (2009).

Evaluation of MEM as Another Metric of Site Connectivity

The MEM approach to assessing connectivity by distance is an enticing approach for studying bog turtles. Searching for new sites in their range remains a conservation priority (North Carolina Wildlife Resources Commission 2018), and the dataset of presence absence data needed to run an MEM will only grow as more effort is expended towards that goal (as long as researchers record search effort even when no turtles are detected and if they individually mark turtles that they detect). In addition, MEMs are easy to integrate with other datasets. They can work with known habitat factors and even incorporate landscape resistance, as shown in my example. Current methods of searching for new populations however cannot adequately investigate how sites are connected through varying distances and resistances. No modeling approach in this study could extract a significant eigenvector, but this result likely has no actual application. Access to private property remains the main difficulty in creating nuanced sampling schemes that have the power to find autocorrelation. In some situations, a single landowner can own multiple sites creating a cluster of sampled sites very close together relative to average distance between sites. Isolated sites are often the result of lack of access in that area, creating large gaps in sampling. Finally, because detection of the species is low (Chapter 1), it takes considerable effort to obtain datasets that can adequately cover a large area. These factors lower the power of the MEM analysis to detect autocorrelation and make model outputs difficult to interpret due to the different scales evaluated (Borcard and Legendre 2002, Brind'Amour et al. 2018). Because of this, I cannot say with confidence if the lack of autocorrelation found on 2019 data is indicative of a distribution that is randomly spread over available habitat, or if the sampling design was inadequate.

If understanding how proximity to other populations effects a site's probability to be occupied is a priority in future studies, a specific sampling design must be adopted. This sampling design should emphasize depth rather than breadth – sampling a smaller area much more thoroughly to account for low overall occurrence. This smaller area should have habitat laid out evenly so that sampling can follow a grid like pattern as much as possible. In this grid structure, sites should be less than 2km from each other, which appears, both in my data and through genetic analysis, to be a distance over which turtles can move (Shoemaker and Gibbs 2013*b*). Finer scales, such as 1km or 0.5km, would allow magnitude of spatial autocorrelation to be plotted via correlogram over the range of movements seen in my data (see Brooks et al.

(2019) for an example). This study design would be able to incorporate habitat variables and barriers to movement using the methods outlined here. Even small gaps in sampling can be accounted for by using supplementary sites (Brind'Amour et al. 2018), allowing some flexibility in inaccessible sites.

Management Implications

The data presented in this study show the utility of long term monitoring to investigate the life history of long lived species. Prior to the unpublished study conducted by Kathy Fleming, on which this study was based, only incidental radio telemetry movements and genetic data were available to infer this species was able to move between sites. This study reports 25 movements, 21 of which were successful and 4 of which were incidentally captured outside of a wetland. Although yearly movement rates appeared low, when standardized by an estimate of generation time of the species, I found evidence of at least 7-9 movements between monitored sites per generation. Throughout the study, the proportion of *G. muhlenbergii* individuals who undertake these movements was low (Figure 3.7), and it may be that these movements are indicative of a special dispersal behavior that could be driven by external factors such as a drought year as reported in Feaga (2010) or a site naturally transitioning to an unsuitable habitat.

Turtles also showed a willingness to cross roads to get to their destination, as the majority of movements examined by a raster layer found that the path of least resistance likely crossed a road. I reported two instances where turtles were found struck by cars, but more investigation is needed to understand how much road mortality is affecting the local demography of the population. As reported in Chapter 1, surrounding impervious surface seems to have a negative impact on presence of turtle populations, stressing the need for future research into the effects of development on turtle demographics.

Finally, I tested a possible metric for studying connectivity between sites in future studies using Moran's eigenvector mapping. This approach, only using presence absence data from surveys, has potential to evaluate connectivity between sites without genetic data or recording movements. Although potential is high, applicability is limited by access to private property, making a regular sampling approach difficult. Until such a sampling design can be implemented in the field, recording movements and genetic data remain the best options for managers interested in studying connectivity in their management units.

Tables

Table 3.1. Summary of Moran's Eigenvector Mapping (MEM) analyses in this study. Three model sets were constructed. The first model set uses raw presence absence data and Euclidean distances between sites. The second model set uses Euclidean distances but the response variable was residuals from a logistic model modeling occurrence probability after accounting for surrounding impervious surfaces. The last model also uses these residuals, but least-cost pathways were calculated between each site to account for barriers to movement. For all model sets, two different weighting functions and three different neighborhood matrixes were compared using R^2-Adj. No model set nor combination of neighborhood matrix and weighting function could produce a significant eigenvector to indicate spatial clustering among occurrences at the scale of the study.

	Neighborhood Matrix	Weighting Function	R^2Adj	P-value
Raw Occurrences				
	Gabriel Graph	Linear	-0.087	0.99
	Gabriel Graph	Concave Down	-0.068	0.99
	Min. Spanning Tree	Linear	0.14	0.79
	Min. Spanning Tree	Concave Down	0.15	0.74
	Delaunay Triangulation	Linear	0.23	0.26
	Delaunay Triangulation	Concave Down	0.27	0.19
Logistic Model Residuals				
	Gabriel Graph	Linear	-0.22	0.99
	Gabriel Graph	Concave Down	-0.2	0.99
	Min. Spanning Tree	Linear	0.03	0.95
	Min. Spanning Tree	Concave Down	0.06	0.92
	Delaunay Triangulation	Linear	0.14	0.56
	Delaunay Triangulation	Concave Down	0.17	0.5
Logistic Model Residuals & Distances Weighted by Least-cost Distance				
	Gabriel Graph	Linear	-0.16	0.99
	Gabriel Graph	Concave Down	-0.15	0.99
	Min. Spanning Tree	Linear	0.0056	0.97
	Min. Spanning Tree	Concave Down	0.0043	0.98

Delaunay Triangulation	Concave Down	0.135	0.58
Delaunay Triangulation	Linear	0.143	0.53

Table 3.2. Summary table of verified movements between habitat patches in this study. N corresponds to the number of times the movement occurred. Potential barriers is defined as any discrete feature of resistance value over 200 in Travis (2018)'s resistance model. Incidental capture refers to any turtle caught outside of a wetland during the monitoring program. Incidental captures were not analyzed using least-cost path analysis. Several other movements were also not examined due to the limited extent of my resistance layer. * No GPS point was recorded for this capture, however Feaga (2010) reports the distance.

N	Euclidean Distance	Least-cost Distance	Movement	Potential Barriers
1	216	260	HR_S <-> HR_N	1 named road with <1000 cars/day
1	300	*Not examined*	OQ <-> PAL	-
3	314	460	RM <-> AG	2 named roads with <1000 cars/day
1	384	*Not examined*	*Incidental capture*	-
4	395	520	RM <-> CG	No obvious barriers
2	558	780	CW <-> ST	1 Driveway
1	585*	*Not examined*	*Incidental capture*	-
1	1085	*Not examined*	CeC <-> CC	-
3	1199	1580	ST <-> CG	1 named road with <1000 cars/day
1	1311	1850	CW <-> DA	1 unnamed road and 1 named Road with <1000 cars/day
1	1503	2180	AG <-> ST	2 named roads with <1000 cars/day
1	1622	2150	PA <-> ROB	2 named roads with <1000 cars/day
1	1622	*Not examined*	*Incidental capture*	-
1	1861	2630	DA <-> ST	1 unnamed road, 1 driveway and 1 named road with <1000 cars/day
1	2523	*Not examined*	*Incidental capture*	-
1	6208	*Not examined*	SK <-> BW	-
1	8999	*Not examined*	AG <-> RC	-

