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Population Dynamics of a Recently Described and Rare Plant Species: Stachys Caroliniana (Lamiaceae)

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Population Dynamics of a Recently Described and Rare Plant Species: *Stachys caroliniana* (Lamiaceae)

by

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DEDICATION

To my parents, who have inspired and guided me to be the person I am today. Also, to Caleb Ellenburg, thank you for believing in me, keeping me grounded, and focused.

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ABSTRACT

Stachys caroliniana J.B. Nelson & D.A. Rayner, a newly described 'hedge-nettle' is found in two locations in South Carolina. The first known collection of this species is from the Santee Coastal Reserve in Charleston County. The second location (the type locality) is from the Tom Yawkey Wildlife Center in Georgetown County, approximately 8 air miles northeast of the first location. This study focused on the population in Georgetown County.

Population dynamics of *Stachys caroliniana* were studied in order to provide management guidelines to South Carolina Department of Natural Resources for this species. This was achieved by assessing suitable habitat conditions, response to disturbance, and reproductive potential. A floristic inventory was taken, as well as a survey for additional populations. The effects of shade, types of flooding and competing species were determined, in addition to evaluating both sexual and asexual reproductive potential. The amount of shade and type of flooding proved to have significant effects on plant density. Weeding treatments to remove competing species did not have a significant effect on population density or proportion of reproductive plants. It was concluded that *S. caroliniana's* main method of reproduction is asexual, through the spreading of rhizomes. No additional populations were found during surveys. Additional studies that should be further explored are discussed.

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LIST OF ABBREVIATIONS

CART .... Classification and Regression Tree

CDP .... Canby's Dropwort Preserve

GIS .... Geographic Information System

HSM .... Habitat Suitability Models

LiDAR .... Light Detection and Ranging

SCDNR .... South Carolina Department of Natural Resources

SCNPS .... South Carolina Native Plant Society

SDM .... Species Distribution Model

TYWC .... Tom Yawkey Wildlife Center

TZ .... Tetrazolium

USCH .... University of South Carolina Herbarium

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Stachys is one of the largest genera in the mint family (Lamiaceae), containing about 300 different species distributed nearly worldwide, excluding Australia and the Indo-Pacific (Nelson & Rayner, 2014). There are about 45 species of *Stachys* in North America, north of Mexico, and about 14 of them inhabit the southeastern United States (Nelson & Rayner, 2014). These southeastern species mostly occupy mesic sites, and at a variety of elevations (Nelson & Rayner, 2014). For this study, one species was focused on in particular, *Stachys caroliniana*, a species newly found and described by J.B. Nelson and D.A. Rayner. Other species in the southeastern United States include: *S. hyssopifolia*, *S. eplingii, S. tenuifolia*, *S. nuttallii*, *S. clingmanii, S. floridana*, and *S. agraria* (Nelson, 1981).

The Santee Coastal Reserve in Charleston, SC, is the first location that *Stachys caroliniana* was found at in 1977. However, at the time it was thought to be a questionable specimen of *Stachys hyssopifolia*; this population was not seen again, until 2016. In 1990 *Stachys caroliniana* was spotted again during a floristic and landscape inventory of the TYWC on Cat Island in Georgetown County, SC, by J.B. Nelson. This location is about 8 air miles northeast of the locality where the first population had been discovered by D.A. Rayner. As described by Nelson and Rayner (2014), this species of *Stachys* differs from all other southeastern US taxa by “its dense, short, uniform stem pubescence, its relatively short calyx lobes, and its white corollas". Figure 1 is a photo of *S. caroliniana* in bloom.

Since the population size is rather small and no other data collection or analysis has been studied for this plant, the conservation status of *S. caroliniana* is very questionable. It is crucial to understand population dynamics for *S. caroliniana* in order to determine effective management techniques to protect this species. This plant may be one of the rarest species in South Carolina, and indeed, the world, and deserves immediate attention.

Figure 1.1: Flowering stems of *S. caroliniana*. Photo by Jim Fowler.

1.1 STUDIES OF RARE PLANTS

Investigating a literature review of rare species in similar habitats and of species in the same genus is helpful when developing a management plan for a newly described species. South Carolina rare and federally endangered species, such as *Schwalbea americana* (Orobanchaceae) and *Oxypolis canbyi* (Apiaceae) have received considerable amounts of attention and research funding, in comparison to *S. caroliniana*. A better understanding of population dynamics has aided in developing management practices to best conserve these species.

Schwalbea americana

Schwalbea americana L. (Orobanchaceae), a Coastal Plain species native to the southeastern United States, is a federally endangered hemiparasite. *S. americana* is commonly known as chaff-seed and is a perennial herb associated with longleaf pine ecosystems dependent upon fire for successful reproduction. Norden (2002) conducted a study to aid in developing a management plan for the recovery of this species. Previous to this study, research of life history stages, such as fire-induced flowering response, seed germination, and seedling establishment were unknown. Flowering is stimulated by above-ground stem removal and increased light availability; Norden (2002) found that successful flowering can be achieved without fire by mowing followed by raking to remove biomass. *S. americana* is capable of producing up to 10,000 viable seeds from each flowering plant. However, seed germination and seedling establishment in the field are reported to be low and unevenly distributed, which proved to be limiting factors for population recovery. In the lab, seeds germinated in garden plots associated with increased earthworm casts, suggesting that germination is more successful in nutrient rich soils. Seeds are capable of remaining viable for at least 8 years in cold storage, providing adequate time for additional studies. Reintroduction of green-house grown seedlings may prove to be the most successful in expanding *S. americana* populations. Individual plants of *S. americana* are long-lived and have the ability to persist in a dormant state, indicating the possibility of remnant populations. Further studies are required for the proper management of this species, such as focusing on species reintroduction into restored habitat, as well as identifying areas where unknown dormant populations could potentially be found (Norden, 2002).

Oxypolis canbyi

Oxypolis canbyi (J.M. Coult. & Rose) Fernald (Apiaceae), commonly known as “Canby’s Dropwort”, is another southeastern native historically ranging from Delaware to South Carolina. This is a perennial herbaceous plant which has received considerable attention as a federally endangered species. This species inhabits embankments of cypress-pine ponds, sloughs, wet meadows, shallow pools, and ditches. Only a small number of populations are currently known for this species’, including Inner Coastal Plains of Delaware, Maryland, the Carolinas and Georgia (Tucker and Dill 1983). Studies of *O. canbyi* have found that its main form of reproduction is asexual, by the vigorous spreading of rhizomes. Larvae of *Papilio polyxenes*, black swallowtail butterfly, may detrimentally impact sexual reproduction of this plant by chewing through the stem just below the inflorescence (CPC, 2017).

Ample conservation efforts have been put into action for the endangered *Oxypolis canbyi*. In 2003, the SCNPS acquired a 52 acre plot of land in Bamberg County, known as Canby’s Dropwort Preserve (CDP). Extensive restoration efforts have been put into place for the successful management of *O. canbyi*. Removal of planted loblolly pines for the conversion back to the natural habitat of a longleaf pine woodland has already begun (SCNPS, 2017). Other restoration activity, such as hardwood removal around the edge of the wetland and thinning out cypress, is being planned. *O. canbyi* thrives best in an open wetland where canopy cover is limited (CPC, 2017). These restorative measures will provide additional knowledge of how to successfully manage other populations of *O. canbyi*.

Rediscovery of *Stachys virgata*

When determining management techniques of a newly described species, it is helpful to examine studies of other rare species of the same genera. *Stachys virgata* was first discovered and collected in Greece between the years 1828 – 1831 and was described by Bory and Chaubard in 1832 (Constantinidis *et al*., 2015). Several botanists recorded the species localities, with the last reported sighting and collection in 1844. Then, in 2005, during an examination of the eastern coastal area of Peloponnisos, Greece, *S.virgata* was rediscovered, 161 years after its last collection. The plants were easily identified as *S. virgata* by their tall erect stems and characteristic inflorescence. A study was quickly initiated to report on the status of the newly rediscovered species. The study was designed to report new localities, define plant morphology, taxonomic relationships, and study the number of chromosomes. The population structure of *S. virgata* was observed during a 10-year period; threats and conservation status were evaluated and used to propose specific conservation measures (Constantinidis *et al*., 2015).

S. virgata flowers from May to July and releases ripe seeds in August. Attempting to reinforce the current population, propagation efforts were made by obtaining cuttings and collecting seeds for germination. However, germination efforts were very unsuccessful and many seeds lacked an embryo, indicating that the seeds were not viable. The cuttings rooted successfully, but when transplanted with one of the existing populations, none survived the following year. Devising management and conservation tactics for *S. virgata* may prove to be difficult due to unsuccessful germination and propagation efforts (Constantinidis *et al*., 2015).

Stachys virgata cannot tolerate competition and is rarely seen in tall and dense vegetation. *S. virgata* grows singly or in small clumps, and is mostly seen along dirt roads, paths, and embankments which have been cleared of other naturally competing vegetation. It is postulated that slight disturbance, such as the occasional fire, could offer new areas for *S. virgata* to colonize. One of the populations was destroyed within 6 years of being discovered, caused by drastic alteration of vegetation and herbicide use due to development within its surrounding habitat. The major threats against *S. virgata* determined from this study were land use changes and human interference.

S. virgata has been assigned to the Endangered category on the IUCN list, due to the species' limited extent of occurrence, its narrow area of occupancy and the high possibility that the current populations could be eradicated from human interference or stochastic events (Constantinidis *et al*., 2015).

1.2 PARAMETERS OF SUCCESSFUL RARE SPECIES MANAGEMENT

Determining parameters such as suitable habitat, response to disturbance, and reproductive potential, is needed to design a management regime of a rare species.

Suitable Habitat

Due to lack of research, rare species are typically poorly understood. Studying the habitat of a particular rare species by performing a habitat assessment provides essential data needed to prescribe advantageous management techniques.

Stachys hyssopifolia Michx. var. *hyssopifolia* is known to grow on the sandy banks of lakes, Carolina bays, sinks, and savannahs. *Stachys hyssopifolia* var. *lythroides* is only found from two sites in Leon County, Florida; both sites are periodically exposed to standing water. One site is exposed to plenty of sunlight within a drained bottomland opening. In comparison, the second population occurs in a well shaded forested area, which is significantly smaller in size. *Stachys hyssopifolia* var. *ambigua* is found in moist sandy soils of savannahs and open marshes (Nelson, 1981). *Stachys eplingii* J.B. Nelson, inhabits forests, bogs, and meadows mainly along the Blue Ridge Mountains. *Stachys tenuifolia* Willd., is found in a variety of habitats, such as bottomlands, roadsides, low swampy woods, and summits. *Stachys nuttallii* Shuttlew. ex Benth., is most commonly found in shaded forested areas of the Blue Ridge Mountains (Nelson, 1981). *Stachys clingmanii* Small, is another species commonly found in the Blue Ridge Mountains, usually below the shaded forest canopy in cool, moist coves.

Stachys floridana Shuttlew. ex Benth., is able to grow in both dry and wet soils, commonly found in disturbed areas, and is considered to be a weedy species. *Stachys agraria* Schltdl. & Cham., is another weedy species that grows in disturbed areas as well and is endemic to the southeast (Nelson, 1981). These species can quickly overtake other lawn and garden plants.

Stachys palustris L., a European native, commonly occurs in moist to wet soils near lakes, ponds, rivers, ditches, and in marshy areas; it is occasionally found on drier roadside banks. *S. palustris* is generally found in open areas, but will sometimes grow in partial shade. In certain areas such as Ireland, *S. palustris* is more frequently found on disturbed ground and is considered a destructive weed (Taylor and Rowland, 2011). This species can also be found sporadically in the northern states of the southeast United States (Nelson, 1981). *Stachys sylvatica* L., another European native, inhabits areas of dry soils and semi-shade such as, hedgerows, thickets, and edges of woods (Wilcock, 1974).

