17 years later: Changes in Vegetation Structure, Burrow Dispersion and Size-class Distribution at a Gopher Tortoise *(Gopherus polyphemus)* Site in Alabama

by

Rebecca C. Pudner

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Approved by

Sharon Hermann, Chair, Assistant Professor of Biological Sciences
John Kush, Research Fellow in Forestry and Wildlife Sciences
Craig Guyer, Professor Emeritus of Biological Sciences

Abstract

There are few studies that reevaluate vegetation structure at a gopher tortoise (Gopherus polyphemus) site after a long period of time in the southern Alabama or Florida panhandle portion of their range. The current study revisits research conducted in 1999 by J.H. Waddle at a study site in Conecuh National Forest in southern Alabama 17 years later. Three types of comparisons of vegetation structure were made: 1999 random points versus 2016 random points, burrows that were active in 1999 and remained active in 2016, and burrows that were active in 1999 but became abandoned in 2016. Compared to 1999 data, in 2016 all plot types had significantly less litter and forbs, and more legumes and shrubs in the understory and more hardwood stems in the midstory. Active burrows that stayed active had no hardwood trees present in either 1999 or 2016. The original study suggested that tortoises were likely selecting the best habitat available for burrow construction in 1999 while this study indicates that habitat may have become more homogenous with few high quality areas to select from.

A 3.5-fold increase of the total number of active and inactive burrows was found in the resampled area, indicating either a robust population or one compensating for declining access to forage by increasing number of locations used. The burrow size distribution shifted to bi-modal from uni-modal, indicating a healthy, recovering population. In addition, shifts in spatial relationships among burrows were considered. I used this burrow survey data to estimate tortoise density and evaluate this population according to requirements of a minimum viable population.

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Chapter 1

Introduction

Long-lived organisms, like turtles, inherently warrant long-term studies (Tuberville et al. 2014). However, the extended lifespan of turtle species makes them particularly difficult to study (Gibbons 1987). Generally, long-lived species often have delayed maturation and can be slow to recover from disturbances (Congdon et al. 1993, Wheeler et al. 2003) These and other related characteristics of long-lived species make it difficult to evaluate anthropogenic impacts and may result in unique conservation challenges (Spencer and Janzen 2010).

An important example of a species of special concern is the gopher tortoise (*Gopherus polyphemus*), an herbivorous, terrestrial turtle restricted to the Coastal Plain of the southeastern United States. This species ranges from eastern Louisiana to southern South Carolina is the only tortoise species east of the Mississippi River (Auffenberg and Franz 1982, Tuberville et al. 2014). It is a fossorial species that may live 50 or more years (J. Goessling, Auburn University, unpublished data), and females in southern Alabama may not mature until they are 9-21 years (Aresco and Guyer 1999a). Gopher tortoises are federally listed as threatened under the Endangered Species Act west of the Tombigbee River in Alabama and a candidate for listing in the eastern part of the range (U.S. Fish and Wildlife 2008; Figure 1). In Alabama alone, less than 6.5% remain of an estimated 2 million tortoises once common in the state (Guyer et al. 2011).

Significance of gopher tortoise burrows- Although gopher tortoise populations are in decline, the species remains ecologically important. Known as both an ecosystem engineer and keystone species of the longleaf pine (*Pinus palustris*) ecosystem, gopher tortoises dig extensive burrows that can provide refuge for more than 330 other species (Kinlaw and Grasmeuck 2012, Catano and Stout 2015; Witz et al. 1991). These commensals include other rare species, such as the gopher frog (*Lithobates capito*), Florida mouse (*Podomys floridanus*), the Florida pine snake (*Pituophis melanoleucus mugitus*) and the eastern indigo snake (*Dymarchon couperi*). Burrows are used by tortoises to avoid predation, escape fire and smoke and maintain constant temperature and humidity (Douglass and Layne 1978). Gopher tortoises often bask in the open, sandy apron of the burrow, as well as occasionally create nests there (Diemer 1986). On average, an individual tortoise will create 2.5 burrows per site per year, and may use up to 10 burrows over a year (Guyer et al. 2012, Eubanks et al. 2003). They often use the same burrows for years and when abandoned, burrows may persist for decades before completely filling in (Guyer and Hermann 1997).

General habitat characteristics- High quality gopher tortoise habitat is defined by a set of metrics that includes soil type, canopy openness, and the historical range of the longleaf pine ecosystem. Tortoises prefer well-drained, deep sandy soils for burrowing (Diemer 1986). Although other ecosystems, such as central Florida scrub, may offer good quality habitat, longleaf pine dominates over much of the species' range. The structure of the longleaf pine ecosystem provides a habitat with a mostly herbaceous groundcover suitable for forage (Auffenberg and Franz 1982, Tuberville et al. 2014) and an open canopy providing abundant sunlight at the ground level for basking and thermoregulation, and nesting (Castellón et al. 2012, Yager et al. 2007, Douglass and Layne 1978).