Figures

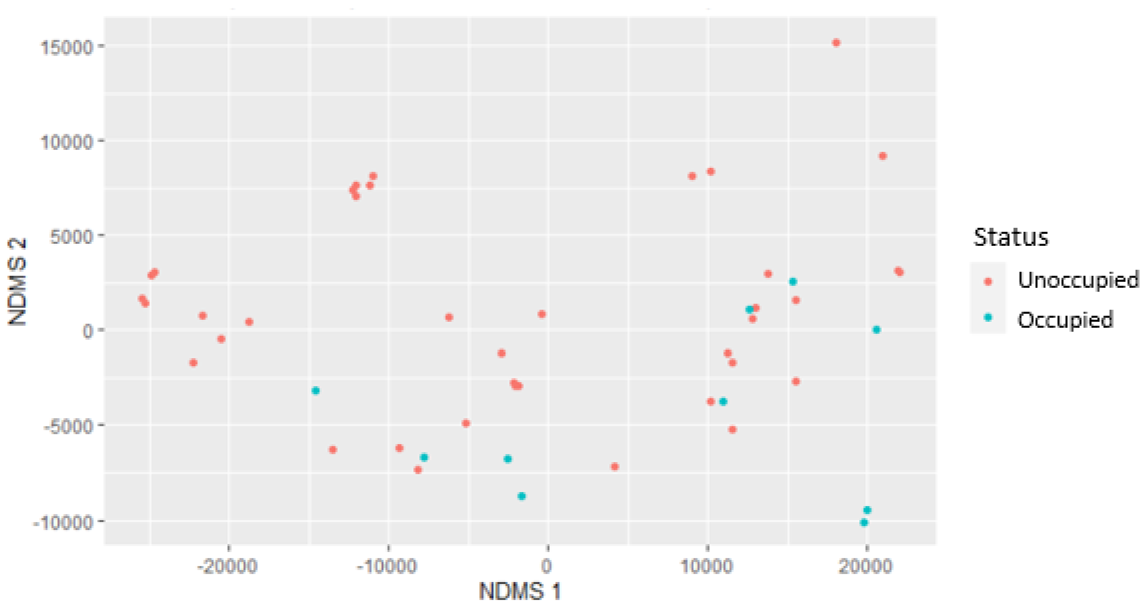

Figure 3.1. Spatial structure of the 49 wetlands in Floyd County, Virginia sampled for spatial patterning in this study. Due to the sensitivity of location information, the sites are presented on a NDMS plot, with distances between sites accounting for resistance values constructed over the study area using Travis et al. (2018)'s resistance model. The Y axis generally corresponds to moving north with increasing value, while NDMS 1 is flipped, moving more west as values increase.

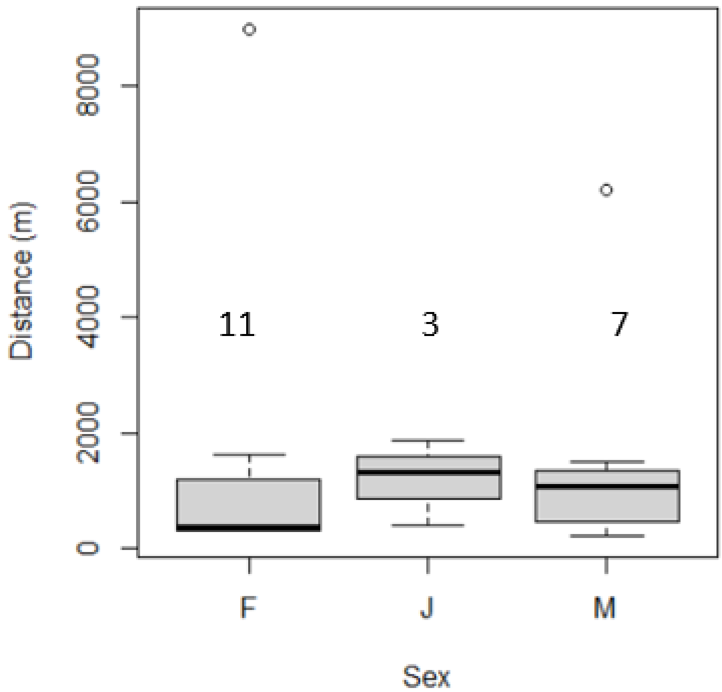

Figure 3.2. Boxplot of all 21 movements documented by recapture of bog turtles marked in Virginia between 1988 and 2020, grouped by the sex of the individual turtle at time of capture before moving. Sample size of each sex displayed above the box of the plot. The Y axis represents the distance of each movement. There was not a significant difference between the distance traveled between males and females. Juveniles were not examined for significance due to small sample size.

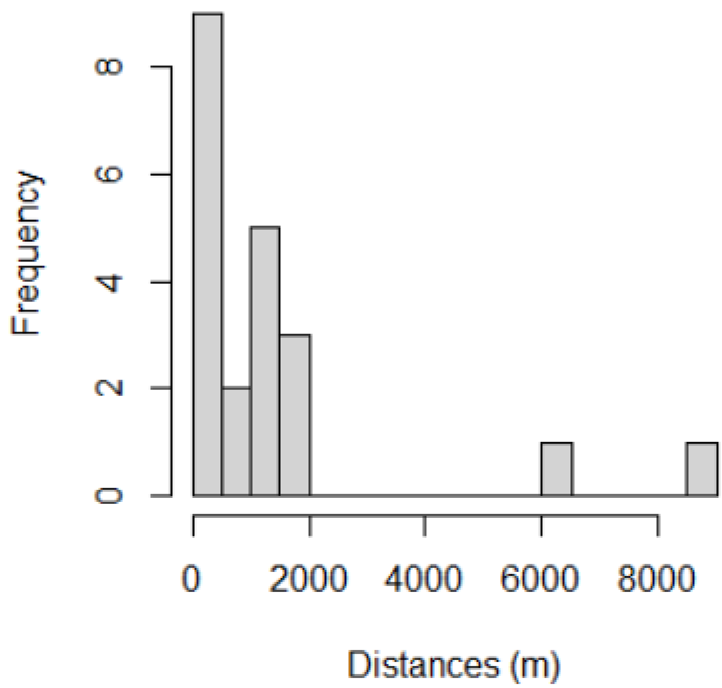

Figure 3.3. Histogram of all movements over the Virginia monitoring program (1988 – 2020) binned by Euclidean distance. The majority of movements recorded were between 0 and 2000 meters, however two movement events at 6200 meters and 9000 meters are notable exceptions.