Defining the characteristics of a rare species' habitat is crucial in predicting other areas that would be able to accommodate its specific needs. Obtaining this information could lead to the location of unknown populations. Even though a site is characteristic of the species' habitat, it is possible that it will not be found in that area due to chance aspects of dispersal or mortality (Wiser *et al*., 1998). However, managers can use these predicted habitat sites as areas to relocate or transfer plants in order to establish sustainable populations.

There are many techniques that have been used over the years for creating models to predict suitable habitat. A species distribution model (SDM) is a tool that can be used for predicting distributions across landscapes by connecting field observations with environmental predictor variables. The chosen environmental variables that are used within the model are based on what influences the species the most, such as limiting factors, disturbances, and resources. SDMs have many applications for species management such as forecasting impacts of climate change on species distribution and for conservation planning (Guisan and Thuiller, 2005).

In a study of a rare forest herb, *Xerophyllum asphodeloides* (L.) Nutt., commonly known as turkeybeard, Bourg *et al*. (2005) used several tools for identifying possible unknown populations by predicting sites that would offer a suitable habitat. These include classification tree analysis, a model within the classification and regression tree (CART) method, in combination with geographic information system (GIS) analysis. Although no previous studies had been conducted for this rare species, field observations by researchers were used to predict variables that would be the most influential for correlating with *X. asphodeloides* population occurrences. The main variables used in this model were elevation, slope, aspect, forest type, and fire frequency. It was found that four of the initial six variables were major factors for identifying suitable habitat sites: elevation, slope, forest type, and fire frequency. This model proved to be successful at identifying previously unknown population sites of this rare species at a landscape scale.

Light detection and ranging (LiDAR) data is another tool often used for providing information of rare species habitat. LiDAR is a remote sensing method, which measures distance to the Earth. The system sends light energy to the ground and measures how long it takes for the emitted light to return back to the sensor (Questad *et al*., 2014). LiDAR can be used to create habitat-suitability models (HSM). In a study by Questad *et al*. (2014), LiDAR was used to determine high-suitability sites and low-suitability sites for restoration and reintroduction of at-risk plant species. The HSM created from LiDAR data proved to accurately identify areas for successful relocations; plant survival was less variable within plots that were considered high-suitability sites (Questad *et al*., 2014).

Disturbance and Potential Threats

Anthropogenic Disturbance

Essentially, there is no place on Earth that has not been directly or indirectly influenced by human activity. In most cases, urbanization has a detrimental effect on species biodiversity; this is a major cause of extinction for native species and is also a source for introducing non-native species (McKinney, 2008). Human actions that directly impact plant survival, such as trampling, mowing, applying herbicides, and implementing fire regimes, can have detrimental effects on rare plants. Indirect anthropogenic impacts, such as habitat modification from pollution, addition of nutrients, fire suppression, hydrology, etc., can sometimes have an even bigger effect on the population as a whole (Bowles and Whelan, 1994). These measures provide the perfect opportunity for species introduction of non-native plants and can change the entire plant community.

Fragmentation is the process of diminishing habitat size and forming multiple isolated patches (Yount *et al*., 1996). Habitat fragmentation is often caused by anthropogenic activity and has a detrimental effect on local populations. Species richness decreases overall since fragmentation restricts immigration and emigration of species between patches (Young *et al*., 1996). There are several components that occur due to habitat fragmentation, such as the formation of edges, matrices, and corridors, all of which have an effect on the species that currently inhabit the area. This is important to note not only for the species of concern, but also, for other flora and fauna that are beneficial to the species, such as pollinators and dispersers.

The greatest impact of fragmentation occurs along the edge of the disturbance and can vary greatly depending on the ecosystem and type of fragmentation. The matrix is the area that occurs between the fragmented patches. The matrix can influence the extinction probabilities of species on the edge and dispersal ability of species between patches. Corridors are tracts of forested habitat within the matrix that provide connectivity between patches and have shown to be beneficial for genetic variation, reducing inbreeding among species and aiding in dispersal. Although some species benefit from fragmentation and new species will colonize at the patch edges, these species are usually invasive or ‘weedy’ and will take over the habitat of the original species.

Stachys palustris cannot tolerate frequent grazing or mowing. This plant has been exposed to herbicides in areas such as croplands where it is considered a weed. However, the plant seems to be partially resistant to most herbicides; the herbicide with the most success at interrupting the growth is Triflusulfuron (Taylor and Rowland, 2011). There has been speculation that *S. floridana* may have a high tolerance for disturbance, as agitation to the tubers seems to encourage new growth of the plant. This is concurrent with it being known as a very weedy plant.

Response to Environmental Variation

Stochastic processes have a greater effect on smaller populations in comparison to larger and more robust population sizes. Chance-events such as floods, hurricanes, wildfires, and drought have a stronger impact on small isolated populations due to the increased probability of local extinction. Even though these events can be detrimental to the species survival, some types of disturbance can be beneficial in moderation. Certainly, this depends on intensity, frequency, and seasonality of the disturbance. The intermediate disturbance hypothesis states that an intermediate level of disturbance promotes a higher biodiversity level rather than no disturbance or disturbance that is too intensive (Roxburgh *et al*., 2004).

As noted earlier, *Stachys virgata* is thought to benefit from fire disturbance due to the creation of open areas for colonization and reducing stress from competing species. When plants are burned, nutrients are released into the atmosphere and ash is created that covers the ground and acts as fertilizer. This immediate increase in nutrients provides resources that are usually limited to plants, such as nitrogen (Fuhlendorf and Engle, 2004).

In response to an experiment by Taylor and Rowland (2011), *S. palustris* formed significantly more rhizomes when grown in moist soil when compared to dry soil; the mean masses ranging from 3.8g – 10.4g in wet soil and 2.8g – 4.6g in drier soil. It was also found that *S. palustris* may be negatively affected by *Ditylenchus destructor* Thorne, an eelworm; this nematode feeds on the plant and creates lesions in the rhizomes. *S. palustris* and *S. sylvatica* are susceptible to fungal infection, *Septoria stachydis* Roberge ex Desm., is known to cause dark brown spots on living leaf tissue. *Neoerysiphe galeopsidis* (DC.) U. Braun, is a mildew that also occurs on the living leaves of those two species (Taylor and Rowland, 2011).

Reproductive Potential

Identifying the reproductive potential of a rare species is imperative for implementing successful conservation strategies. All of the previously mentioned species of *Stachys* are perennials (the above ground portion of the plant dying back in the winter and the rhizomes producing new stems in the next growing season), with the exception of *S. agraria*, which is an annual. For perennials, the underground plant parts are usually tuberous-thickened, often sending out rhizomes that produce more stems above ground, aiding with increasing the plant's surface area. Annual *Stachys* species often have thin, fibrous root systems.

Most southeastern *Stachys* start to produce new aerial stems in early May and will begin to flower June through August, producing nutlets in mid-late summer. The timing will vary among different species, but this a general guideline of their growing season.

Not all species of *Stachys* reproduce in the same way. Some species only reproduce vegetatively such as *S. sieboldii*, while other members, *S. palustris* and *S. sylvatica*, reproduce vegetatively as well as by seed (Legkobit and Khadeeva, 2004). Rhizomes serve as an agent in biotype dispersal for *S. palustris* and are able to adequately maintain a population with no dependence on seedlings (Taylor and Rowland, 2011). Other, such as *S. ocymastrum* can only reproduce by seed (Legkobit and Khadeeva, 2004).

Pollination is widely variable within *Stachys*. Some species, such as *S. germanica*, *S. olympica*, and *S. palustris*, are self-compatible and are able to self-pollinate, while others, such as *S. cooleyae*, *S. lanata*, and *S. sylvatica*, strictly rely on bees and other insects for pollination. They are usually nectar rich which increases visitation by bees that influence pollination (Gill, 1980; Kochieva *et al*., 2006).

Determining seed viability is crucial in understanding plant demographics and population dynamics of the species. Seed viability refers to the seed's ability to germinate under favorable conditions and produce a seedling. There are many ways to test for seed viability, but some are more time consuming than others. These include germinating seeds, preforming tetrazolium (TZ) staining tests, and seed crush tests.

In order to germinate seeds, soil flats or petri dishes are often used in a greenhouse where settings can be manipulated in order to mimic favorable growing conditions. Seed viability would be tested by counting the seedlings that emerge from the soil, but this procedure can take months or even years to complete. Since this method can often become very lengthy, some seeds that were once viable may die or become nonviable during the study from causes such as fungal infection (Borz *et al*., 2007; Sawma and Mohler, 2002).

TZ testing is a quicker procedure to determine seed viability compared to the germination test. For this test, 2,3,5 triphenyl tetrazolium chloride is added to water to form a diluted solution of either 1% or 0.1%. Seeds are first prepared by soaking in water overnight and then dissected. The dissected seeds are placed in the TZ solution ranging from a few hours to 24 hours. Once the tissue within the seed reacts with the TZ solution, an analyst can determine seed viability by the color of the stained tissues. Depending on size, the seeds can be examined under a microscope, or if large enough, with the naked eye. Viable tissues will produce a red color which indicates that the tissues resisted the penetration of tetrazolium. If the seed tissue is weak, then an abnormal color will be produced and if the tissues are dead, they do not stain and usually remain white. TZ staining is a widely accepted method of assessing seed viability (Borza *et al*., 2007; Sawma and Mohler, 2002).

The seed crush test determines seed viability by applying pressure to the seed, usually with forceps. If the seed is easily crushed under gentle pressure, then it is categorized as nonviable. In a seed viability study of *Chenopodium album, Amaranthus retroflexus*, *Amaranthus hybridus* and *Abutilon theophrasti*, it was found that there was

20% more variation in detecting seed viability when compared to the TZ test. Although this method is much less laborious, it is not as accurate as the TZ test and is only recommended for studies in which some error is acceptable (Borza *et al*., 2007).

1.3 PURPOSE OF STUDY

Studies on *S. caroliniana* population dynamics, such as life history stages, response to disturbance, reproductive potential, and seed viability, have not been published or performed. Determining the ecology of this species is imperative for its survival.

The purpose of my research was to study the population dynamics of *Stachys caroliniana* to aid South Carolina Department of Natural Resources in devising management guidelines for this species. This was achieved by assessing suitable habitat conditions, response to disturbance, and reproductive potential.

Suitable habitat was determined by conducting a floristic inventory and testing three hypotheses related to habitat suitability and disturbance.

Hypothesis 1: As the amount of shade increases, the plant population density will also increase. This is based on my initial observation of the population in December, 2015.

Hypothesis 2: After evaluating the response to type of flooding, it will be found that flooding from saltwater will be more harmful to the population than freshwater flooding. This is based on the detrimental effects that increased salinity can have on freshwater wetland plants.

Hypothesis 3: There will be a significant difference between areas where competing species have been weeded compared to areas that have not. Generally, *Stachys* cannot tolerate growing in thick dense vegetation.

An assessment of *S. caroliniana's* reproductive potential, including both sexual and vegetative means, was performed. Seeds were collected in late August, to determine seed viability of this species; the TZ stain method was used.

Hypothesis 4: After evaluating *S. caroliniana's* reproductive potential, it will be found that the species main method of reproduction is asexual by the spreading of rhizomes. This is predicted because there is only one known population on the island.

In addition, a survey for unknown *S. caroliniana* populations was performed. Additional populations would most likely be found along the freshwater Canal Complex, which is adjacent to Hume Pond.

CHAPTER 2

BACKGROUND

2.1 BIOGRAPHY OF TOM YAWKEY

Tom Yawkey inherited a 20,000 acre preserve in Georgetown County from his uncle, William Yawkey, when he passed away in 1919. Yawkey was a naturalist, studying and enjoying the nature and wildlife on his preserve. Each day spent on the preserve, he recorded all of his observations in a journal. These observations were then used to make management decisions for the land; he would study the changes that occurred from implementing new management and would note how it affected the wildlife (Lee, 2016).

In the 1960's, Yawkey began experimenting with his land to find out which best management practices led to an increase of wildlife. Yawkey and his friend, Phil Wilkinson, started with waterfowl research by managing two ponds, each with their own water control structure so they could change the water regimes and measure for differences in water chemistry, water column, plant responses, invertebrate responses, and other important environmental factors. The idea was to invite graduate students, doctoral candidates, and waterfowl researchers from all over the United States to visit and conduct research. Yawkey promoted conservation of migratory waterfowl, shore birds, and wild turkeys by prohibiting hunting on his land (Lee, 2016).