Habitat quality and decline- Although it is well-understood that appropriate habitat conditions are critical for viable gopher tortoise populations, detailed information on this topic is often lacking or is described in general terms. Studies that estimate population densities often include site descriptions but less commonly provide measurements of vegetation components or habitat structure (e.g. Ashton et al. 2008, Castellón et al. 2012, Hermann et al. 2002, Jones and Dorr 2004, Yager et al. 2007). In these and other studies that estimate population densities site descriptions are often provided but and it is less common that measurements of vegetation components and/or habitat structure are provided and studies that target Alabama or the Florida panhandle are limited. Although these studies are somewhat narrow in scope, they provide guidance for the current study, which focuses on a site in south Alabama

An awareness that habitat is critical to gopher tortoises has not yet resulted in efforts to promote a range-wide effort to improve conditions. In fact, habitat loss is a significant factor in the decline of the gopher tortoise (Diemer 1986). Longleaf pine ecosystems have been lost from 97% of its historical range, and persist only in isolated, often small fragments (Frost 1993, Ware et al. 1993), severely limiting available gopher tortoise habitat. Although the lower Coastal Plain of the southeastern United States historically burned every 2-4 years (Guyette et al. 2012), in the modern landscape, the remaining habitat is frequently excluded from fire, altering the open canopy structure (Van Lear et al 2005). Tortoises in these degraded sites usually abandon their burrows as canopy cover increases (Aresco and Guyer 1999b). Auffenberg and Franz (1982) reported that after 16 years with no fire at a site, all tortoises will likely be extirpated

Long-term studies- While there are studies that evaluate changes in gopher tortoise demography over long periods of time (e.g. Ashton et al. 2008, Diemer Berish et al 2012) there appears to be only one that also provides information on long-term quantitative habitat changes (McCoy et al. 2006), and none that reevaluated population and habitat in the Alabama/Florida panhandle area. Studies that document changes in habitat spanning only a few seasons or even years may fail to capture the long-term effects on gopher tortoise population size and demographics (Yager et al. 2007). In addition, lag time and legacy effects of previous habitat changes or anthropogenic effects may not be accounted for (Tuberville et al. 2014). The work described in the current thesis compares data from two points in time that span 17 years. This much longer-term study provides information previously unavailable for this portion of the gopher tortoise range.

Current research- The purpose of the current study was to investigate changes in vegetation structure at a gopher tortoise site first sampled in 1999, and tortoise responses to these changes 17 years later. In Chapter 2, I provide information on reevaluated vegetation structure and burrow activity status at both a subset of burrows that were active in 1999 and random control points at this site in Conecuh National Forest in southern Alabama (Waddle 2000; Figure 1). I present three comparisons that illustrate changes in vegetation over time: random points in 1999 and newly created random points in 2016, burrows that were active in both 1999 and active in 2016, and burrows that were active in 1999 but became abandoned by 2016. In Chapter 3, I provide information on population viability and present the results of repeating a complete survey of active and inactive burrows. I compare burrow density and burrow dispersion between the sampling years, as well as estimate tortoise density. Burrow size-class distribution was compared with previously published data from this same site collected at nearly the same time period (Guyer et al. 2012). With so little appropriate habitat remaining, it is increasingly important to assess the relationship between vegetation structure and tortoise populations, so that this information can be utilized by biologists and land managers to both maintain quality habitat and implement restoration techniques in areas of past and potential habitat.

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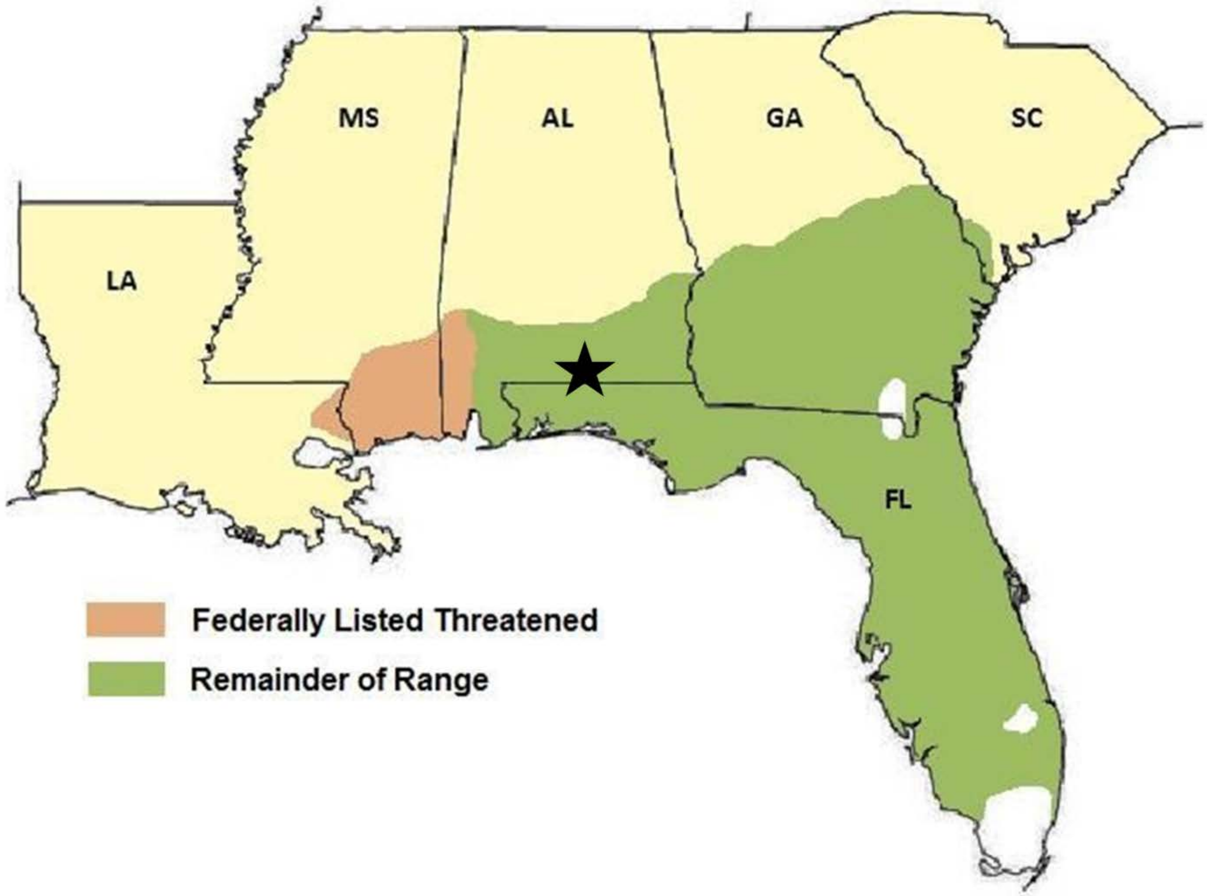