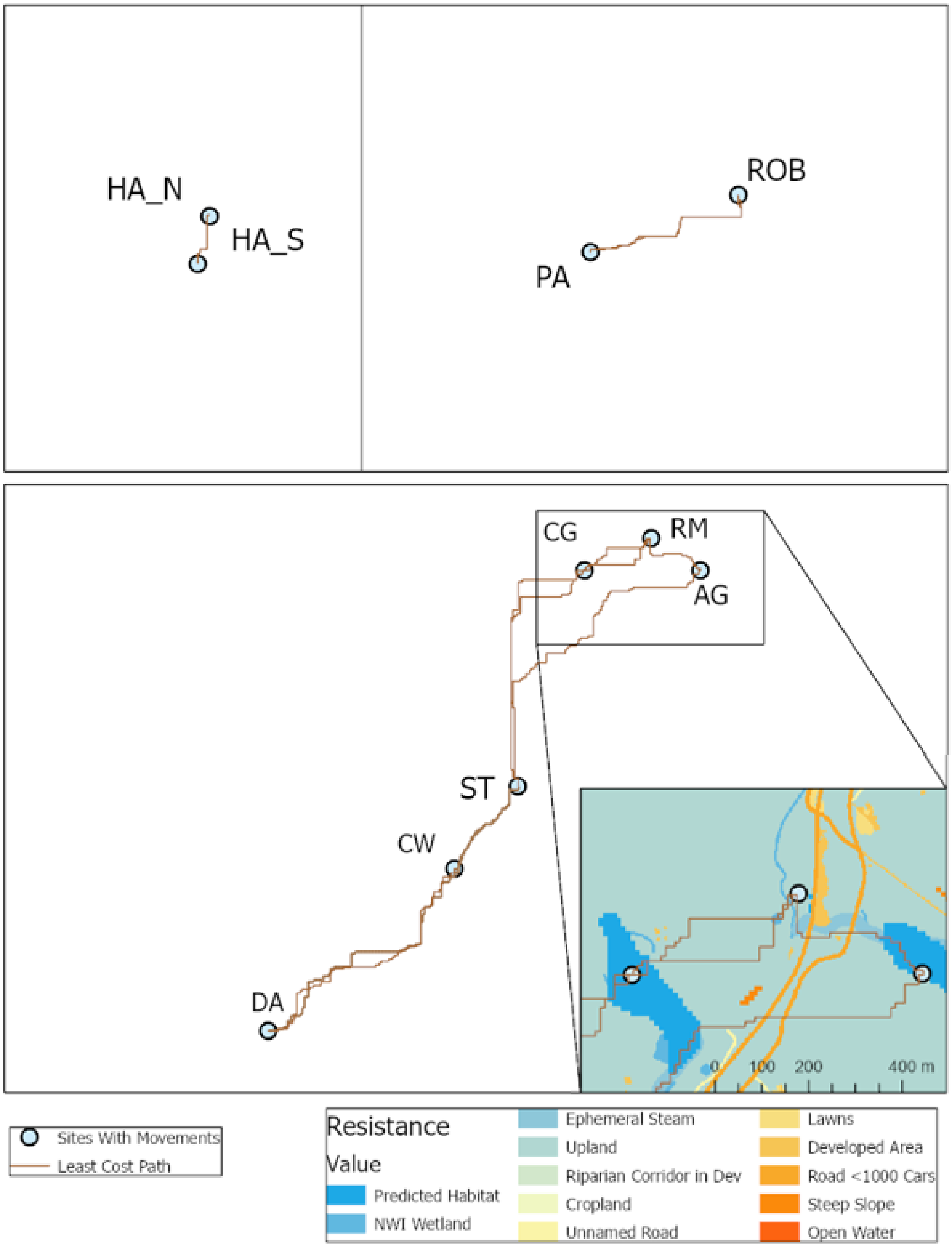

Figure 3.4. Map of least-cost movements between sites for 17 out of 21 movements. Because of location sensitivity, resistance is only shown for one small subset of sites. Resistance is color coded by its resistance value in the map. Average least-cost distance was on average 35% longer then the straightline distance between sites for each movement. In 13 movements, a resistance value of over 200 was crossed, all of which were roads and driveways.

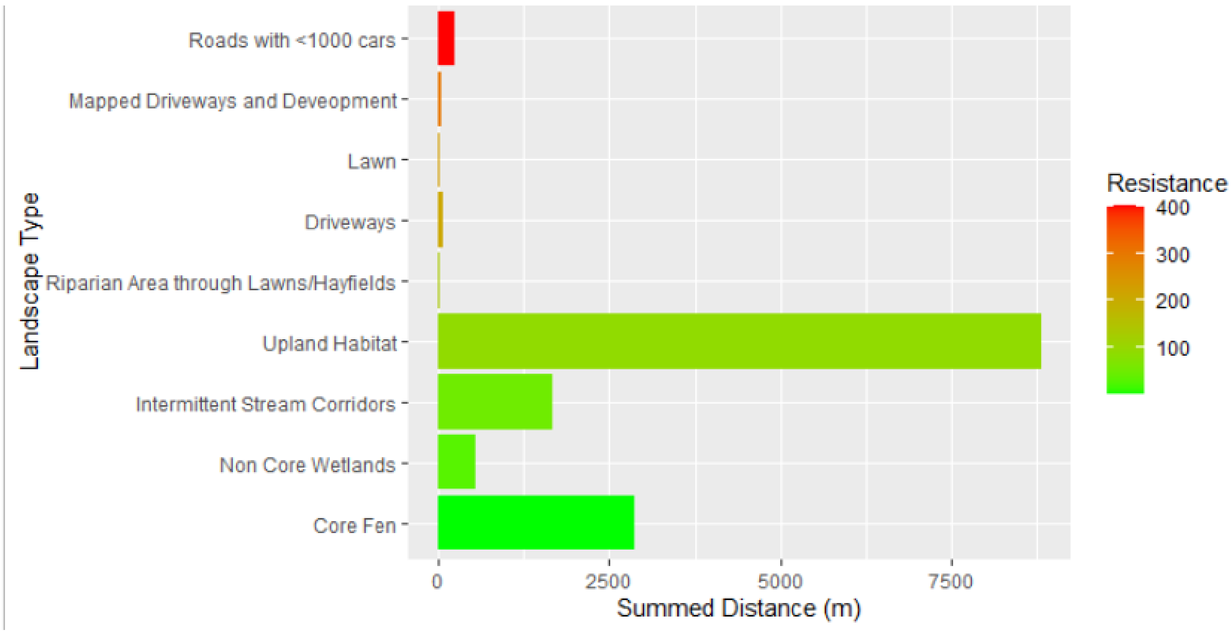

Figure 3.5. Barchart of the sum length of matrix values crossed in the 9 calculated least-cost pathways, representing 17 discrete movement events by bog turtles. Upland Habitat was the most traversed habitat type, followed by Core Fen area and Intermittent Stream Corridors. Areas of relatively high resistance (>=200) were crossed in 13 out of 17 movements.

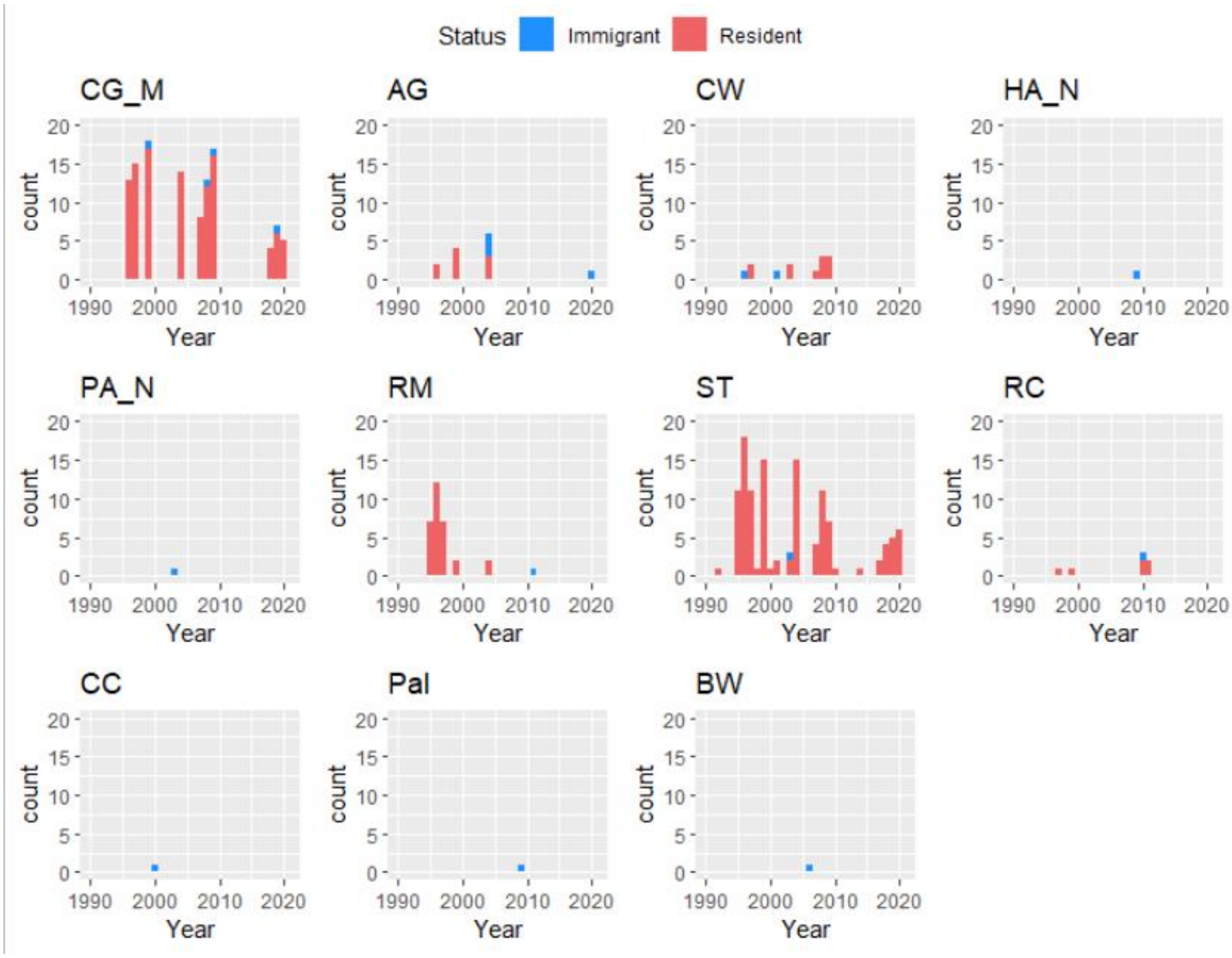

Figure 3.6. Proportion of recaptures for every site that received a migrant from another site over the course of the Virginia bog turtle monitoring program. Turtles captured at these sites without marks are not counted in these figures. Sites in this study were not sampled every year, nor was effort standardized per year. Recaptures were also filtered to one capture per year per site for any given individual, to reduce recapture bias.