2.2 HISTORY OF THE TOM YAWKEY WILDLIFE CENTER

In 1976 the Yawkey Foundation 1, now known as the TYWC, was established per Yawkey's will. This area consists of approximately 20,000 acres and was donated to the South Carolina Department of Natural Resources, in 1976. This donation was intended to preserve the areas that Mr. Yawkey grew up loving and to this day is considered to be one of the greatest contributions to the conservation of wildlife in North America. This area includes a variety of habitats, such as salt marshes, marine wetlands, forests and sandy beaches. These undisturbed areas create a diverse environment that hundreds of species including migratory birds, alligators, sea turtles, and other endangered and rare species inhabit. The mission of the TYWC is "to remain protected and undisturbed, and to be a place of research, study, and education". To stay true to their mission, the Wildlife Center grew in its area of research by partnering with Clemson University in 2014. However, Clemson University has had access to the Wildlife Center since 1994, serving as an outdoor lab for sea turtle conservation, as well as leading education courses for k-12 students from all over South Carolina. The Medical University of South Carolina is conducting research on one of the most dynamic projects at the Wildlife Center on a long-term American alligator study; while the Woods Hole Oceanographic Institute is studying how climate changes might be affecting hurricanes (Yawkey Foundation, 2015). To contribute to the knowledge of SC Coastal Plain plant life, Nelson (1990) performed a floristic inventory of the TYWC. The TYWC is vastly expanding its research program and cooperation with outside organizations to improve land management techniques.

The TYWC is made up of 3 islands, North, South and Cat (Figure 2.1). In Yawkey's will, he set forth stipulations for the management of this land implying that it should be managed as if he were still caring for it himself and must only be used for wildlife management, education, and research. Each island is managed differently; North Island is to be left undeveloped, damaging activities that would compromise the natural primitive state are prohibited as it is a designated barrier island of wilderness. South Island is managed as a waterfowl preserve that prohibits any hunting activity. The rest of the land on Cat Island is managed for migratory birds, native game, and other wildlife species.

Cat Island was formed by sand ridges and inter-dune swales. Over the last few centuries, the swales have been characterized as freshwater forested wetlands. Hume Pond is considered a cypress swamp, as there are many old-growth cypress trees around the pond.

During the rice culture era, in the 1820’s, a dam was constructed just to the south of the lower pond (Figure 2.2). In 1942, Santee Cooper completed work on the hydroelectric project on the Santee River. As a result freshwater from the Santee River was diverted into the Cooper River and out through Charleston Harbor. This caused saltwater to move farther upstream into normally freshwater wetlands, and killed most of the cypress trees in the pond.

Yawkey’s alteration of the wetland, in the 1960's, reverted Hume Pond back to freshwater. However, it has been infiltrated by saltwater during storm events, such as Hurricanes Hugo and Matthew.

Figure 2.1: Map of the Tom Yawkey Wildlife Center.

Figure 2.2: Map of study area. The location of the dam constructed in the 1820's is shown in red.

2.3 HISTORY OF *STACHYS CAROLINIANA*

S. caroliniana was first collected in 1977 by D.A. Rayner at the Santee Coastal Reserve in Charleston County, South Carolina on the south side of the Santee River. The population was located between pine flatwoods and a freshwater marsh. Unfortunately, due to the lack of location description, this population was not found again, until the summer of 2016 (Nelson, 2016).

In 1990, when Nelson rediscovered the plant during a floristic inventory at the TYWC, the location was very similar to the habitat where it was previously found in Charleston County. This population was found along the edge of a freshwater cypress swamp, Hume Pond, in partial shade on Cat Island. In 2014 *S. caroliniana* was formally described and recognized as a new hedge-nettle, native and presumably endemic to the South Carolina coast (Nelson and Rayner, 2014).

Unfortunately, even with stipulations put forth, the area where *S. caroliniana* is found has been subject to certain management techniques that could be harmful for the species. These include herbicide applications, mowing, trampling from visitors and maintenance workers, as well as frequently prescribed fires. For the duration of the study, all herbicide use, mowing, and fire regimes were halted due to the unknown effects that these disturbances may have.

In 2016 a staff member of the Santee Coastal Reserve observed a plant that seemingly resembled *S. caroliniana*. J.B. Nelson was immediately notified and after visitation, he confirmed that indeed the plant was *S. caroliniana*. This small population most likely represents the original population that D.A. Rayner had visited in 1977. Figure 2.3 shows a map of the locations where *S. caroliniana* is found.

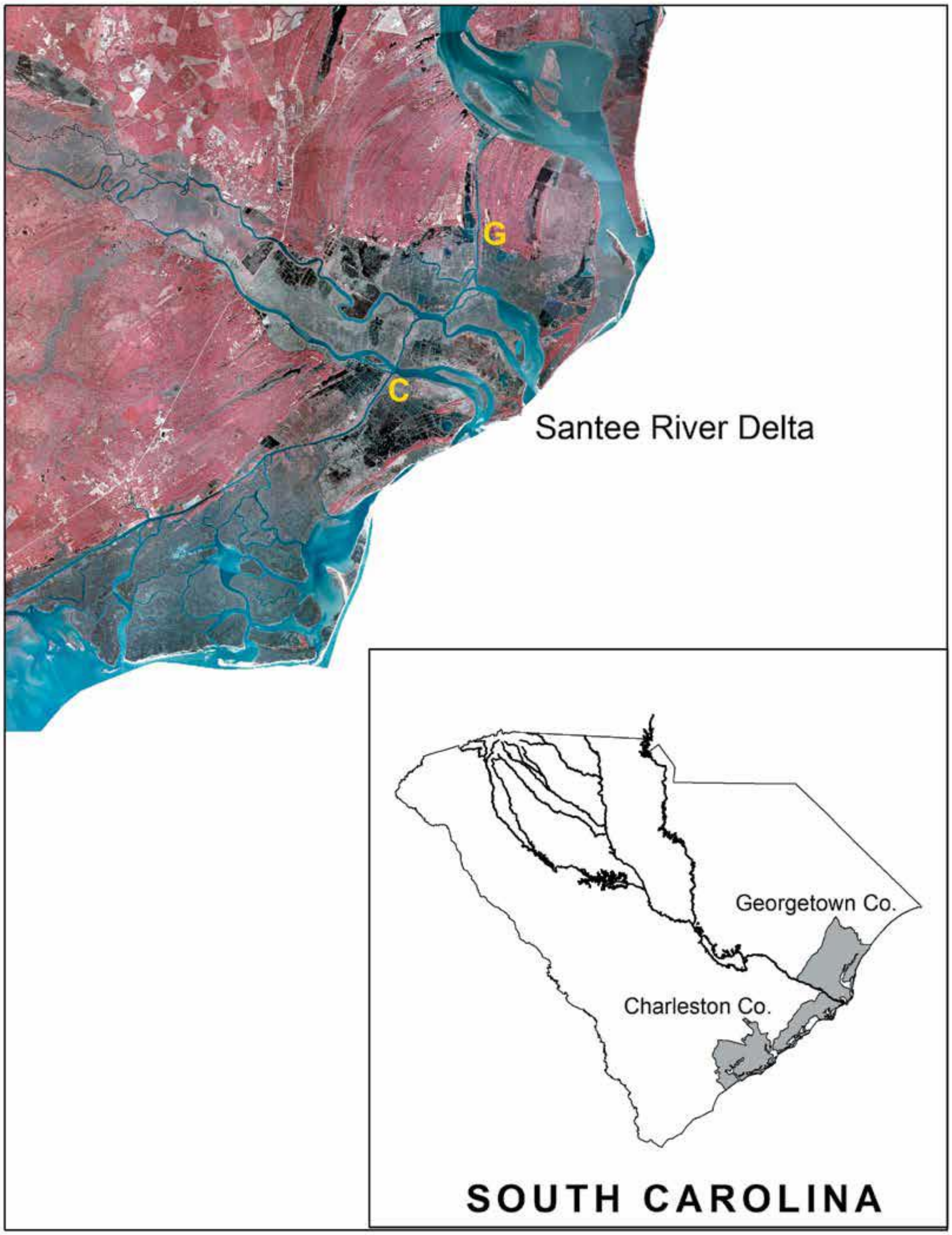

Figure 2.3: Location of known populations of *Stachys caroliniana*. G = Georgetown County (type locality), C = Charleston County, Rayner collection. Inset map indicates borders of South Carolina, Georgetown County, Charleston County and the drainage of the Santee River. Map from Nelson and Rayner (2014).

CHAPTER 3

METHODS

3.1 DATA COLLECTION

Data collection began in December 2015 following the October flood from hurricane Joaquin, in which the entire population was flooded with 2 feet of freshwater. It was essential to obtain data at this time since the biology of this plant is poorly known.

During the initial population count, the area was divided into six subpopulations A-F, shown in Figure 3.1, to obtain stem counts and the density of each subpopulation was calculated. This process was repeated again in the growing season of 2016 (May - July). To remove competing species, three of the subpopulations (A, B, and F) were 'weeded' with gardening scissors (Figure 3.2). Subpopulations C, D, and E were not weeded to serve as controls. In addition to obtaining stem counts, each plant was categorized as: vegetative, budding, flowering, seeding, or dead. Areal coverage of each subpopulation was also taken to determine if shade influences the survival of *S. caroliniana*.

Another flood occurred in October 2016. Hurricane Matthew created a saltwater surge which inundated subpopulations E and F with saltwater; no other subpopulations were submerged. Due to road inaccessibility, an assessment of the population was delayed until early December.

In the growing season of 2017 (May - August), an initial population count was taken and the weeding of subpopulations A, B and F were continued. Population counts and categorizations were repeated throughout the growing season until August 2017. Bar graphs were created of each subpopulation to show categorization of plants after each trip to the TYWC. In mid-August seeds were collected for further study.

3.2 ASSESSMENTS AND STATISTICAL ANALYSES

Suitable Habitat

A floristic inventory of the surrounding area of Hume Pond was conducted to aid in describing the habitat type. Jim Lee, the educational coordinator at the TYWC, as well as J.B. Nelson, aided in surveying and identifying these species. In addition, during each trip made to the TYWC for data collection, a survey was made along the freshwater canal complex to identify additional *S. caroliniana* populations. A map of the area surveyed is shown in Figure 3.3. In order to assess the impact of shade on this species, population density was compared among subpopulations that were categorized as having low, medium, or high shade. An analysis of variance (ANOVA) was used to determine if there was a significant difference among the three levels of shade.

Response to Disturbance

To determine *S. caroliniana's* response to certain types of disturbance, two factors were tested: response to flooding by both freshwater and saltwater as well as response to competing species.

The difference in proportion of dead plants after flooding was compared between freshwater inundation in December 2015 and saltwater inundation in December 2016. Data from subpopulations E and F were only used for this analysis, since they were the only subpopulations both inundated by freshwater and again with saltwater the next year.

A Welsh two-sample t-test was used to determine if there was a significant difference between the two types of flooding.

To assess response to competing species, the difference in population density from July 27, 2016 to August 1, 2017 was compared between weeded and non-weeded subpopulations. The difference in proportion of reproductive plants was also compared between weeded and non-weeded subpopulations. A Welsh two-sample t-test was used to determine if there was a significant difference between the types of treatment for plant density. A Wilcoxon rank sum test was used to determine if there was a significant difference between the types of treatment and proportion of reproductive plants.

Reproductive Potential

In mid-August, marking the end of the 2017 growing season, 40 seeds were collected from various subpopulations. A seed viability test was performed using a tetrazolium assay. A 1% TZ solution was made by adding 1 gram of 2,3,5 triphenyl tetrazolium chloride in 100 ml of distilled water and stored in an amber colored bottle. The seeds were first soaked in distilled water for 24 hours, then each seed was dissected using a scalpel to expose the embryo. One half of the seed was placed into a petri-dish of the TZ solution and the other half was discarded. After 24 hours in the TZ solution, the dissected seeds were observed under a dissecting microscope to determine viability.