Figure 1. Study site location designated with a star within the range of the gopher tortoise. Adapted from http://www.gophertortoisecouncil.org/gt.

Chapter 2

Changes in Vegetation Structure at a Gopher Tortoise Site after 17 Years

Introduction

Studies have correlated vegetation and habitat characteristics with current gopher tortoise populations and burrow status (Hermann et al. 2002, Jones and Dorr 2004, Castellón et al. 2012, McCoy et al. 2013.)   In addition to sandy soil types, tortoises use habitats that have an open canopy, open midstory and exposed mineral soil. Frequent application of prescribed fire is used to manage these conditions at such sites (Jones and Dorr 2004, Aresco and Guyer 1999b, Ashton et al. 2008). Gopher tortoises are historically associated with the longleaf pine (*Pinus palustris*) ecosystem (Jones and Dorr 2004) which is characterized by a savanna structure of a sparse overstory with an herbaceous understory. Groundcover plants are of particular interest to those studying tortoise habitat because gopher tortoises are exclusively herbivorous (MacDonald and Mushinksy 1988). The closure of the canopy, or the reduction in distance between overstory trees and corresponding reduction in sunlight that penetrates to the forest floor, has been found to be the primary driver of burrow abandonment in pine plantations (Aresco and Guyer 1999a), however tortoises have been shown to persist on sites that are actively managed for timber production for more than 15 years, utilizing the most open areas available which tend to be roadsides and other ruderal areas (Diermer Berish et al. 2012).

Few studies have correlated gopher tortoise response to habitat changes (Aresco and Guyer et al. 1999a, Yager et al. 2007). However, no long-term research has repeatedly sampled a gopher tortoise site beyond a time frame of ten years (McCoy et al. 2006), and none specifically in the southern Alabama/Florida panhandle area of their range. My study at

Conecuh National Forest (CNF) in south Alabama utilized a 17-year-old vegetation and tortoise activity data from Waddle (2000) in addition to a new dataset collected during summer 2016 to address the following questions: 1) What changes in vegetation structure occurred and 2) How did the status of active tortoise burrows change over time?

Methods

Study Site **–** This study was conducted on the Conecuh National Forest (CNF) at a sandhill site located in Covington County, AL, near the town of Bradley in southwest Covington County (Figure 1). The sandhill is naturally bounded by Blackwater Creek to the west and north, with private agricultural land to the east, and a seasonally wet drain to the south. The 2016 study area was restricted to 23.11 hectares north of the drainage. Soil type at the site is Troup loamy sand (NRCS Soil Survey Staff, 2016), a priority gopher tortoise soil (Guyer et al. 2011).

By the 1960s, the site had been logged and only scattered large longleaf pine trees remained (J.R. Lint, CNF, pers. comm. in Aresco and Guyer 1999a). In 1973 it was clear-cut, site-prepped and planted with slash pine (*Pinus elliottii*). The first thinning likely occurred in 1993, and it was thinned again in 2007 (Steve Johnson, CNF, pers. comm.). Tortoise study sites in CNF, including this site, were burned during the winter every 3-4 years from 1985-1996 (J.R. Lint, CNF, pers. comm. in Aresco and Guyer 1999a) and burned in the growing season between 1997 and 2008 every 3-4 years (Guyer et al. 2012). The study site was last burned in April 2013 and is expected to be burned during the 2016-2017 dormant season (Steve Johnson, CNF, pers. comm.).