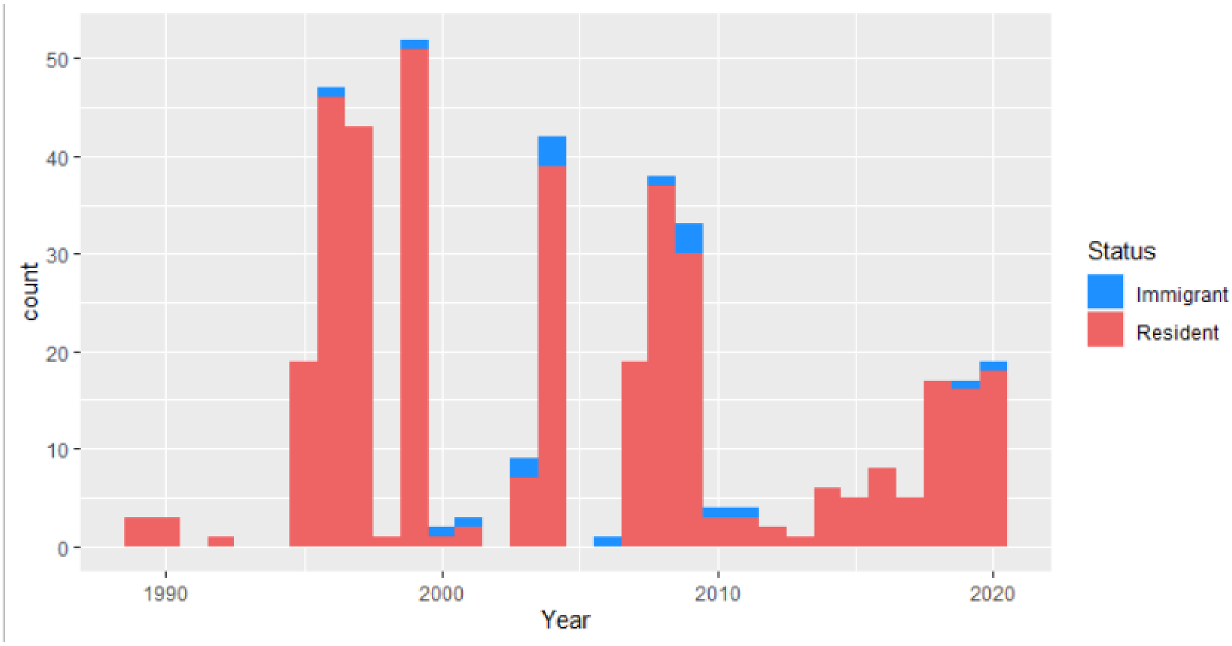

Figure 3.7. Proportion of recaptures for every site that either had a turtle leave or migrate into it from another site over the course of the Virginia bog turtle monitoring program. Sites in this study were not sampled every year, nor was effort standardized per year. Low apparent capture rates at the beginning of the program reflect the lack of marked turtles for recapture. Recaptures were also filtered to one capture per year per site for any given individual, to reduce recapture bias.

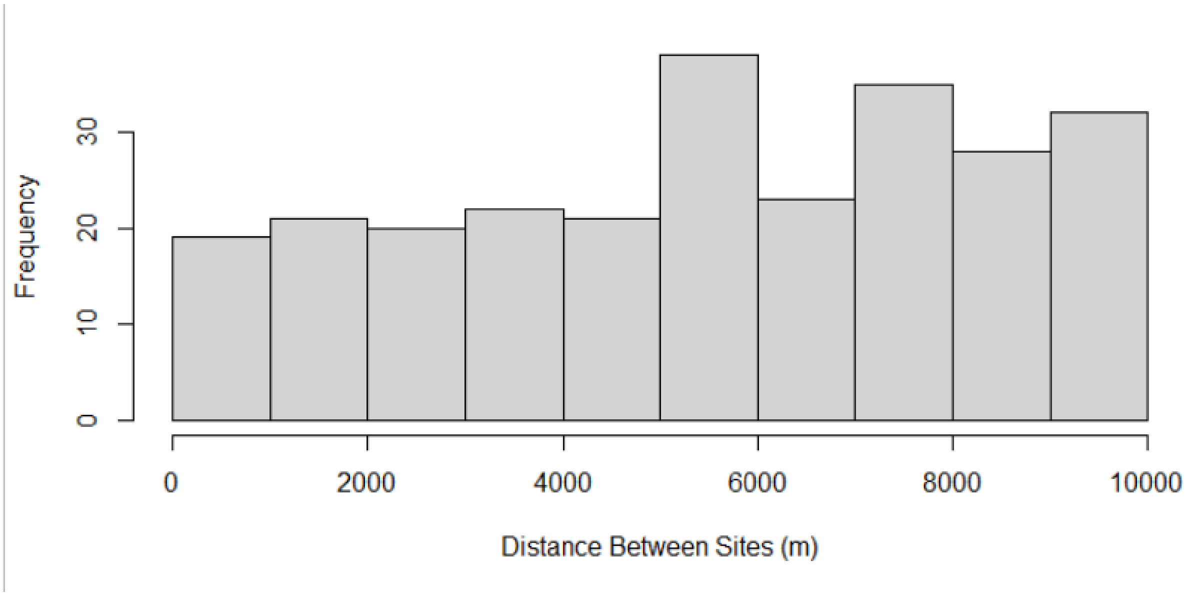

Figure 3.8. Histogram plotting distances between sites using a distance based neighborhood matrix using 10 km as the cut off. 10km was chosen as it was the first value found that connected all sites to at least one other after iteratively changing the distance by 1 km. Distances between sites followed a roughly uniform pattern between 0 and 10 km, with a slight bias towards longer distances. A distribution of distances like this would make it difficult to interpret any spatial eigenvectors, as multiple spatial processes could be occurring.

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Appendix A: Chapter 1 Supplemental Materials

Description of Weather Codes Used During Surveys

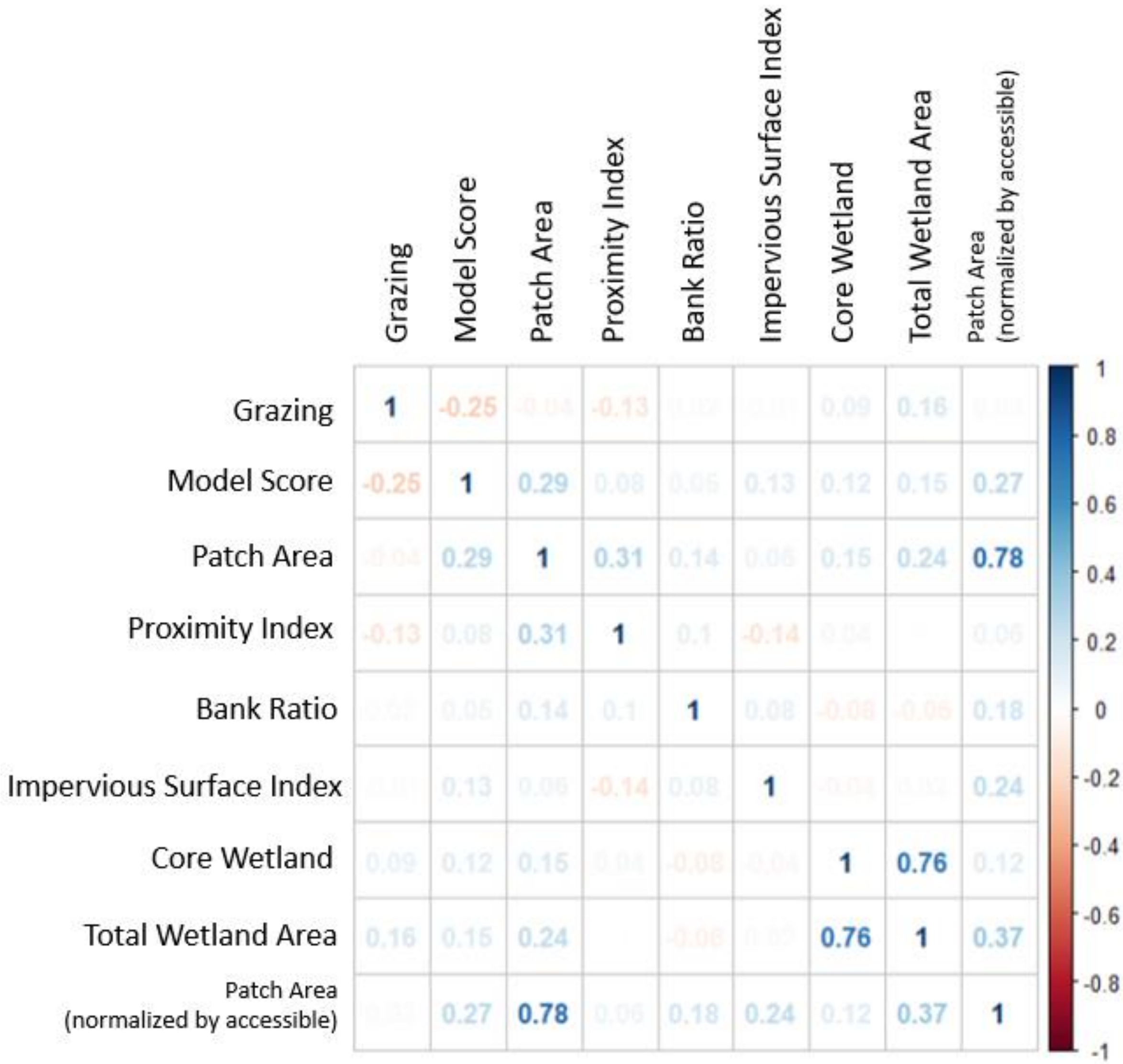
During the start of each survey, the current weather was denoted on the following scale. Scale values were assigned based on perceived effect on detection. This accounted for both difficulty of searching in the conditions as well as their hypothesized effect on turtle activity.