To assess the plants ability to reproduce vegetatively, multiple plants were dug up for root and rhizome analysis. Previously collected and mounted specimens from the University of South Carolina Herbarium (USCH) were also used in the analysis.

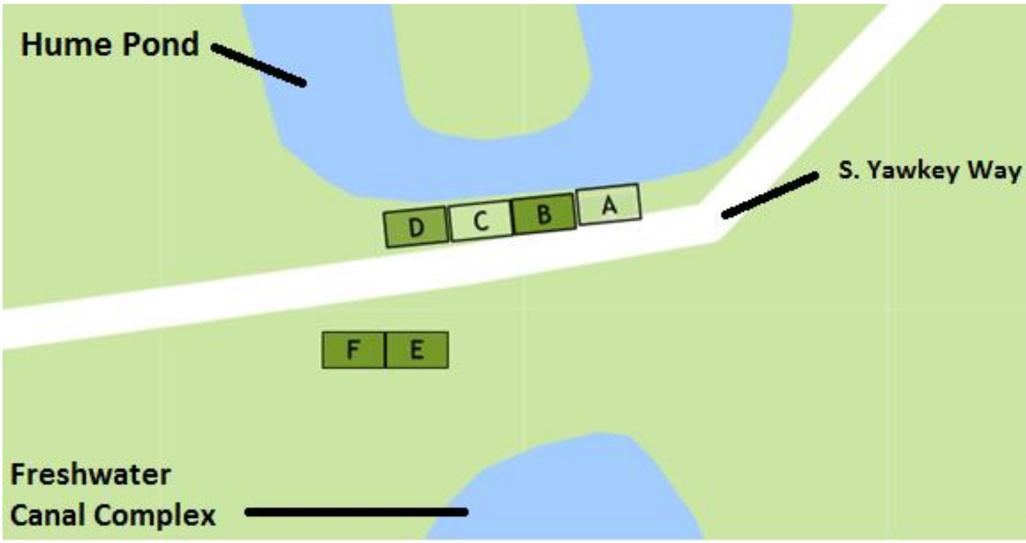

Figure 3.1: Map of subpopulations A-F. The subpopulations are shaded to represent the amount of areal coverage over each area. Dark green represents high shade, the intermediate green represents medium shade, while light green represents low shade.

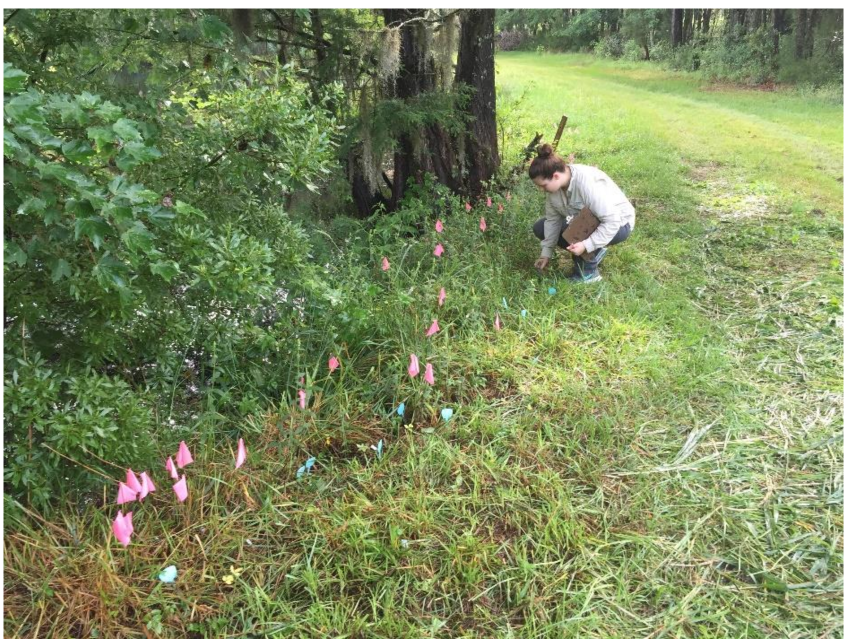

Figure 3.2: Weeding and obtaining stem counts of subpopulation B, Shelby Moody pictured. Photo by Caleb Ellenburg.

Figure 3.3: Map of Hume Pond and Freshwater Canal Complex. The area outlined in red is where *S. caroliniana* grows. The area outlined in purple is the section that was surveyed for additional populations of *S. caroliniana*.

CHAPTER 4

RESULTS

In order to show the different life stages of *S. caroliniana*, the data collected throughout the duration of the study is shown in Figures 4.1 – 4.6. Budding and flowering begins in late May and early June for the medium to high shaded subpopulations of B and D-F, which is shown in Figures 4.2 and 4.4 – 4.6 respectively. For the low shaded areas A and C, shown in Figures 4.1 and 4.3, the budding and flowering begins in July.

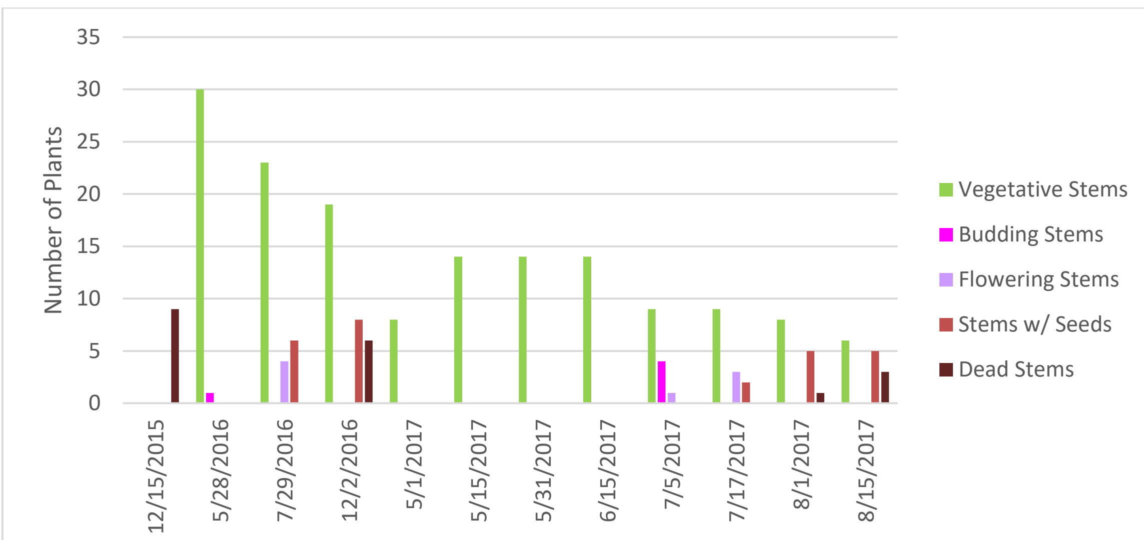

Figure 4.1: Subpopulation A. Plants were counted and categorized during each visit to the TYWC. Data was collected from December 15, 2015, through August 15, 2017. This area was weeded and has a low level of shade.

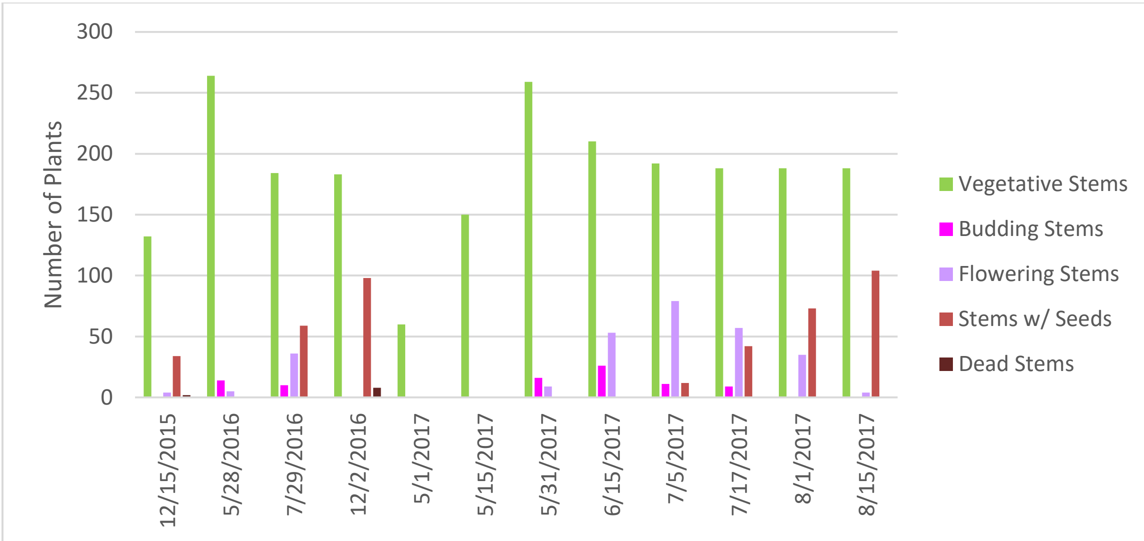

Figure 4.2: Subpopulation B. Plants were counted and categorized during each visit to the TYWC. Data was collected from December 15, 2015, through August 15, 2017. This area was weeded and has a medium level of shade.

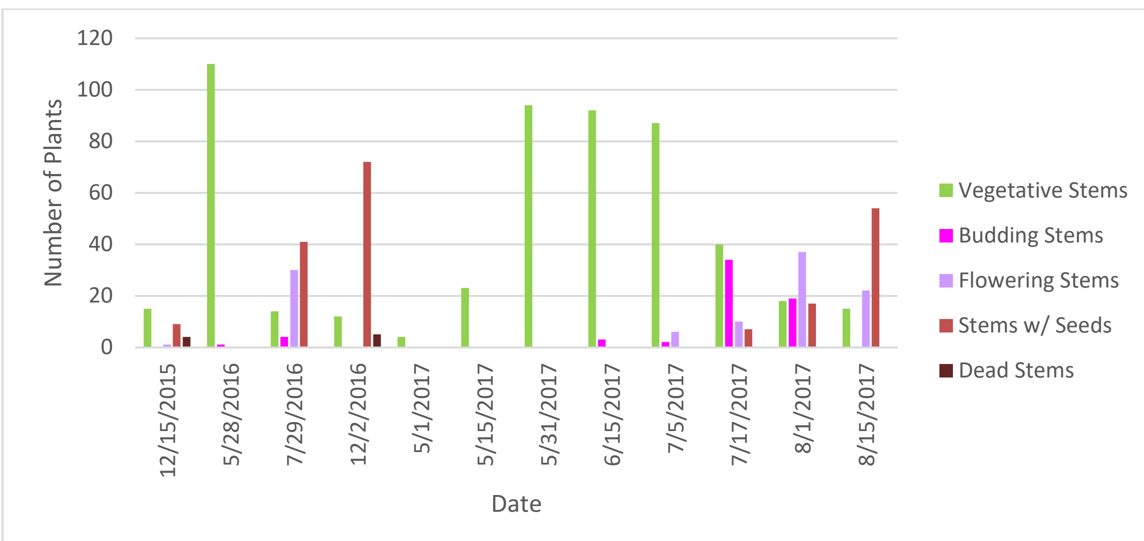

Figure 4.3: Subpopulation C. Plants were counted and categorized during each visit to the TYWC. Data was collected from December 15, 2015, through August 15, 2017. This area was not weeded and has a low level of shade.