Currently, the canopy of the study site is open and dominated by both longleaf and slash pine. Occasional hardwood hammocks with mature water oaks (*Quercus nigra)*, flowering dogwood (*Cornus florida*) and darlington oak (*Quercus hemispherica*) are scattered throughout. The habitat is patchy, with some open areas of bare sand, including an area with a continuous carpet of wiregrass (*Aristida beyrichiana*). A conspicuous midstory of dense sand live oak (*Quercus geminata)* and dwarf live oak (*Quercus minima*) occurs in patches throughout the site with heights varying from less than 1 meter to over 3 meters. Typical sandhill oaks such as bluejack oak (*Quercus incana*), turkey oak (*Quercus laevis*) and sandpost oak (*Quercus margaretta*) compose the remaining midstory species, with occasional small thickets of *Vaccinium spp*. and yaupon (*Ilex vomitoria*).

Previous Sampling- I used data provided by Hardin Waddle and published in his thesis (Waddle 2000). Waddle studied gopher tortoises at the same CNF site, however his work included an additional area south of the drain in his 1999 study (Figure 1). At the time of his 1999 sampling, both areas shared the same management history, however the southern area was clearcut in 2011 and not included in my study. In 1999, he measured the width of each tortoise burrow at the site and the location was mapped with a GPS. Status of each burrow was recorded as being active, inactive, or abandoned, using the definitions of Mushinsky and McCoy (1994). Data describing vegetative structure were collected at 50 active burrows marked with a metal tag, and at 50 randomly chosen points (not tagged) at least 10m from the nearest burrow. At each burrow or random point Waddle measured aspects of the groundcover, midstory, and overstory vegetation. A 1m x 1m sampling frame with 100 uniformly distributed points was used to characterize the understory. For tortoise burrows, the entrance to the burrow was considered to be a single point with the direction of the burrow entrance creating an axis through this point generating front, back, right, and left directions. The sampling frame was placed to four positions, each 3 m from the central point and representing the four directions.

For random points, the “front” position was chosen randomly and the other 3 positions placed 90 degrees apart. At each point within the sampling frame, plants subtending a sample point were categorized as wiregrass, other grasses, legumes, other forbs, woody vines, shrubs, longleaf pine, bracken fern (*Pteridium aquilinum*), litter or bare ground. These count data were converted to percent cover. Midstory was evaluated within a 10m diameter circular plot with the same center point for each burrow. Woody stems greater than 1m tall but less than 10cm diameter at breast height (DBH; 1.37m) were tallied by species. Canopy cover was estimated using a Type-C spherical densitometer held at a height of 1m directly over the burrow entrance or at each random point. These count data were converted to percent cover (Lemmon 1956). All trees within the circular plot that were 10 cm DBH or greater were tallied by species. The distance from the burrow or random point was also recorded for the nearest three trees 10 cm DBH or greater.

2016 Vegetation Sampling- I repeated the vegetation sampling regime instituted by Waddle (2000), with the following modifications. I confined data collection to a section of the study site bounded by Blackwater Creek to the north and west, and by a seasonally wet drain to the south (Figure 1). I did this to focus on an area not dissected by any obvious habitat that is inappropriate for tortoises (the drain forming the southern boundary), or with a different management history (clearcut south of the drain). I defined the sample area using a polygon feature in ArcMap 10.4.1 (ESRI, Redlands, CA). In 2016, I used GPS coordinates to navigate to the 24 burrows from 1999 that were in the newly defined polygon. Preliminary surveys indicated success relocating the old tagged burrows by use of maps and coordinates, followed by discovery of the tag with a metal detector. These preliminary surveys demonstrated that coordinates proved consistently accurate within approximately 1 meter of tags.

Six of these relocated burrows were judged to be active, one was judged to be inactive and 17 were judged to be abandoned. Coordinates were not available for the random points sampled in 1999, nor were they permanently marked. Consequently, ArcMap10.4.1 was used to create a new set of 25 random points buffered at least 20m from one another within the defined sampling polygon. Using the same criteria as Waddle (2000), I omitted two random points because they fell within 10m of an active or inactive burrow.

Because Waddle (2000) found no significant difference in understory vegetation among the four understory plot positions (front, back, left, and right) at burrows and I revisited burrows that had become completely filled with soil in the intervening 17 years, I oriented the four understory subplots along north-south and east-west axes (Figure 2). In two instances a tree inhibited placement of the frame and the plot was moved to 4m from the burrow or plot center.

Although Waddle (2000) tallied midstory stems according to species, I tallied stems into two categories, "all pine" and "hardwood". I combined the 1999 data using the same method. I measured the DBH of and the distance to the three nearest trees to plot center. If there were fewer than three trees within the plot I measured the three nearest to burrow/plot center beyond the plot boundary. For analysis of these data I used three categories: longleaf pine, slash pine and all hardwood species.

Statistical Analyses- To assess changes at the site over time, a one-way analysis of variance (ANOVA) was used to compare understory vegetation structure and canopy cover at 1999 random points with 2016 random points (Minitab 17 Statistical Software, State College, PA). The new random points were confined to the 2016 sampling area, while the 1999 points were dispersed throughout both areas, which at that time had been managed as one stand by CNF foresters. Paired t-tests were used to compare understory vegetation structure and canopy cover at 1999 active burrows that remained active in 2016, and 1999 active burrows that were abandoned by 2016. Understory percent cover and canopy cover data were arcsine-square root transformed to normalize these proportion data.