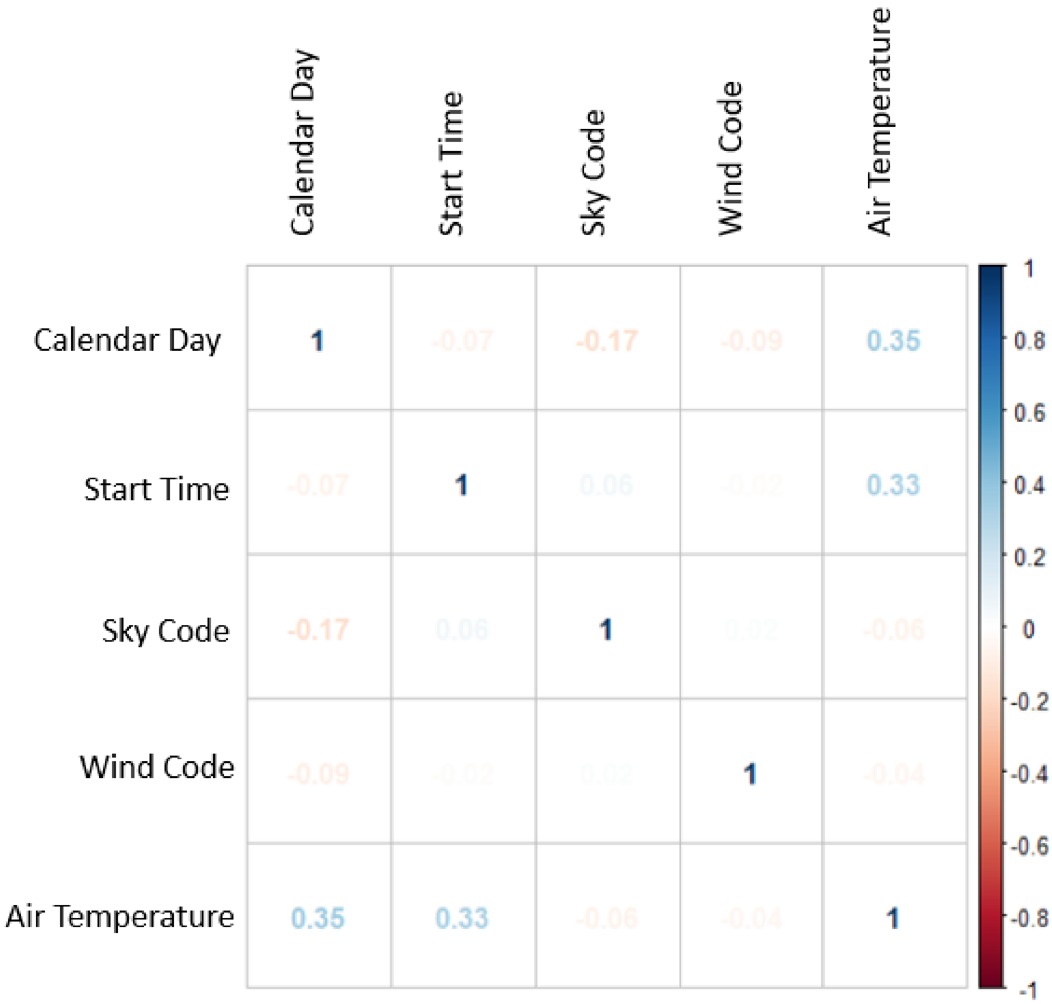
Sky Code

1 Clear Sky	**2** Partly Cloudy (25-70%)
3 Cloudy or overcast (70-100%)	**4** Fog/Mist
5 Drizzle	**6** Rain
7 Snow	**8** Hail/Severe Storm

Correlation Plots of Measured Variables

Prior to modeling, correlation plots were constructed to verify that no factors were correlated. Some factors were found to be highly correlated, but in almost all cases, this was due to them being derived from the same data set. Time of day and temperature are an important exception, but this effect is to be expected as temperature typically increases throughout the day. Although not discussed in the paper, I report here both the predicted patch area and the area of the predicted patch I was able to access. This way if lack of access masked a correlation, it would have been uncovered with these plots.


Grazing
Model Score
Patch Area
Proximity Index
Bank Ratio
Impervious Surface Index
Core Wetland
Total Wetland Area
Patch Area
(normalized by accessible)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1


Calendar Day
Start Time
Sky Code
Wind Code
Air Temperature
1
-0.07
-0.17
-0.09
0.35
0.06
0.02
0.33
-0.06
-0.04
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Full Model Tables from Occupancy Model Selection

These tables summarize the models evaluated for both detection and occupancy. Since total area was found to affect detection prior to the entire suite of models being evaluated, it was left as a fixed effect in all models. For occupancy, average patch score from the habitat model and patch area were not in this final evaluated set of models, as they failed to improve AICc over the null in previous comparisons.