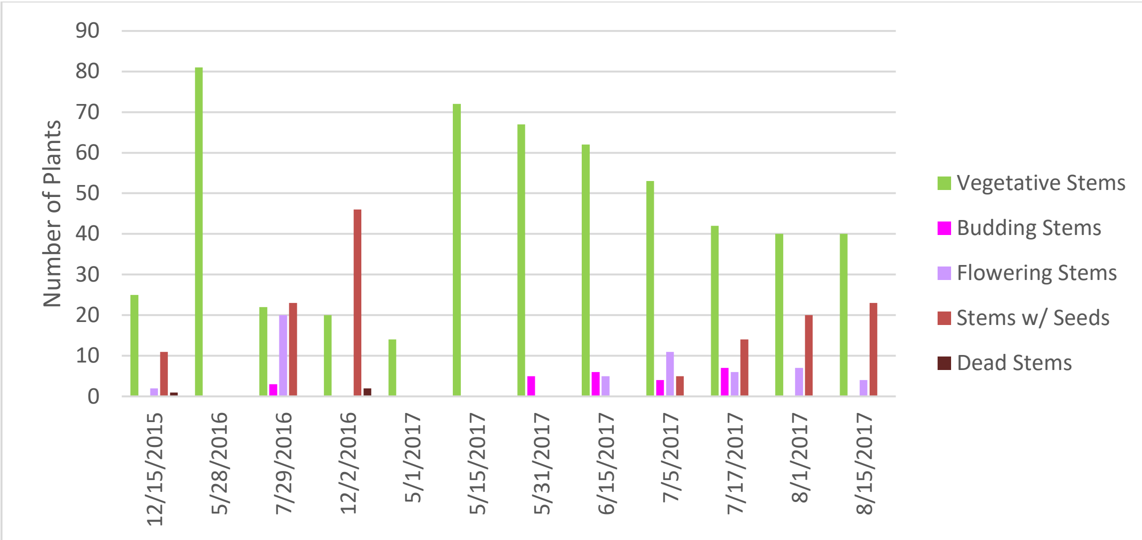

Figure 4.4: Subpopulation D. Plants were counted and categorized during each visit to the TYWC. Data was collected from December 15, 2015, through August 15, 2017. This area was not weeded and has a medium level of shade.

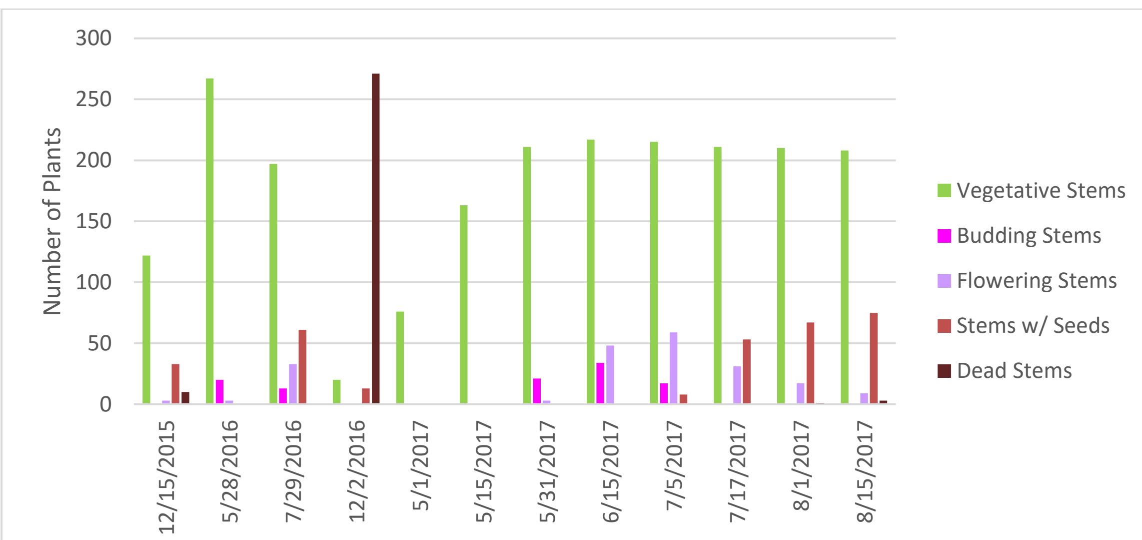

Figure 4.5: Subpopulation E. Plants were counted and categorized during each visit to the TYWC. Data was collected from December 15, 2015, through August 15, 2017. This area was not weeded and has a high level of shade.

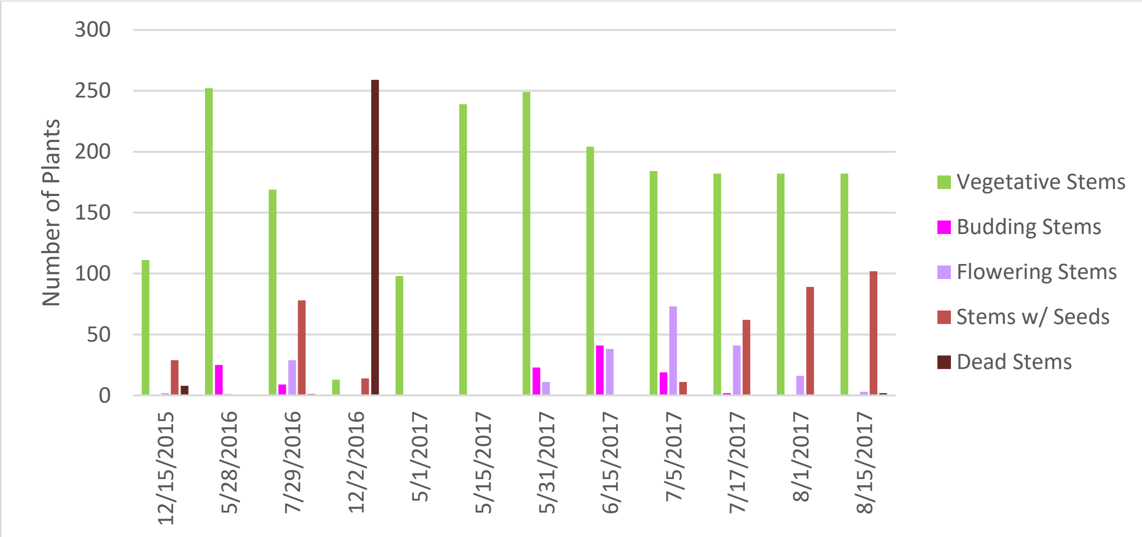

Figure 4.6: Subpopulation F. Plants were counted and categorized during each visit to the TYWC. Data was collected from December 15, 2015, through August 15, 2017. This area was weeded and has a high level of shade.

Suitable Habitat

The floristic inventory of the surrounding area of *Stachys caroliniana* is displayed in Table 1 below; the name of the species, family, and common name is included in the table. This study accounted for 50 vascular plant families, 70 genera and 88 species.

The survey for additional *S. caroliniana* populations did not lead to the finding of any other populations along the freshwater canal complex. Figure 3.3 shows a map of the area that was surveyed and its proximity to the population of *S. caroliniana* that was studied.

Amount of shade had a significant effect on plant density, $F_{2,3}$=15.39, p-value = 0.0265. Plant density increased with increasing shade (Figure 4.7).

Table 4.1: Floristic inventory. Plants of the area surrounding the population of *S. caroliniana* were identified. Hume Pond at the TYWC, Georgetown County, SC. Nomenclature follows that of Tropicos.org (2017).

Species	**Family**	**Common Name**
Sesuvium portulacastrum (L.) L.	Aizoaceae	Sea purslane
Echinodorus cordifolius (L.) Griseb.	Alismataceae	Creeping burhead
Sagittaria graminea Michaux var. *graminea*	Alismataceae	Grass-leaved arrowhead
Sagittaria latifolia Willdenow	Alismataceae	Arrowhead
Liquidambar styraciflua L.	Altingiaceae	Sweetgum
Alternanthera philoxeroides (Mart.) Griseb.	Amaranthaceae	Alligator weed
Rhus copallinum L. var. *copallinum*	Anacardiaceae	Winged sumac
Toxicodendron radicans (L.) Kuntze	Anacardiaceae	Poison Ivy
Hydrocotyle ranunculoides L.	Apiaceae	Buttercup hydrocotyle
Ilex opaca Aiton	Aquifoliaceae	American holly
Ilex vomitoria Aiton	Aquifoliaceae	Yaupon
Peltandra virginica (L.) Schott & Endler	Araceae	Green arrow arum
Baccharis halimifolia L.	Asteraceae	Sea myrtle
Cirsium Mill.	Asteraceae	Thistle
Eupatorium capillifolium (Lam.) Small ex Porter & Britton	Asteraceae	Dog Fennel
Mikania scandens (L.) Willd.	Asteraceae	Hempweed
Bignonia capreolata L.	Bignoniaceae	Cross-vine
Woodwardia areolata (L.) Moore	Blechnaceae	Netted chain-fern
Woodwardia virginica (L.) J. E. Smith	Blechnaceae	Virginia chain-fern
Spergularia marina (L.) Griseb.	Caryophyllaceae	Salt-marsh sand spurrey
Stellaria media (L.) Cyrillo	Caryophyllaceae	Chickweed
Ipomoea muricata (L.) Jacq.	Convolvulaceae	Moonvine
Melothria pendula L.	Cucurbitaceae	Creeping cucumber
Taxodium distichum (L.) Rich.	Cupressaceae	Bald-cypress
Carex albolutescens Schwein.	Cyperaceae	Greenwhite sedge
Carex hyalinolepis Steud.	Cyperaceae	Shoreline sedge
Carex venusta Dewey	Cyperaceae	Darkgreen sedge
Dulichium arundinaceum (L.) Britton	Cyperaceae	Three-way sedge

Rhynchospora glomerata (L.) Vahl	Cyperaceae	Clustered beaksedge
Rhynchospora macrostachya Torrey	Cyperaceae	Tall beaksedge
Lyonia lucida (Lam.) K. Koch	Ericaceae	Evergreen swamp-fetterbush
Lyonia mariana (L.) D. Don	Ericaceae	Staggerbush
Triadica sebifera (L.) Small	Euphorbiaceae	Chinese tallow
Chamaecrista fasciculata (Michx.) Greene	Fabaceae	Partridge pea
Clitoria ternatea L.	Fabaceae	Butterfly pea
Quercus alba L.	Fagaceae	White Oak
Quercus laurifolia Michaux	Fagaceae	Laurel oak
Quercus nigra L.	Fagaceae	Water oak
Quercus phellos L.	Fagaceae	Willow oak
Limnobium spongia (Bosc) Steudel	Hydrocharitaceae	Frog's-bit family
Hypericum denticulatum Walter	Hypericum	Coppery S. John's wort
Persea palustris Mill.	Lauraceae	Red bay
Utricularia inflata Walter	Lentibulariaceae	Inflated bladderwort
Utricularia juncea Vahl	Lentibulariaceae	Rushlike bladderwort
Decodon verticillatus (L.) Elliott	Lythraceae	Water willow
Magnolia virginiana L.	Magnoliaceae	Sweet bay
Hibiscus moscheutos L.	Malvaceae	Mallow- rose
Myrica cerifera L.	Myricaceae	Wax myrtle
Nyssa sylvatica Marsh. var. *biflora* (Walt.) Sarg.	Nyssaceae	Swamp blackgum
Osmunda cinnamomea L.	Osmundaceae	Cinnamon-fern
Pinus serotina Michaux	Pinaceae	Pond pine
Pinus taeda L.	Pinaceae	Loblolly Pine
Arundinaria gigantea (Walt.) Chapman	Poaceae	Switch cane
Dichanthelium acuminatum (Sw.) Gould & Clark var. *densiflorum* (Rand & Red.) Gould & Clark	Poaceae	Witchgrass
Dichanthelium scoparium (Lam.) Gould	Poaceae	Velvet witchgrass
Paspalum dilatatum Pior.	Poaceae	Dallisgrass
Sacciolepis striata (L.) Nash	Poaceae	Cupscale grass
Tripsacum dactyloides (L.) L.	Poaceae	Gammagrass
Nymphaea odorata Aiton	Polygalaceae	Water-lily
Rumex verticillatus L.	Polygalaceae	Swamp-dock