Differences in the midstory stems at random plots in 1999 and 2016 were analyzed using a one-way ANOVA (Minitab 17 Statistical Software, State College, PA). A paired t-test was used to compare midstory hardwood stems at burrows active in 1999 with the same burrows that remained active in 1999 because no hardwood stems were found in either sampling year. A one-way repeated measure ANOVA design was used to describe midstory changes at the burrows that were active in 1999 that became abandoned in 2016 (SAS Institute, Inc., Cary, NC). These midstory count data were square root transformed to normalize them.

The nearest three trees to plot center or burrow for both 1999 and 2016 sample years were categorized as “longleaf pine”, “slash pine” or “hardwood”. A chi-square was used to evaluate changes in the tree species composition at random points in 1999 and random points in 2016. I used a paired-design t-test to compare the proportion of the tree composition that was longleaf pine and slash pine at those burrows that were active in 1999 and were active in 2016 (Minitab 17 Statistical Software). No hardwood species were in the nearest three trees in either sampling year for this dataset and therefor omitted from the comparisons. I included the hardwood species category in the same analysis with those burrows that were active in 1999 but were found to be abandoned in 2016.

Due to the 2007 thinning, a one-way ANOVA was used to evaluate how the mean distance from plot center to the nearest three trees at random points in both sampling years had changed. Changes in mean DBH at each point were evaluated using the same method. Paired t-tests were used to make the same two comparisons at active burrows that remained active, and at active burrows that became abandoned. These data were square root transformed to normalize them.

To visualize changes in multivariate space, I used a principal-components analysis (PCA) of a covariance matrix (R Version 3.3.2, www.r-project.org, accessed 1 February 2017). Canopy cover, understory, midstory data, and mean DBH and mean distance from plot center of the nearest three trees were included in the PCAs. For these analyses, I combined the understory vegetation categories “wiregrass” and “other grass” into a single “grass” category. I also omitted the midstory category “all pine” to avoid zero values. For these principal components analysis, 0.01 was added to remaining zeros. Canopy and understory percent cover data were arcsine-square root transformed to normalize them. Midstory data and tree DBH and distance were standardized by dividing each value by the maximum value in the category and then arcsine square root transformed to normalize them. I included the 1999 random points and 2016 random points to characterize changes to the vegetation over time at the site. I also included the 1999 data from all burrows I resampled in 2016, and the data from those same burrows in 2016 categorized as still active or now abandoned.

Results

Changes in Vegetative Structure of the Site- In the understory, percent cover of litter (F = 5.26, P = 0.025; Table 1; Figure 3), percent other grasses (F = 4.46, P = 0.038), and percent other forbs (F =6.62, P =0.012) were greater at random plots in 1999 than at random plots in 2016. Percent cover of bare ground (F = 27.8, P = 0.000) was more than six times greater at 1999 plots than 2016 plots. Mean percent cover of legumes (F = 4.48, P =0.038) was 1.5 times greater in 2016, while percent cover of understory shrubs (F = 19.19, P =0.000) was almost two times greater in 2016. There were 19 times as many hardwood stems in the midstory at 2016 random plots compared to 1999 random plots (F = 258.06, P = 0.000). Canopy cover was significantly greater at 1999 random plots than 2016 random plots (F = 27.8, P =0.000; Figure 4). Tree species composition changed significantly between sample years (χ^2=14.57, df =2, P =0.0007), being dominated by slash pine in 1999 and relatively evenly distributed between hardwoods, slash pine, and longleaf pine in 2016. Distance to the three nearest trees increased from 1999 to 2016 (F = 61.04, P = 0.001, Figure 3) as did DBH (F = 29.99, P = 0.001).

Changes for Active Burrows That Remained Active- In the understory, litter was significantly greater in 1999 (T = 3.43, P = 0.014) and other forbs were almost 2.5 times greater (T = 2.7, P = 0.035; Table 2) than in 2016. In 2016, shrub cover in the understory was greater than two times the shrub cover in 1999 (T = -7.99, P =0.000). There were no pine species stems in the midstory in either sample year, and the number of hardwood stems in the midstory increased significantly between 1999 and 2016 (T = -12.27, P = <0.000). Canopy cover was significantly greater in 1999 than 2016 (T = 3.72, P = 0.010). There were no hardwood species among the nearest three trees at these active burrows in both 1999 and 2016, although there was significantly more slash pine in 1999 (T = 358, P = 0.012; Table 5) and a relatively even number of slash and longleaf pine in 2016 (T = -3.26, P = 0.017). Both mean distance to the three nearest trees (T = -3.50, P = 0.013; Table 2b, Figure 12) and DBH (T = -5.44, P = 0.002; Figure 13) increased between sampling years.