Detection

p(Int)	psi(Int)	p(Grazing)	p(Calendar Day)	p(Sky Code)	p(Start Time)	p(Temp)	p(Site Size)	p(Grazing*Calendar Day)	df	AICc	delta	weight
-1.58	-0.40	NA	NA	NA	NA	NA	0.54	NA	3	124.64	0.00	0.17
-2.10	-0.46	+	NA	NA	NA	NA	0.49	NA	4	124.86	0.23	0.15
-1.60	-0.40	NA	NA	NA	NA	0.14	0.53	NA	4	126.67	2.04	0.06
-1.61	-0.38	NA	NA	-0.09	NA	NA	0.54	NA	4	126.88	2.25	0.06
-1.59	-0.40	NA	NA	NA	0.02	NA	0.53	NA	4	126.97	2.34	0.05
-1.58	-0.40	NA	-0.01	NA	NA	NA	0.53	NA	4	126.98	2.34	0.05
-2.08	-0.46	+	NA	NA	NA	0.09	0.49	NA	5	127.18	2.54	0.05
-2.09	-0.48	+	-0.05	NA	NA	NA	0.49	NA	5	127.26	2.62	0.05
-2.10	-0.45	+	NA	-0.05	NA	NA	0.50	NA	5	127.28	2.65	0.05
-2.10	-0.46	+	NA	NA	0.01	NA	0.49	NA	5	127.31	2.67	0.05
-1.62	-0.38	NA	NA	-0.10	NA	0.14	0.53	NA	5	129.00	4.36	0.02
-1.58	-0.42	NA	-0.06	NA	NA	0.16	0.52	NA	5	129.06	4.42	0.02
-1.59	-0.40	NA	NA	NA	-0.04	0.15	0.53	NA	5	129.10	4.46	0.02
-1.61	-0.37	NA	NA	-0.09	0.04	NA	0.53	NA	5	129.31	4.67	0.02
-1.60	-0.38	NA	-0.01	-0.09	NA	NA	0.54	NA	5	129.32	4.69	0.02
-2.13	-0.46	+	-0.33	NA	NA	NA	0.48	+	6	129.33	4.69	0.02
-1.58	-0.40	NA	-0.01	NA	0.02	NA	0.53	NA	5	129.42	4.78	0.02
-2.07	-0.49	+	-0.09	NA	NA	0.13	0.48	NA	6	129.59	4.96	0.01
-2.09	-0.45	+	NA	-0.05	NA	0.09	0.49	NA	6	129.70	5.06	0.01
-2.08	-0.46	+	NA	NA	-0.03	0.10	0.49	NA	6	129.72	5.08	0.01
-2.10	-0.47	+	-0.05	-0.05	NA	NA	0.50	NA	6	129.78	5.14	0.01
-2.09	-0.48	+	-0.05	NA	0.00	NA	0.49	NA	6	129.81	5.17	0.01
-2.10	-0.45	+	NA	-0.05	0.02	NA	0.50	NA	6	129.83	5.19	0.01
-1.60	-0.40	NA	-0.07	-0.10	NA	0.17	0.53	NA	6	131.47	6.84	0.01
-1.62	-0.38	NA	NA	-0.09	-0.03	0.15	0.54	NA	6	131.54	6.91	0.01
-1.57	-0.43	NA	-0.07	NA	-0.07	0.18	0.53	NA	6	131.56	6.93	0.01
-2.12	-0.47	+	-0.36	NA	NA	0.11	0.48	+	7	131.82	7.18	0.00
-1.61	-0.38	NA	-0.01	-0.09	0.04	NA	0.53	NA	6	131.86	7.22	0.00
-2.14	-0.45	+	-0.33	-0.06	NA	NA	0.49	+	7	131.95	7.32	0.00
-2.13	-0.46	+	-0.33	NA	0.00	NA	0.49	+	7	131.99	7.35	0.00
-2.06	-0.50	+	-0.11	NA	-0.07	0.15	0.50	NA	7	132.21	7.57	0.00
-2.07	-0.48	+	-0.09	-0.06	NA	0.13	0.49	NA	7	132.21	7.58	0.00
-2.09	-0.45	+	NA	-0.05	-0.03	0.10	0.50	NA	7	132.35	7.72	0.00
-2.10	-0.47	+	-0.05	-0.05	0.01	NA	0.50	NA	7	132.44	7.81	0.00
-1.59	-0.41	NA	-0.08	-0.10	-0.05	0.19	0.54	NA	7	134.11	9.47	0.00
-2.12	-0.46	+	-0.36	-0.06	NA	0.11	0.48	+	8	134.56	9.92	0.00
-2.11	-0.48	+	-0.38	NA	-0.07	0.14	0.49	+	8	134.56	9.93	0.00
-2.14	-0.45	+	-0.33	-0.06	0.01	NA	0.49	+	8	134.74	10.10	0.00
-2.07	-0.49	+	-0.11	-0.05	-0.06	0.15	0.50	NA	8	134.96	10.33	0.00
-2.11	-0.47	+	-0.38	-0.05	-0.06	0.13	0.49	+	9	137.44	12.81	0.00

Occupancy

p(Int)	psi(Int)	p(Total Habitat)	psi(Bank Ratio)	psi(Grazing)	psi(Proximity Index)	psi(Core Wetland)	psi(Impervious Index)	df	AICc	delta	weight
-1.56	-0.71	0.55	NA	NA	NA	NA	-1.29	4	121.72	0.00	0.22
-1.41	-1.19	0.49	NA	NA	NA	0.54	-1.39	5	122.64	0.92	0.14
-1.56	-0.70	0.56	0.42	NA	NA	NA	-1.30	5	123.81	2.10	0.08
-1.55	-0.74	0.54	NA	NA	0.11	NA	-1.26	5	124.05	2.34	0.07
-1.57	-0.55	0.55	NA	+	NA	NA	-1.28	5	124.12	2.40	0.07
-1.58	-0.40	0.54	NA	NA	NA	NA	NA	3	124.64	2.92	0.05
-1.40	-1.18	0.49	0.34	NA	NA	0.55	-1.43	6	124.88	3.16	0.05
-1.39	-1.22	0.48	NA	NA	0.12	0.54	-1.38	6	125.06	3.34	0.04
-1.41	-1.00	0.49	NA	+	NA	0.56	-1.38	6	125.11	3.39	0.04
-1.46	-0.70	0.48	NA	NA	NA	0.43	NA	4	125.81	4.09	0.03
-1.68	-0.09	0.57	1.17	NA	NA	NA	NA	4	126.19	4.48	0.02
-1.55	-0.73	0.55	0.37	NA	0.08	NA	-1.29	6	126.32	4.60	0.02
-1.57	-0.57	0.56	0.41	+	NA	NA	-1.30	6	126.34	4.62	0.02
-1.55	-0.66	0.54	NA	+	0.10	NA	-1.26	6	126.60	4.88	0.02
-1.56	-0.45	0.53	NA	NA	0.20	NA	NA	4	126.62	4.90	0.02
-1.60	-0.20	0.54	NA	+	NA	NA	NA	4	126.89	5.18	0.02
-1.40	-1.00	0.50	0.34	+	NA	0.58	-1.42	7	127.47	5.75	0.01
-1.39	-1.21	0.49	0.31	NA	0.09	0.55	-1.41	7	127.47	5.75	0.01
-1.40	-1.09	0.49	NA	+	0.10	0.55	-1.37	7	127.69	5.97	0.01
-1.44	-0.74	0.47	NA	NA	0.20	0.42	NA	5	127.89	6.17	0.01
-1.47	-0.67	0.49	0.25	NA	NA	0.41	NA	5	128.09	6.37	0.01
-1.46	-0.49	0.48	NA	+	NA	0.44	NA	5	128.12	6.41	0.01
-1.69	0.14	0.58	1.17	+	NA	NA	NA	5	128.53	6.81	0.01
-1.67	-0.13	0.57	1.09	NA	0.08	NA	NA	5	128.60	6.88	0.01
-1.55	-0.65	0.55	0.37	+	0.07	NA	-1.28	7	128.97	7.25	0.01
-1.57	-0.35	0.53	NA	+	0.19	NA	NA	5	129.04	7.32	0.01
-1.40	-1.06	0.49	0.32	+	0.07	0.57	-1.41	8	130.21	8.50	0.00
-1.44	-0.73	0.48	0.18	NA	0.18	0.41	NA	6	130.35	8.63	0.00
-1.44	-0.61	0.47	NA	+	0.18	0.43	NA	6	130.39	8.68	0.00
-1.47	-0.46	0.49	0.25	+	NA	0.42	NA	6	130.51	8.79	0.00
-1.68	0.09	0.57	1.13	+	0.04	NA	NA	6	131.06	9.35	0.00
-1.45	-0.58	0.48	0.18	+	0.16	0.42	NA	7	132.96	11.24	0.00