Pontederia cordata L.	Pontederiaceae	Pickeral-weed
Potamogeton foliosus Raf. var. *foliosus*	Potamogetonaceae	Leafy pondweed
Potamogeton pusillus L.	Potamogetonaceae	Slender pondweed
Rubus trivialis Michx.	Rosaceae	Dewberry
Cephalanthus occidentalis L.	Rubiaceae	Buttonbush
Diodia virginiana L.	Rubiaceae	Virginia -buttonweed
Mitchella repens L.	Rubiaceae	Partridge berry
Ruppia maritima L.	Ruppiaceae	Ditch-grass
Azolla caroliniana Willd.	Salviniaceae	Eastern mosquito-fern
Acer rubrum L.	Sapindaceae	Red maple
Saururus cernuus L.	Saururaceae	Lizard's tail
Gratiola pilosa Michaux	Scrophulariaceae	Hedge-hyssop
Smilax laurifolia L.	Smilacaceae	Blaspheme vine
Smilax rotundifolia L.	Smilacaceae	Greenbrier
Smilax smallii Morong	Smilacaceae	Jackson vine
Solanum carolinense L.	Solanaceae	Horse-nettle
Taxodium ascendens Brong.	Taxodiaceae	Pond cypress
Taxodium distichum (L.) Rich.	Taxodiaceae	Bald cypress
Gordonia lasianthus (L.) Ellis	Theaceae	Loblolly bay
Thelypteris palustris Schott	Thelypteridaceae	Fern
Typha latifolia L.	Typhaceae	Common cat-tail
Boehmeria cylindrica (L.) Sw.	Urticaceae	False-nettle
Viola lanceolata L.	Violaceae	Strap-leaved violet
Ampelopsis arborea (L.) Koehne	Vitaceae	Pepper-vine
Parthenocissus quinquefolia (L.) Planch.	Vitaceae	Virginia creeper
Vitis aestivalis Michaux	Vitaceae	Summer-grape
Vitis rotundifolia Michaux	Vitaceae	Muscadine

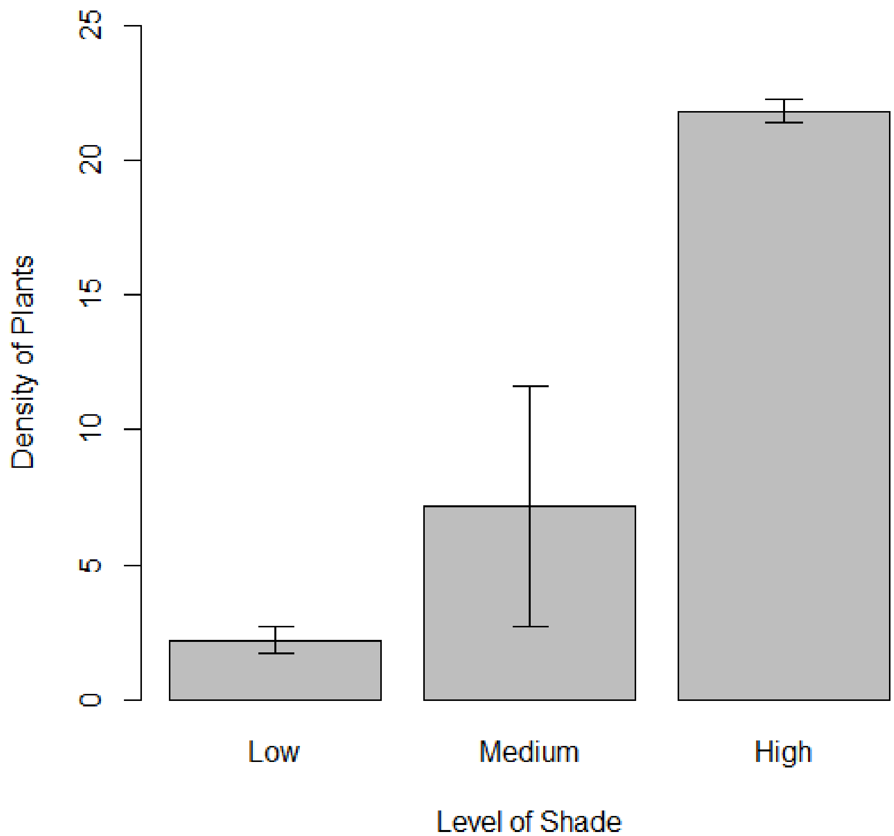

Figure 4.7: Density of plants compared to level of shade. Error bars representing standard error.

Response to Disturbance

The proportion of dead plants after freshwater and saltwater inundation differed significantly, t = -109.06, p-value = 0.0012. Saltwater flooding has a considerably greater effect on the *S. caroliniana* population (Figure 4.8).

The difference in plant density between weeded and non-weeded subpopulations were not significantly different, t = 0.407, p-value = 0.719. The difference in weeded subpopulations from 2016 to 2017 had a lower density of plants compared to non-weeded areas (Figure 4.9).

The difference in proportion of reproductive plants between weeded and non-weeded subpopulations were not significantly different W = 1, p-value = 0.2. The difference in weeded subpopulations from 2016 to 2017 had a higher proportion of reproductive plants compared to non-weeded areas (Figure 4.10).

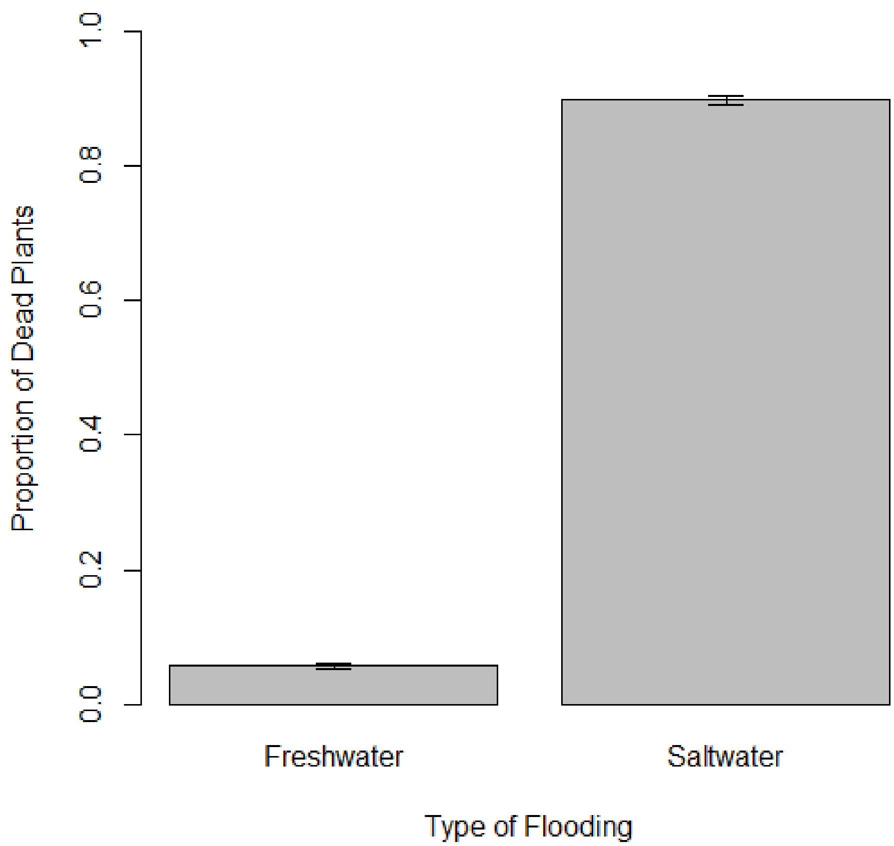

Figure 4.8: Proportion of dead plants after flooding. The freshwater flood was caused by Hurricane Joaquine in October 2015, while the saltwater flood was caused by a saltwater surge from Hurricane Matthew in October 2016. Error bars representing standard error.

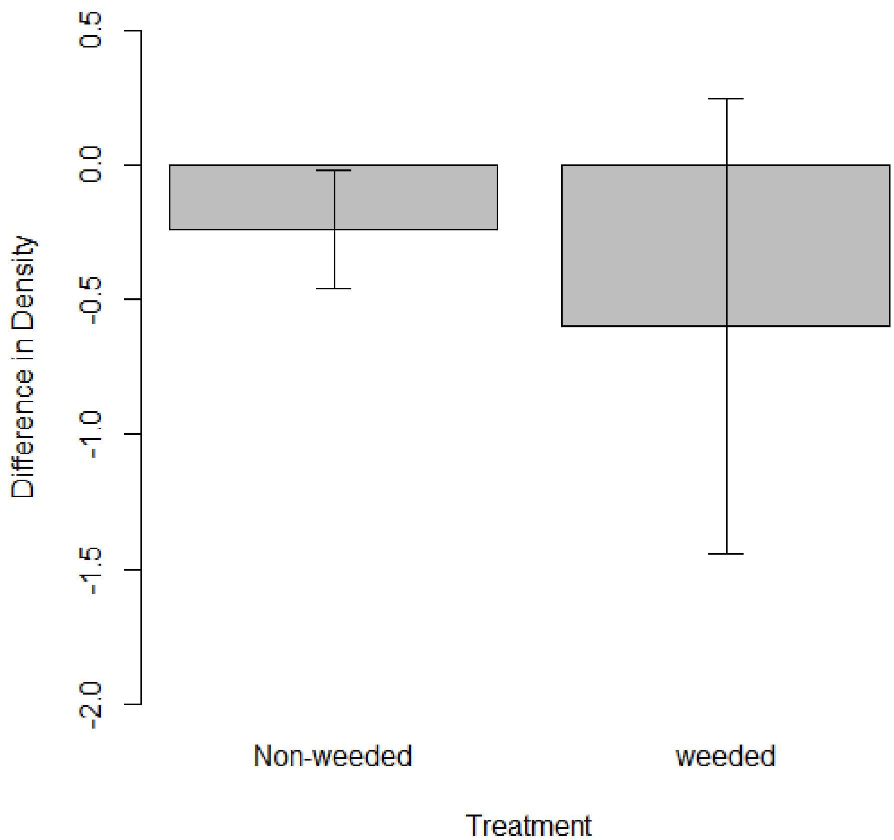

Figure 4.9: Difference in density of plants after the weeding or non-weeding treatment from 2016 to 2017. Error bars representing standard error.

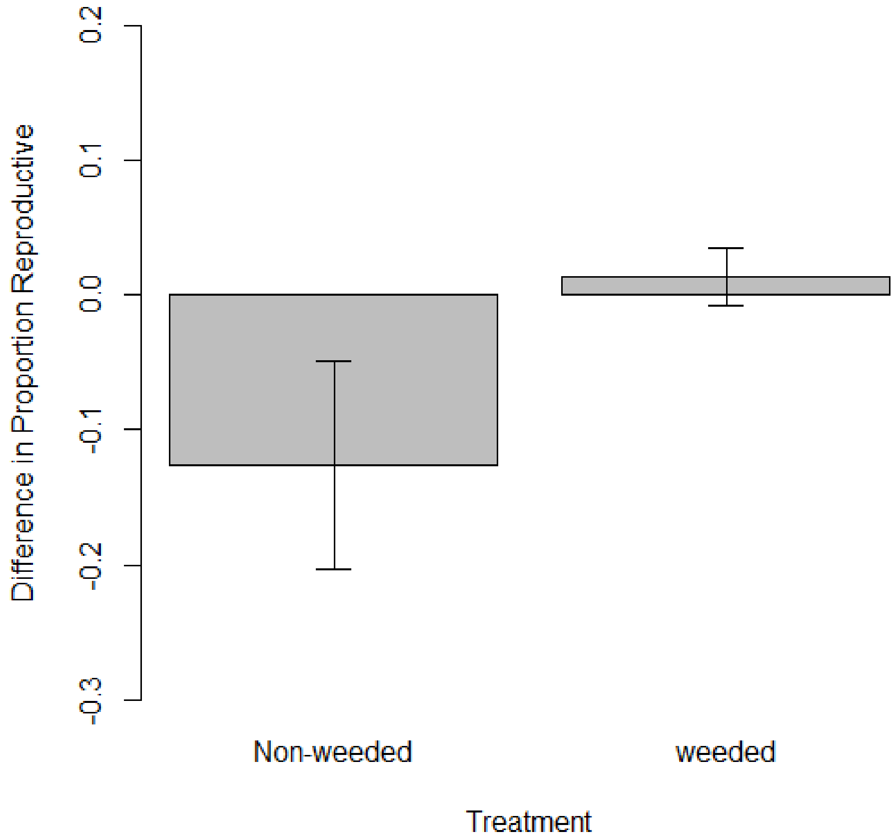

Figure 4.10: Difference in proportion of reproductive plants after weeding or non-weeding treatment from 2016 to 2017. Error bars representing standard error.