Changes for Active Burrows That Became Abandoned- Burrows that were active in 1999 and abandoned by 2016 had significantly more litter (T = 3.09, P = 0.007; Table 6) and other forbs (T = 2.13, P = 0.049) in 1999 than when they were sampled in 2016. The percent cover of bare ground at burrows in 1999 was almost 6.5 times greater than in 2016 (T = 5.26, P =

0.000). When these burrows were sampled in 2016, there was more than two times the cover of both legumes (*T* = -2.43, *P* = 0.027) and shrubs (*T* = -5.17, *P* = 0.000) than in 1999. The number of stems in the midstory varied significantly between 1999 and 2016 (*F* = 20.6 *P* = <0.0001) increasing more over time for hardwoods than for pines (F = 17.0; P <.0001), and the proportion of stems in each category (slash pine, longleaf pine, and hardwood) changed significantly between sample years (*F* = 54.11 *P* = <0.001). Burrows in 1999 had significantly more canopy cover (*T* = 3.42, *P* = 0.004) than when the same burrows were assigned their abandoned status in 2016 (Figure 4). The proportion of slash pine among the nearest three trees was 2.6 times the proportion in 1999 (*T* = 6.99, *P* = 0.000; Table 8), while the proportion of longleaf pine was 3.4 times greater in 2016 (*T* = -4.26, *P* = 0.001). Distance to the three nearest trees (*T* = -4.23, *P* = 0.001; Figure 4) and DBH (*T* = -6.97, *P* = 0.000, Figure 4) increased from 1999 to 2016, with trees almost doubling in diameter.

Principal Components Analyses- All Random, All Active and Abandoned- Samples from 1999 and 2016 separated strongly on PC1, an axis along which shrub percent cover, midstory hardwood abundance, and distance and DBH of the nearest three trees load positively and canopy cover loads negatively. This axis explains 49.7% of the variation in vegetation composition, with 2016 data achieving high values along PC1 and 1999 achieving low values. PC 2 loads heavily and positively on litter percent cover and negatively on grass percent cover (Table 9). This axis explains 19.6% of the variation in vegetation composition, with sites at active burrows achieving low values along PC 2 and sites lacking burrows achieving high values. Burrows that were active in 1999 and we also active in 2016 had an increase in understory shrubs, midstory hardwood, and a decrease in grass, forbs, bracken fern, bare ground, litter and canopy. Those changes were less dramatic for those burrows that were active in 1999 and abandoned when surveyed in 2016.

Discussion

Habitat changes- High quality longleaf pine systems are composed of a sparse overstory and lush understory, while lacking a discernable midstory (Castellón et al. 2012, Oswalt et al. 2012). Management activities at the study site were designed, in part, to remove previously planted slash pine so that natural regeneration of longleaf could replace the offsite slash pine canopy. This management activity also was designed to reduce canopy cover to restore understory growth so that habitat quality could be improved. The results of both the one-way ANOVA tests for random samples and the PCA summarizing all habitat variables indicate that the 2007 thinning was effective in reducing canopy cover, altering tree composition, and widening spacing between trees. My data document that canopy cover was reduced from 72% in 1999 to 41% in 2016. Distance from burrow or random plot center to the nearest three trees increased approximately 2.5 m and DBH increased about 10 cm, documenting that management activities reduced tree density and the remaining trees grew over time. Because these trees create canopy coverage, these variables all measure similar features of the overstory and document that management goals for those variables were met. Similarly, when the nearest three trees in 2016 were compared to those in 1999, there was a species shift from mostly slash pine to a greater proportion of longleaf pine. Because the site was planted in slash pine in 1973 following a clearcut, these longleaf pine trees are the result of natural seedlings present at the time of the clearcutting.

Habitat management activities did not yield the lush understory and sparse midstory expected of habitat restoration. Components of vegetation structure are correlated, and most explicitly linked are the understory and overstory structure. Changes in the overstory will affect the understory (Harrington 2007). Litter cover in the understory declined between samplings due to the reduction in overstory (Harrington and Edwards 1999). However, a significant increase in midstory hardwoods, shrubs in the understory, and a decrease in grasses and forbs indicate that the other pine savanna structural components were not maintained throughout the time leading up to resampling in 2016. It is possible that the herbaceous understory initially flourished immediately following the 2007 thin. However, application of prescribed fire to the site on a 3-4-year rotation was not frequent enough to prevent hardwood encroachment and promote native groundcover and woody stems tend to resprout (Brockway and Outcalt 2000, Drewa et al. 2003, Yager et al. 2007). Understory shrubs have approximately doubled in each of the sampling groups. Historically, southern Alabama would have burned slightly more frequently, on a 2-4 year fire return interval (Guyette et al. 2012).

Legumes were found to be significantly greater or approaching significantly greater at all 2016 plot types in all comparisons with data from 1999, while other forage groups (other forbs, wiregrass and other grass) declined. Gopher tortoises ingest legumes for their relatively high nutritional value, and juvenile gopher tortoises show a preference for plants in *Fabaceae* (MacDonald and Mushinsky 1988). In 1999 legume cover was greater at burrows when compared to 1999 random points. Because the understory habitat provided more plant functional groups suitable for forage in 1999 the tortoises selected these areas for their burrows and did not have to forage widely. However, it is possible that there are more legumes at all plot types throughout the site in 2016 because tortoises are dispersing the seeds over greater distances.