Appendix B: Full Species x Site List for Floral Inventories

Species	A	B	C	D	E	F	G	H	I	J	K	L
Achillea millefolium	*	*	*	-	-	*	*	-	*	-	*	-
Acorus calamus	-	*	-	-	-	*	*	*	-	-	*	-
Agrimonia parviflora	-	*	*	-	*	*	*	*	*	*	*	*
Agrostis gigantea	-	-	-	-	-	-	*	-	*	*	-	*
Agrostis perennans	*	*	-	-	*	-	*	-	-	*	-	*
Aletris farinosa	-	-	-	-	-	-	-	-	*	-	-	-
Alnus serrulata	-	*	-	*	*	-	*	*	*	-	*	*
Amphicarpa bracteata	-	*	-	*	*	*	*	*	-	*	*	*
Apios americana	-	-	-	-	-	*	-	-	*	-	*	-
Apocynum cannabinum	-	*	*	-	*	*	-	-	-	*	-	*
Arisaema triphyllum	-	-	-	-	-	-	-	-	*	*	-	-
Aronia prunifolia	-	-	-	-	-	-	-	-	*	-	-	-
Arthraxon hispidus	-	-	*	*	-	-	*	-	*	*	*	*
Asclepias incarnata var. pulchra	-	*	*	-	-	*	*	*	*	*	*	-
Asclepias syriacus	*	*	-	-	*	*	*	*	*	*	-	*
Asclepias tuberosa	-	-	-	-	-	-	-	-	-	-	*	-
Asplenium platyneuron	-	-	-	-	*	-	-	*	-	-	-	-
Baptisia tinctoria	-	-	-	-	-	-	-	-	-	-	*	-
Bidens spp.	-	-	-	-	-	-	*	-	-	*	-	-
Boehmeria cylindrica	-	-	-	-	-	-	*	-	-	-	-	-
Calystegia sepium	-	-	-	-	-	-	*	-	-	*	*	-
Campanula aparinoides	-	-	-	*	-	*	*	-	-	*	-	-
Carex annectens	-	-	-	*	-	-	-	*	-	-	-	-
Carex atlantica	*	*	-	*	*	*	-	*	*	*	*	*
Carex debilis	-	*	-	-	*	-	-	-	-	-	*	*
Carex frankii	-	-	-	-	-	-	-	-	-	-	*	*
Carex gynandra	*	*	-	*	-	*	-	*	*	-	-	*
Carex intumescens	-	-	-	*	-	-	-	-	*	-	-	-
Carex leptalea	-	-	*	-	-	*	-	-	*	*	-	-
Carex lurida	*	*	*	*	*	*	*	*	*	*	*	*
Carex scoparia	*	*	*	*	*	*	*	-	*	*	*	*
Carex stricta	-	-	*	-	-	-	-	-	-	-	-	-
Carex swanii	-	-	-	-	-	*	-	-	-	-	-	-
Carex tribuloides	-	-	-	-	-	-	-	-	-	*	-	-
Carex trichocarpa	-	*	-	-	-	-	*	-	-	*	*	-
Carex vulpinoidea	*	*	*	*	*	*	*	*	*	*	*	*
Cicuta maculata	*	*	*	-	*	*	*	-	*	-	-	*
Cinna arundinacea	-	*	-	-	-	-	-	-	-	*	-	-
Cirsium discolor	-	-	-	-	-	-	*	-	-	*	-	-
Clematis virginiana	-	-	-	-	*	*	*	-	-	*	*	-
Climacium americanum	-	-	-	-	-	-	-	*	-	-	-	-
Cornus amomum	-	*	*	-	-	*	*	-	*	*	-	-

Species	A	B	C	D	E	F	G	H	I	J	K	L
Crataegus spp.	-	-	-	-	-	*	-	-	-	-	-	-
Cuscuta spp.	-	-	*	-	-	-	*	*	*	*	-	-
Cyperus strigosus	-	-	*	*	*	*	*	*	*	*	*	*
Daucus carota	*	-	-	-	-	*	*	-	-	*	*	*
Desmodium glabellum	-	-	-	-	-	-	-	-	-	-	*	-
Dichanthelium acuminatum	-	-	-	-	-	-	*	-	-	-	-	-
Dichanthelium clandestinum	*	*	*	*	*	*	*	*	*	*	*	-
Dichanthelium lucidum	-	-	-	-	-	-	-	-	*	-	-	-
Dichanthelium microcarpon	-	*	*	*	*	*	*	*	*	*	*	*
Dryopteris cristata	-	-	-	*	*	*	-	*	*	-	*	*
Dulichium arundinaceum	-	-	-	-	-	-	-	-	-	*	-	-
Echinochloa crusgalli	-	-	-	-	-	-	*	-	-	-	-	-
Elaeagnus angustifolia	-	-	-	-	-	-	*	-	-	*	-	-
Elaeagnus umbellata	-	-	-	-	-	-	*	-	-	-	*	-
Eleocharis obtusa	*	-	-	-	-	*	*	*	*	-	*	*
Eleocharis tenuis var. pseudoptera	-	-	-	-	-	-	-	-	*	*	-	-
Eleocharis tenuis var. tenuis	*	*	*	*	*	-	*	-	*	*	-	-
Elymus repens	-	-	-	-	-	-	*	-	-	-	-	-
Epilobium coloratum	-	-	*	-	-	-	*	-	-	*	-	-
Epilobium leptophyllum	-	*	-	-	-	-	-	-	-	-	*	-
Erigeron spp.	-	-	-	-	-	-	-	-	-	-	*	-
Eupatorium perfoliatum	*	*	*	*	-	*	*	*	*	-	*	*
Eutrochium fistulosum	*	*	-	-	-	*	*	*	*	-	*	-
Fallopia scandens	-	-	-	-	-	*	-	-	-	*	-	-
Galinsoga quadriradiata	-	-	-	-	*	-	-	-	-	-	-	-
Galium asprellum	*	*	-	*	*	*	*	*	*	*	-	*
Galium tinctorium	-	*	*	*	*	*	*	-	*	*	*	*
Geum canadense	-	-	-	-	-	-	*	-	-	-	-	-
Glyceria canadensis	-	-	*	-	-	*	-	-	*	-	-	-
Glyceria melicaria	-	-	-	-	-	-	-	-	*	-	-	-
Glyceria striata	-	*	-	-	-	-	*	-	*	*	-	*
Gratiola neglecta	*	-	-	-	-	-	-	-	-	-	-	-
Helenium autumnale	-	*	-	-	*	*	*	-	*	*	-	*
Holcus lanatus	*	-	-	*	-	*	*	-	*	*	*	*
Hydrocotyle americana	*	*	-	*	*	*	*	*	*	*	*	*
Hypericum canadense	-	-	-	*	-	-	-	-	*	-	-	-
Hypericum densiflorum	-	-	-	-	-	*	-	-	-	-	-	-
Hypericum fraseri	-	-	*	-	-	*	-	-	-	-	-	-
Hypericum mutilum	*	-	*	*	-	*	*	*	*	-	*	*
Hypericum prolificum	-	-	-	-	-	*	-	-	-	-	-	-
Hypericum punctatum	-	*	-	-	-	-	-	-	-	-	-	-
Hypoxis hirsuta	-	-	-	-	-	-	-	-	*	-	-	-
Impatiens capensis	*	-	*	*	*	*	*	*	*	*	*	*
Ipomoea purpurea	-	-	-	-	-	-	*	-	-	-	-	-
Iris pseudacorus	-	-	-	-	-	-	*	-	-	*	-	-
Juncus acuminatus	*	-	-	-	-	-	-	-	-	-	-	-
Juncus canadensis	-	-	-	-	-	-	-	-	*	-	-	-