Reproductive Potential

Analyzing a TZ test was conducted by examining the color produced by the seed. A deep red stain indicates that tissues are normal, abnormal tissues stain a lighter color, while no stain denotes dead tissues. After observing the dissected seeds under the microscope, I found that of the 40 seeds tested 9 stained red, 12 turned light pink/ peach and 19 did not stain at all. This results in 22.5% normal tissues, 30% abnormal tissues and 47.5% dead tissues. Figure 4.11 shows examples of dead (seed A and B), normal (seed C) and abnormal tissues (seed D).

After examining the underground structure of *S. caroliniana* I found that in addition to plant roots, several plants also had rhizomes. These vigorous, slender, and very pale rhizomes produce new plants in the following growing season. Figure 4.12 is a specimen from the USCH collection and shows the underground structure of *S. caroliniana*.

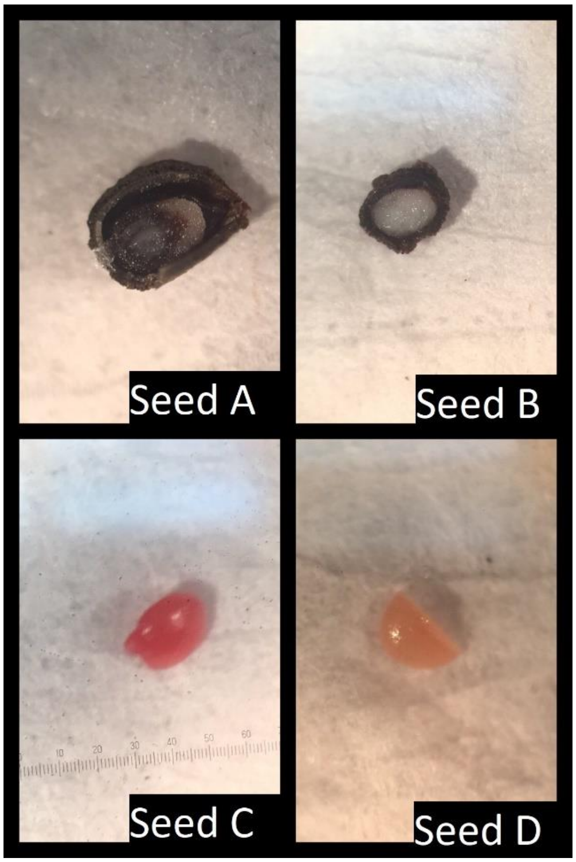

Figure 4.11: Results of a tetrazolium chloride staining test. Seed A and B are examples of seeds with dead tissues, seed C has living tissue and seed D has abnormal tissues.

Figure 4.12: *S. caroliniana* herbarium specimen USCH 98306. Rhizomes were found in the underground plant structure.

CHAPTER 5

DISCUSSION

The number of plants in subpopulation A drastically declined from 2016 to 2017. Plant density decreased by 2.28 stems/m^2. This is believed to be a result from weeding an area with a low level of shade. Clearing out competing species from this subpopulation removed most of the cover and protection given to *S. caroliniana*. This exposed the plants to the natural elements of the environment.

Subpopulation B was also weeded and the number of plants slightly increased between 2016 and 2017. Plant density increased by 0.27 stems/m^2. However, this area provided more shade compared to subpopulation A, suggesting that the canopy of the overstory was able to shelter *S. caroliniana* from adverse environmental elements. It is probable that given more time for this study, plant density would significantly increase in this area.

The number of plants in subpopulation C did not differ from 2016 to 2017. This area was exposed to the same level of shade as subpopulation A, but it was not weeded. This implies that competing species in this area acted as a protective barrier for *S. caroliniana*. If the study was continued over a longer timeframe, it is likely that competing species would eventually become too dense and it would choke out *S. caroliniana*.

Subpopulation D was not weeded and had medium level of shade. The number of plants slightly declined from 2016 to 2017. Plant density decreased by 0.04 stems/m^2.

The combination of shade abundance in addition to competing species could become detrimental to this subpopulation in future years.

Subpopulation E and F were both exposed to high levels of shade, but subpopulation E was not weeded. The number of plants from 2016 to 2017 in E slightly decreased while F slightly increased. Plant densities changed -0.67 stems/m^2 and 0.22 stems/m^2 respectively. This suggests that the removal of competing species is beneficial for *S. caroliniana* in high shaded areas.

Suitable Habitat

The plant community surrounding *S. caroliniana* represent habitat types of hardwood sloughs and freshwater ponds. Obtaining a floristic inventory of the area surrounding a rare plant is crucial when determining the species' habitat. Knowing the type of habitat the species flourishes in, may aid in locating other areas where the species could be found.

Influence of Shade

The first hypothesis was supported from the results. This test determined that there was a statistically significant difference of population density among the three levels of shade. The bar graph in Figure 3 confirmed that plant density was greater in areas with higher amounts of shade.

Overstory trees are able to provide essential shade, nutrients, and protection for the facilitation of understory herbs (Callaway and Walker, 1997). As described by Belsky (1994), benefits of shade include reducing temperatures and evapotranspiration of species that grow beneath tree crowns. Tree litter and feces of animals inhabiting the trees increase nutrients by fertilizing the soil, enabling increased herbaceous productivity (Belsky, 1994).

Ellison and Houston (1958) recognized the positive effects that an overstory, in their study predominantly made of aspen, can have on understory plants. Herbaceous productivity was much higher under the canopy of aspen compared to the adjacent open grassland. The type of overstory species, number of trees, as well as root density, greatly impact the ability to facilitate understory plants. Callaway *et al*. (1991) found that biomass of understory species correlates inversely with root biomass from trees. Trees with low fine-root biomass in the upper soil horizon facilitate greater understory biomass, while high root-biomass in the upper soil horizon limits the growth of understory species.

Response to Disturbance

Type of Flooding

The test comparing the effect of different types of flooding on *S. caroliniana* supports the second hypothesis. There is a statistically significant difference between the proportion of dead plants after freshwater and saltwater inundation. As shown in Figure 4.8, saltwater inundation killed nearly all of the above ground stems, whereas freshwater had a much lesser effect.

Saltwater intrusions are expected to increase in areas that are historically freshwater environments as sea level rises. Neubauer (2013) studied the environmental responses of a freshwater marsh to elevated salinity and increased water inputs. Environmental responses contrasted between saltwater intrusions and increased freshwater flow. The net ecosystem production decreased by 55% when salinity was increased, while net ecosystem production increased by 75% when exposed to additional freshwater. However, there was no change in net ecosystem production when both salinity and freshwater inputs were increased (Neubauer, 2013).

Freshwater wetland plants are negatively affected by saltwater intrusion. It is evident that the increased concentration of ions and metabolic products reduce plant growth and productivity. Neubauer (2013) also found that species composition drastically changed in plots that were exposed to elevated salinity; species richness was two times greater in the control plots, which contained freshwater. Plant species richness and diversity are generally higher in freshwater wetlands compared to brackish and salt marshes (Neubauer, 2013).

Increased inundation from freshwater typically leads to a decrease in plant photosynthesis and productivity due to stress from reduced O_2 in the soil. Certain species grow better when exposed to increased water input, while others had a negative response. This is dependent on the individual species' tolerance to flooding. Overall, species richness was not greatly affected from increased flooding, there were no noticeable differences when compared to control plots (Neubauer, 2013).

Competing Species

The difference in plant density from 2016 to 2017 was compared between non-weeded and weeded subpopulations. The t-test determined that there was no statistically significant difference in plant density between weeding treatments. The difference in proportion of reproductive plants from 2016 to 2017 was also compared between the non-weeded and weeded subpopulations. Wilcoxon rank sum test indicated that there is no statistically significant difference in proportion of reproductive plants between weeding treatments. These results refute the third hypothesis that states weeding will be beneficial to the species survival. From this study, it can be concluded that there is no evidence that removing competing plant species affects the survival of *S. caroliniana*. In order to get an accurate response to competitive species, the weeding treatment analysis should extend over a longer trial period.

Competitive interaction of plant species is a very complex process. A study by Callaway and Walker (1997) found that the life stage of the plant greatly effects a species' response to other plants. Many studies have shown that seedlings benefit from shelter that is provided from another adult species. However, when the beneficiary species are older and require more resources, competitive interactions between the species will arise. The species that once supported plant growth of another, may hinder that species' ability to continue to grow (Callaway and Walker, 1997).

Reproductive Potential

Seeds with normal tissues are considered to be viable and seeds that have abnormal or dead tissues are considered to be non-viable. The TZ test revealed that only 22.5% of the seeds tested were viable and 77.5% were non-viable. After analyzing the underground structure of *S. caroliniana,* it was evident that many plants possessed rhizomes. These rhizomes run horizontally and give rise to new stems in the next growing season. The fourth hypothesis is proven correct, it is concluded that *S. caroliniana* mainly reproduces vegetatively by the spreading of rhizomes. Knowing the reproductive potential of *S. caroliniana* will aid in implementing management decisions for this plant.

5.1 FUTURE STUDIES

The population that was recently rediscovered at the Santee Coastal Reserve in Charleston County needs to be revisited. Population assessments that were studied for *S. caroliniana* at the TYWC also need to be evaluated for this location. Observing suitable habitat, types of disturbance and reproductive potential will aid in determining overall population dynamics of *S. caroliniana*.

Additional seed viability tests need to be studied for *S. caroliniana*; only a small random sample of 40 seeds were tested from the Georgetown County population. A larger sample size would yield more accurate results of seed viability. Furthermore, it would be interesting to determine if there is variability in seeds among subpopulations. Seed germination tests in either seed flats or petri dishes should also be conducted to test the accuracy of the TZ staining method. It would also be interesting to test the effect that increased salinity in soils has on the success of germination of *S. caroliniana*. Seeds should also be collected and tested from the population at the Santee Coastal Reserve location.

S. caroliniana grows in a marginally disturbed area along a wetland that is frequented by vehicles and foot-traffic of staff. It is probable that this species benefits from intermediate disturbance. Prescribing a low intensity fire would remove competitive species and could allow *S. caroliniana* to expand its population size. Certainly, this disturbance should not occur during the growing season of the plant, but in the few months before the plants emerge. A study of optimal time of year for fire regimes would benefit the management of this species.

As stated earlier, SDMs, LiDAR and GIS can be used to predict sites that would be appropriate to introduce and translocate rare species as well as detect locations of unknown populations. LiDAR images should be obtained for the two locations of *S. caroliniana* and used to help create a HSM. This model should be used as a guide for locating areas to survey for additional populations. They would most likely be found near other freshwater wetlands on the TYWC and along the Santee River.

Translocations include removing and transplanting the species from an original population, which may be problematic if there is only a single, small source population. On the other hand, plant introduction refers to establishing a new population from *ex situ* material, such as seedlings grown in a greenhouse. Introduction strategies have been used by conservationists to enhance the probability of a species survival. Establishing several new populations greatly reduces the chance of extinction. First, propagule type has to be determined, either plant directly from seed or transplant green-house grown plants (Guerrant and Kaye, 2007). In an introduction study of several plants, Guerrant and Kaye (2007) found that transplants had a higher success rate than just sowing seed. However, the latter is less time consuming and a more cost effective method.

For *S. caroliniana* it would be ideal to implement the plant introduction method before translocating plants from their original site, since only one population is known. However, this will depend on the success of additional studies of seed germination. Since this species mainly reproduces vegetatively, then translocating individual plants from the original population could be successful. There are several other factors that also need to be determined, such as how many plants are needed to successfully establish a new population, and how many total populations should be planted. Dispersal ability of the species is also important to understand so that optimal distance between the populations can be determined.