Because forage is scarcer, tortoises must increase the distance of their foraging excursions (Guyer et al 2012) and due to the thick nature of their seed coats, digestion by gopher tortoise increases germination of legumes. Seeds from tortoise scat are typically deposited near burrows (Boglioli et al. 2000). However, seeds of plant genera not found near tortoise burrows have been documented in tortoise scat (Birkhead et al. 2005). If the tortoises are making longer foraging excursions, it is likely that tortoise scat is more evenly dispersed and legumes that germinate from these scats would be not be found concentrated at burrows (Diemer 1986). Further study of faunal dispersal of legume seeds in the longleaf pine ecosystem is warranted (Hainds et al. 1999).

At this site, these changes over time are likely the result of management practices between samplings, specifically the thinning in 2007 and prescribed fire frequency. At the present time, this site is scheduled for an herbicide treatment of the hardwood midstory along with repeated prescribed fire (USFS 2017.). This combination of treatment should be more effective than prescribed fire alone at restoring grasses and forbs in the understory (Brockway and Outcalt 2007).

Burrow activity and selection- Tortoises are most likely to abandon burrows due to increases in overstory and are most likely to select open grassy areas to dig new burrows (Aresco and Guyer 1999a, McCoy et al. 2013). Because my study examined some active burrows that remained active across 17 years of time and others that were abandoned by 2016, my data allow evaluation of similar patterns of the effects of vegetation on placement and abandonment of burrows.

I would expect that burrows that were active in both 1999 and 2016 had few changes in their vegetation structure over the years. However, my data indicate the burrows that stayed active had more understory litter and forbs in 1999 than in 2016. These burrows also had significantly fewer hardwood stems in the midstory in 1999, and no pine stems were found in the midstory at these burrows in either 1999 or 2016. In the overstory, these burrows had significantly more canopy cover in 1999, prior to the 2007 thinning. Nearest tree composition at these burrows shifted from mostly slash pine to 52% longleaf pine, with no hardwood tree species among the nearest three trees at these burrows in either 1999 or 2016. A lack of hardwood trees and retention of nearby pine species may be advantageous to tortoises, as pine needles are more pyrogenic than hardwood litter, and would carry fire throughout the vicinity of the burrows. While in 2016 these burrows had a more open and widely spaced overstory, hardwood shrubs have begun to fill both the understory and midstory.

Burrows that were active in 1999 but eventually become abandoned by 2016 show additional differences in vegetation structure. When these burrows were active they had an understory characterized by more herbaceous cover and fewer shrubs. There was more canopy cover in 1999 and an increase in hardwood shrubs in the midstory by the time they were judged to be abandoned in 2016. These burrows also had hardwood trees amongst the nearest three trees in both 1999 and 2016, which may be associated with the fact that they were eventually abandoned.

In 1999, the tortoises had diverse habitat to select from and their burrows were established in areas with the least canopy and shrubs, and most grasses, forbs and legumes (Waddle 2000). Waddle’s finding is supported by management recommendations for gopher tortoises (Diemer 1986). However, both ANOVA and PCA indicate that in 2016 the habitat was more homogenous and 2016 active burrows had more in common with the available habitat than active burrows did in 1999. This finding indicates that, although canopy cover has been reduced, the habitat is more even and the tortoises now have fewer lush open-midstory areas to select for burrow construction. While I would expect that there would be few differences in the vegetation characteristics of active burrows at the same site regardless of the sample year, the abundance of forbs at the seven burrows that were active/inactive in 2016 declined since they were first sampled in 1999, while shrubs and midstory hardwoods increased. All results support the conclusion that areas of high quality habitat previously available are no longer available.

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Table 1. Comparison of mean percent cover ± SE of 10 understory functional groups, midstory stems/ 10m diameter plot and canopy cover at random points in 1999 (n=50) and new random points (n=23) in 2016 (Waddle 2000). Significantly greater means are bolded ($P < 0.05$, one-way ANOVA).

1999 Random Points & New 2016 Random Points			
	1999 Mean	2016 Mean	P-value
Bare ground (% cover)	**2**.21 ± 0.34	0.36 ± 0.24	<0.000*
Litter (% cover)	**50.35** ± 2.09	41.85 ± 3.19	0.025*
Wiregrass (% cover)	0.07 ± 0.07	0.24 ± 0.18	0.235
Other grasses (% cover)	**15.67** ± 1.39	11.36 ± 2.02	0.038*
Legumes (% cover)	0.82 ± 0.26	**1.23** ± 0.29	0.038*
Bracken fern (% cover)	3.59 ± 0.76	1.58 ± 0.51	0.15
Other forbs (% cover)	**6.14** ± 0.67	3.51 ± 0.68	0.012*
Woody vines (% cover)	1.68 ± 0.28	2.48 ± 1.14	0.672
Shrubs (% cover)	19.49 ± 1.68	**37.37** ± 4.43	<0.000*
Longleaf pine (% cover)	0.00 ± 0.00	0.01 ± 0.01	0.142
Midstory hardwood stems	3.80 ± 0.94	**72.09** ± 9.48	<0.000*
Midstory all pine stems	0.34 ± 0.15	0.09 ± 0.09	0.327
Canopy (% cover)	**71.55** ± 2.16	41.01 ± 5.44	<0.000*

Table 2. Comparison of the vegetation at burrows that were active in both 1999 and 2016 (n=7), Means of percent cover ± SE of 10 understory functional groups and percent canopy cover are compared. Wiregrass was absent at plots in both sampling years. Significantly greater means are bolded (*P* < 0.05, paired t-test).