Species	A	B	C	D	E	F	G	H	I	J	K	L
Juncus dudleyi	-	-	-	-	-	-	-	-	*	-	-	-
Juncus effusus	*	*	*	*	*	*	*	*	*	*	*	*
Juncus marginatus	*	-	-	*	-	-	-	-	*	-	-	-
Juncus tenuis	*	-	*	*	-	*	-	*	*	*	*	*
Lauracea spp.	-	-	-	-	-	*	-	-	-	-	-	-
Leersia oryzoides	*	*	*	*	*	*	*	*	*	*	*	*
Lespedeza spp.	-	-	-	-	-	*	-	-	-	-	*	-
Lilium grayi	-	-	-	-	*	-	-	-	*	-	-	-
Lindernia dubia	-	-	-	-	-	-	*	-	*	-	-	-
Linum striatum	-	-	-	-	-	-	-	-	*	-	*	-
Linum virginianum	-	-	-	-	-	-	-	-	-	-	*	-
Lobelia inflata	-	-	-	-	-	-	-	-	-	-	*	-
Lonicera japonica	-	-	-	-	-	-	-	-	-	*	-	-
Lonicera morrowii	-	-	-	-	-	-	*	-	-	-	-	-
Ludwigia alternifolia	-	-	*	*	-	*	*	*	*	*	*	*
Ludwigia palustris	*	-	-	*	-	*	*	*	*	-	*	-
Lycopus uniflorum	*	-	*	*	*	*	*	-	*	*	*	*
Lycopus virginicus	-	-	-	-	-	-	*	-	*	*	-	*
Lysimachia ciliata	-	-	*	-	-	*	*	-	*	-	-	-
Lysimachia nummularia	-	-	-	-	-	-	-	-	*	-	-	-
Lythrum salicaria	-	-	-	-	-	-	-	-	-	-	*	-
Melanthium virginicum	-	-	-	-	-	-	-	-	*	-	-	-
Mentha arvensis	-	-	*	-	-	-	-	-	-	-	-	-
Microstegium vimineum	-	-	-	-	*	*	*	-	-	*	-	-
Mimulus ringens	*	-	*	*	*	*	*	*	*	-	*	*
Monarda fistulosa	-	-	-	-	-	*	-	-	-	-	-	-
Muhlenbergia schreberi	-	-	-	-	-	-	-	-	-	*	-	-
Myosotis scorpioides	-	-	-	-	-	-	-	-	-	*	-	-
Myosoton aquaticum	-	-	-	-	-	*	*	-	-	-	-	-
Oenothera fruticosa	-	-	-	-	-	-	-	-	*	-	-	-
Onoclea sensibilis	*	*	*	-	*	*	*	*	*	*	*	*
Osmunda cinnamomea	-	*	*	*	*	*	-	-	-	-	-	-
Oxalis dillenii	-	*	*	*	*	*	*	-	*	*	*	*
Oxalis stricta	-	-	-	-	-	-	-	-	-	*	-	-
Oxypolis rigidior	*	*	-	-	-	-	-	-	*	-	-	-
Panicum anceps	-	-	-	-	-	-	*	-	-	-	-	-
Parthenocissus quinquefolia	-	-	-	-	-	-	-	*	-	-	-	-
Penthorum sedoides	-	-	-	-	-	-	-	*	-	-	-	*
Persicaria hydropiper	*	-	-	*	*	-	*	*	*	*	*	*
Persicaria hydropiperoides	*	*	*	-	-	-	*	-	*	*	*	*
Persicaria maculosa	-	-	-	-	-	-	*	-	*	-	*	*
Persicaria saggitata	*	*	*	*	*	*	*	*	*	*	*	*
Phalaris arundinacea	-	*	-	-	-	-	*	-	*	*	-	-
Phleum pratense	-	-	-	-	-	-	-	-	-	-	-	*
Physocarpus spp.	-	-	-	-	-	-	-	-	*	-	-	-
Pilea pumila	-	-	-	-	*	*	*	*	-	*	-	*
Platanthera flava var. herbiola	-	-	-	-	-	-	-	-	*	-	-	-

Species	A	B	C	D	E	F	G	H	I	J	K	L
Poa pratensis	-	-	-	-	-	-	-	-	-	*	-	-
Polygala cruciata	-	-	-	-	-	-	-	-	*	-	-	-
Polygala sanguinea	-	-	-	-	-	-	-	-	*	-	*	-
Polystichum acrostichoides	-	-	-	-	*	-	-	-	-	-	-	-
Potentilla canadensis	-	-	-	-	-	*	-	-	*	-	-	-
Potentilla simplex	*	*	-	*	*	*	*	*	-	*	*	*
Prunella vulgaris	-	-	*	*	*	*	*	*	*	-	*	*
Pycnanthemum muticum	-	-	*	-	-	-	-	-	-	-	-	-
Pycnanthemum tenuifolium	-	*	-	-	-	-	-	-	*	*	-	-
Rhododendron maximum	-	-	-	*	-	-	-	-	-	-	-	-
Rhynchospora spp.	*	-	*	*	-	-	-	-	*	-	*	*
Rorippa palustris	-	-	-	-	-	-	*	-	-	-	-	-
Rosa multiflora	-	-	-	-	-	-	*	*	-	*	-	-
Rosa palustris	-	*	*	*	-	*	*	*	*	*	*	*
Rubus allegheniensis	-	-	-	-	-	-	-	-	*	*	-	-
Rubus hispidus	-	-	-	*	-	-	-	-	*	-	*	-
Rubus occidentalis	-	-	-	-	-	-	*	-	-	-	-	-
Rudbeckia hirta	-	-	-	-	-	*	-	-	-	-	-	-
Rudbeckia laciniata	-	-	-	-	-	-	*	-	*	-	-	-
Rumex crispus	*	-	-	-	*	-	-	*	-	-	*	*
Sagittaria latifolia	*	*	*	*	*	*	*	*	*	*	*	-
Salix nigra	-	-	-	-	-	-	*	-	-	-	-	-
Salix sericea	-	*	*	-	*	-	*	-	-	*	*	-
Schoenoplectus tabernaemontani	-	-	*	*	-	*	*	*	-	-	-	*
Scirpus cyperinus	-	*	-	-	*	*	*	*	*	*	-	*
Scirpus expansus	*	*	*	*	*	*	*	*	*	*	*	*
Scirpus georgianus	-	*	-	-	-	-	*	-	*	*	*	*
Scirpus polyphyllus	-	-	-	*	-	-	-	-	-	-	-	-
Scutellaria integrifolia	-	-	-	-	-	-	-	-	*	-	-	-
Scutellaria laterifolia	-	-	-	-	-	-	-	*	-	-	-	-
Securigera varia	-	-	-	-	-	-	*	-	-	-	-	-
Sellaginella apoda	-	-	-	-	-	-	-	-	*	-	-	-
Silphium perfoliatum var. connatum	-	-	-	-	-	-	*	-	-	-	-	-
Sisyrinchium atlanticum	*	-	-	*	-	-	-	-	*	-	-	-
Smilax rotundifolia	-	-	-	*	-	-	-	-	-	-	-	-
Solanum carolinense	*	*	*	*	-	*	*	*	-	*	*	*
Solanum dulcamara	-	-	-	-	-	-	*	-	-	*	-	-
Solidago altissima	-	*	-	-	-	*	*	-	-	*	-	*
Solidago gigantea	*	*	*	*	*	*	*	*	*	*	*	-
Solidago patula	-	*	*	*	*	*	-	-	*	*	*	*
Solidago rugosa	*	*	*	*	*	*	*	*	*	*	-	-
Spiraea alba	-	-	*	-	-	*	-	-	*	*	*	*
Spiraea tomentosa	-	*	*	-	-	*	-	-	*	-	*	-
Spiranthes spp.	-	-	-	-	-	-	-	-	-	-	*	-
Stellaria longifolia	*	-	-	*	-	*	*	*	*	*	-	-
Stellaria media	*	-	-	-	-	-	-	-	-	-	-	-
Symphoricarpos orbiculatus	-	-	-	-	-	*	-	-	-	-	-	-

Species	A	B	C	D	E	F	G	H	I	J	K	L
Symphyotrichum dumosum	-	-	*	-	-	-	-	-	-	-	*	-
Symphyotrichum puniceum	-	*	*	-	-	-	*	-	*	*	-	*
Symplocarpus foetidus	*	*	*	*	*	*	*	*	*	*	-	*
Teucrium canadense	-	-	-	-	-	-	*	-	-	-	-	-
Thalictrum pubescens	-	-	-	-	-	-	-	*	-	*	-	-
Thalictrum revolutum	-	-	*	-	-	*	-	*	*	*	-	-
Thelypteris palustris	*	*	*	-	*	*	-	-	*	*	*	*
Toxicodendron radicans	-	-	-	-	-	-	*	*	-	-	*	-
Trifolium pratense	-	-	-	*	-	*	-	-	-	-	*	*
Typha latifolia	*	*	-	-	-	*	-	-	-	-	*	-
Veratrum viride	-	-	-	-	*	*	-	-	*	-	-	-
Verbascum thapsus	-	-	*	-	-	-	-	-	-	-	-	-
Verbena hastata	*	*	*	*	*	*	*	*	-	*	*	*
Verbesina alternifolia	-	-	-	-	-	-	*	*	-	*	-	-

Letter on Appendix B	Name	Turtles	VDWR Number
A	Gully Fen	N	125
B	S Creek Fen	Y	134
C	Fenced Fen	Y	142
D	CG	Y	2
E	C Creek Fen	N	136
F	EPC	N	98
G	OF Creek Wetland	N	151
H	FP Fen	Y	128
I	Witchgrass Fen	Y	126
J	Iris Fen	N	139
K	SK	Y	19
L	T Creek Fen	N	135