CHAPTER 6

CONCLUSION: MANAGEMENT RECCOMENDATIONS FOR *S. CAROLINIANA*

The survival of the *S. caroliniana* J.B. Nelson & D.A. Rayner, population located at the Tom Yawkey Wildlife Center is dependent upon South Carolina Department of Natural Resources, as the managers of one of the most rare and potentially endangered plant species on the southeastern coast of the United States. Steps should be taken in order to federally recognize and protect *S. caroliniana* under the Endangered Species Act. Based on the knowledge of other *Stachys* species, as well as information obtained from this study, prescriptive management recommendations are given below.

Continual Monitoring – Since there are only two known locations of *S. caroliniana*, the continued monitoring of this small population is imperative for the species' survival.

Additional Research – Studies including seed germination, introduction and translocation of plants, as well as response to disturbance needs to be investigated. Surveys for unknown populations should be organized for North, South, and Cat Island, as well as along the upstream stretches of the Santee River. Initial areas to inspect ought to be characteristic of suitable habitat, such as impoundments, hardwood sloughs, borrow pits, and ponds. Surveying in the summer months would be ideal, since plants will be in bloom and easier to recognize. A select number of areas that are thought to be suitable for *S. caroliniana* should be managed for experimental translocation studies. These areas would be ideal periodic monitoring and quantitative studying.

Site Preparation – Moderate disturbance may be beneficial to increasing the size of the population. Prescribing a fire regime or administration of weeding competitive species in the months leading up to the emergence of *S. caroliniana* should to be considered.

Restricted Grounds Maintenance – The area where *S. caroliniana* is located has been subject to substantial mowing and herbicide use in the past. Mowing should not occur during the growing season of the species and herbicide use should be eliminated from routine maintenance all together.

Limited Access – Access to the TYWC is already heavily restricted from the public. However, during educational tours or maintenance work from staff, the population of *S. caroliniana* should be protected from vehicular disturbance, as well as foot-traffic.

Public Awareness – S. caroliniana has not received the attention that is warranted for the rediscovery of a rare plant. Educating the public is crucial for raising awareness for *S. caroliniana*, not only about the importance of conserving this species, but the significance of sustaining biodiversity as a whole.

CHAPTER 7

CONSERVATION SIGNIFICANCE

Stachys species are extremely diverse, studies have found variation of pharmacological properties of the biologically active substances among *Stachys* (Kartsev and Stepanichenko, 1994). Essential oils of many *Stachys* species have been studied for their medicinal properties. In a study of twenty-two *Stachys* species from Turkey, Goren *et al*. (2011) found that certain species of *Stachys* can be used as antibacterial agents. In Anatolia and Iran, some *Stachys* are used in herbal teas, “mountain tea”, to treat stomach disorders or are applied as tonics to treat skin. *S. inflata* is used in Iranian folk medicine and is thought to aid in infection, asthma, rheumatic and inflammatory disorders. Digestive disorders are treated with *S. lanvandulifolia*, while *S. recta* is used in Iran as a healing agent for wounds (Goren *et al*., 2011).

Of the 37 species of *Stachys* that grow in the Russian Federation, 12 are used in medicine (Legkobit and Khadeeva, 2004). Above ground stems and leaves from *S. officinalis* are used to treat bronchial asthma. In China and Japan, *S. seboldii* has been cultivated and used as an annual crop (Kocheiva *et al*., 2006). The underground tubers of *S. seboldii* are edible and also provide antibacterial properties (Legkobit and Khadeeva, 2004).

There are many other valuable features of *Stachys*, including cultivation for ornamental purposes (Kocheiva *et al*., 2006; Legkobit and Khadeeva, 2004). *S. lanata* and *S. germanica* are commonly used in floriculture. The fatty oils produced from seeds of various *Stachys* are used in varnish production. Natural insecticides and paints can be obtained from the leaves of *S. sylvatica* (Kocheiva *et al*., 2006). Nearly all of species of *Stachys* produce nectar, such as *S. palustris*, providing a valuable nectar source for insects (Taylor and Rowland, 2011).

The conservation of *S. caroliniana* is extremely important due to the unknown services that this species may be able to provide, including medicinal uses. Not only is it important to conserve this species for anthropogenic use, but for biodiversity as well.

Importance of Biodiversity

If additional research of *S. caroliniana* concludes that the species does not provide any direct services, such as medicinal use, this species still contributes to biodiversity as a primary producer, which represents the basal component of most ecosystems and provides several ecosystem services. Studies show a positive trend of ecosystem functioning as biodiversity increases (Loreau *et al*., 2001). Even in certain ecosystems where high biodiversity is not required to maintain ecosystem processes, it may be important for maintaining them under stochastic events. The insurance hypothesis suggests that high biodiversity creates a ‘buffer’ against environmental change, this is because various species respond differently to these changes. Variation among species insures ecosystems against declines in their functioning (Loreau *et al.*, 2001).

In a healthy ecosystem, plants help produce goods such as food, fuel, fresh water, regulate climate, resistance to disturbance, water flow, erosion control, and sediment retention. Environmental services such as soil formation, nutrient cycling, and primary production support the production of other ecosystem services (Costanza *et al.*, 1997). Non-material services can also be appreciated from ecosystems as well, including cultural, educational, scientific, and recreational, just to name a few (Costanza *et al*., 1997).

REFERENCES

Belsky, J. (1994). Influences of Trees on Savanna Productivity: Tests of Shade, Nutrients, and Tree-Grass Competition. *Ecology*, 75(4), 922-932.

Borza, J.B., Westerman, P.R. and Liebman, M. (2007). Comparing Estimates of Seed Viability in Three Foxtail (*Setaria*) Species Using the Imbibed Seed Crush Test with and without Additional Tetrazolium Testing. *Weed Technology*, 21, 518-522.

Bourg, N.A., McShea, W.J. and Douglas, G.E. (2005). Putting a CART Before the Search: Successful Habitat Prediction for a Rare Forest Herb. *Ecological Society of America*, 86(10), 2793-2804.

Bowles, Marlin and Whelan, Christopher. (1994). Restoration of Endangered Species: Conceptual Issues, Planning and Implementation. *Cambridge University Press*, *New York*, 159-160.

Callaway, R.M. and Walker, L.R. (1997). Competition and Facilitation: A Synthetic Approach to Interactions in Plant Communities. *Ecology*, 78(7), 1958-1965.

Callaway, R.M., Nadkarni, N.M. and Mahall, B.E. (1991). Facilitaiton and interference of Quercus douglasii on understory productivity in central California. *Ecology*, 72, 1484-1499.

Center for Plant Conservation (CPC). (2017). *Oxypolis canbyi*. https://saveplants.org/national-collection/plant-search/plant-profile/?CPCNum=3064

Constantinidis, T., Kalpoutzakis, E. and Kougioumoutzis, K. (2015). The rediscovery of *Stachys virgata* (Lamiaceae), a rare endemic of Peloponnisos, Greece: taxonomy, distribution, karyology and conservation. *Phytotaxa,* 218 (3), 241-252.

Costanza, R., d'Arge, R., Groot, R., Farber, S., Grasso, M., Hannon, B. *et al*. (1997). The Value of the World's Ecosystem Services and Natural Capital. *John Wiley & Sons*, 118-120.

Ellison, L. and Houston, W.R. (1958). Production of herbaceous vegetation in openings and under canopies of western aspen. *Ecology*, 39, 338-345.

Fuhlendorf, S. D. and Engle, D. M. (2004). Application of the fire–grazing interaction to restore a shifting mosaic on tallgrass prairie. *Journal of Applied Ecology*, 41, 604–614.

Gill, L.S. (1980). Cytotaxonomy of the Genus Stachys L. in Canada. *Caryologia*, 33(4), 473-481.

Goren, A., Piozzi, F., Akcicek, F., Kilic, T., Carikci, S., Mozioglu, E. and Stezer, W. (2011). Essential oil composition of twenty-two Stachys species (mountain tea) and their biological activities. *Phytochemistry Letters*, 4(4), 448-453.

Guerrant, E. and Kaye, T. (2007). Reintroduction of rare and endangered plants: common factors, questions and approaches. *Australian Journal of Botany*, 55, 362-370.

Guisan, Antoine and Thuiller, Wilfried. (2005). Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, 8, 993-1009.

Kartsev, V.G. and Stepanichenko, N.N. (1994). Chemical Composition and Pharmacological Properties of Plants of the Genus *Stachys*. *Chemistry of Natural Compounds*, 30(6), 695-696.

Kochieva, E.Z., Ryzhova, N.N., Legkobit, M.P. and Khadeeva, N.V. (2006). RAPD and ISSR Analyses of Species and Populations of the Genus *Stachys*. *Russian Journal of Genetics*, 42(7), 723-727.

Lee, Jim. (2016). Personal Communication.

Legkobit, M.P., and Khadeeva, N.V. (2004). Variation and Morphogenetic Characteristics of Different Stachys Species during Microclonal Propagation. *Russian Journal of Genetics*, 40(7), 743-750.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J., Hector, A., Hooper, D. (2001). Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. *Science*, 294, 804-808.

McKinney, Michael L. (2008). Effects of urbanization on species richness: A review of plants and animals. *Urban Ecosystems*, 11(2), 161-176.

Nelson, J.B. (1981). Stachys (Lamiaceae) in southeastern United States. *Sida,* 9(2), 104-123.

Nelson, J.B. and Rayner, D.A. (2014). A new hedge-nettle (*Stachys*: Lamiaceae) from South Carolina. *U.S.A. J. Bot. Res. Inst. Texas*, 8(2), 431 – 440.

Nelson, J.B. (2016). Personal Communication.

Neubauer, S.C. (2013). Ecosystem Response of a Tidal Freshwater Marsh Experiencing Saltwater Intrusion and Altered Hydrology. *Estuaries and Coasts*, 36, 491-507.

Norden, Heather. (2002). Species Biology and Life History of the Federally Endangered *Schwalbea americana*. 1-91.

Questad, E.J., Kellner, J.R., Kinney, K., Cordell, S., Asner, G.P., Thaxton, J., *et al*. (2014). Mapping habitat suitability for at-risk plant species and its implications for restoration and reintroduction. *Ecological Applications*, 24(2), 385-395.

Roxburgh, S.H., Shea, K., and Wilson, J.B. (2004). The Intermediate Disturbance Hypothesis: Patch Dynamics and Mechanisms of Species Coexist. *Ecology*, 85(2), 359-371.

Salmaki, Y., Zarre, S. and Jamzad, Z. (2008). Nutlet micromorphology and its systematic implication in *Stachys* L. (Lamiaceae) in Iran. *Wiley InterScience*, 119, 607-621.

Sawma, J.T. and Mohler, C.L. (2002). Evaluating Seed Viability by and Unimbibed Seed Crush Test in Comparison with the Tetrazolium Test. *Weed Technology*, 16, 781-786.

South Carolina Native Plant Society (SCNPS). (2017). Lisa Mathews Memorial Bay. http://scnps.org/activities/matthews-bay/

Taylor, K. and Rowland, P. (2011). Biological Flora of the British Isles: *Stachys palustris* L. *Journal of Ecology*, (99), 1081-1090.

Tropicos.org. (2017). Missouri Botanical Garden. http://www.tropicos.org

Tucker, A.O. and Dill, N.H. (1983). Nomenclature, Distribution, Chomosome Numbers, and Fruit Morphology of *Oxypolis canbyi* and *O. filiformis* (Apiaceae). *Systematic Botany*, 8(3), 299-304.

Wilcock, C. (1974). Population Variation, Differentiation and Reproductive Biology of *Stachys palustris* L. *S. sylvatica* L. and *S.* x *ambigua* SM. *New Phytol*., (73), 1233-1241.

Wiser, Susan K., Robert K. Peet, and Peter S. White. (1998). Prediction of Rare-Plant Occurrence: A Southern Appalachian Example. *Ecological Appliactions*, 8(4), 909-920.

Yawkey Foundations. (2015). Thomas A. Yawkey. http://yawkeyfoundations.org./thomas_yawkey.html

Young, A., Boyle, T., and Brown, T. (1996). The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution*, 11(10), 413-418.