1999 Active Burrows and Paired 2016 Active Burrows			
	1999 Mean	2016 Mean	P-value
Bare ground (% cover)	4.07 ± 1.33	1.21 ± 0.68	0.110
Litter (% cover)	**48.32** ± 2.80	32.21 ± 4.75	0.014*
Wiregrass (% cover)	0.00 ± 0.00	0.00 ± 0.00	-
Other grasses (% cover)	17.43 ± 2.65	12.39 ± 2.51	0.198
Legumes (% cover)	0.46 ± 0.17	2.25 ± 0.79	0.082
Bracken fern (% cover)	1.14 ± 1.06	2.57 ± 1.34	0.470
Other forbs (% cover)	**7.93** ± 0.96	3.29 ± 0.89	0.035*
Woody vines (% cover)	0.46 ± 0.18	2.00 ± 1.48	0.448
Shrubs (% cover)	20.18 ± 4.42	**44.05** ± 7.04	<0.000*
Longleaf pine (% cover)	0.00 ± 0.00	2.64 ± 2.60	0.303
Canopy (% cover)	**53.94** ± 3.31	30.31 ± 8.35	0.010*

Table 3. Nearest three tree species composition at 1999 active burrows and the same burrows in that remained active in 2016. Hardwood species were not present in either year. Significantly greater values are bolded (P < 0.05, paired t-test).

1999 Active Burrows and Paired 2016 Active Burrows				
	T	P-value	1999 Mean	2016 Mean
% Longleaf pine	-3.26	0.017*	18.95	**52.15**
% Slash pine	358	0012*	**81.04**	47.43

Table 4. Comparison of the vegetation at burrows that were active in 1999 but were abandoned in 2016 (n=17), Means of percent cover ± SE of 10 understory functional groups and percent canopy cover are compared. Wiregrass and longleaf pine were absent from plots in both sampling years. Significantly greater means are bolded (*P* < 0.05, paired t-test).

1999 Active Burrows and Paired 2016 Abandoned Burrows			
	1999 Mean	2016 Mean	P-value
Bare ground (% cover)	**6.235** ± 1.33	0.97 ± 0.37	<0.000*
Litter (% cover)	**45.81** ± 3.42	35.87 ± 3.41	0.007*
Wiregrass (% cover)	0.00 ± 0.00	0.00 ± 0.00	-
Other grasses (% cover)	22.19 ± 3.25	16.62 ± 2.47	0.061
Legumes (% cover)	0.941 ± 0.20	**2.257** ± 0.69	0.027*
Bracken fern (% cover)	2.42 ± 0.92	3.588 ± 1.28	0.317
Other forbs (% cover)	**5.088** ± 0.61	4.15 ± 0.83	0.049*
Woody vines (% cover)	0.529 ± 0.23	0.485 ± 0.38	0.432
Shrubs (% cover)	16.79 ± 4.07	**35.75** ± 5.64	<0.000*
Longleaf pine (% cover)	0.00 ± 0.00	0.00 ± 0.00	-
Canopy (% cover)	**61.03** ± 2.66	41.11 ± 5.14	0.004*

Table 5. Nearest three tree species composition at 1999 active burrows and the same burrows that were abandoned in 2016. Significantly greater values are bolded ($P < 0.05$, paired t-test).

1999 Active Burrows and Paired 2016 Abandoned Burrows				
	T	P-value	1999 Mean	2016 Mean
% Longleaf pine	-4.26	0.001*	15.61	**52.76**
% Slash pine	6.99	<0.000*	**80.19**	31.12
% Hardwoods	-1.83	0.086	3.92	15.5

Table 6. Eigenvalues and loading values of the first two principal components of understory, midstory, canopy cover and the mean distance and DBH of the three nearest trees of all random plots, and data from those plots that were active both in 1999 and 2016 and burrows that were active in 1999 but abandoned in 2016.

All random, 1999 active, 2016 active and 2016 abandoned		
Eigenvalues and loading values	**PC1**	**PC2**
Eigenvalue	0.148	0.058
Variation explained (%)	49.66	19.55
Loading values		
Bare ground (%)	-0.045	-0.033
Litter (%)	-0.257	0.274
All grass (%)	-0.097	-0.165
Legumes (%)	0.022	-0.017
Bracken fern (%)	-0.007	-0.040
Forbs (%)	-0.0533	-0.023
Woody vines (%)	0.009	-0.022
Shrubs (%)	0.409	-0.012
Midstory hardwood (stems/plot)	0.578	0.581
Mean distance of the 3 nearest trees	0.356	0.106
Mean DBH of the 3 nearest trees	0.293	0.060
Canopy cover (%)	-0.453	0.736

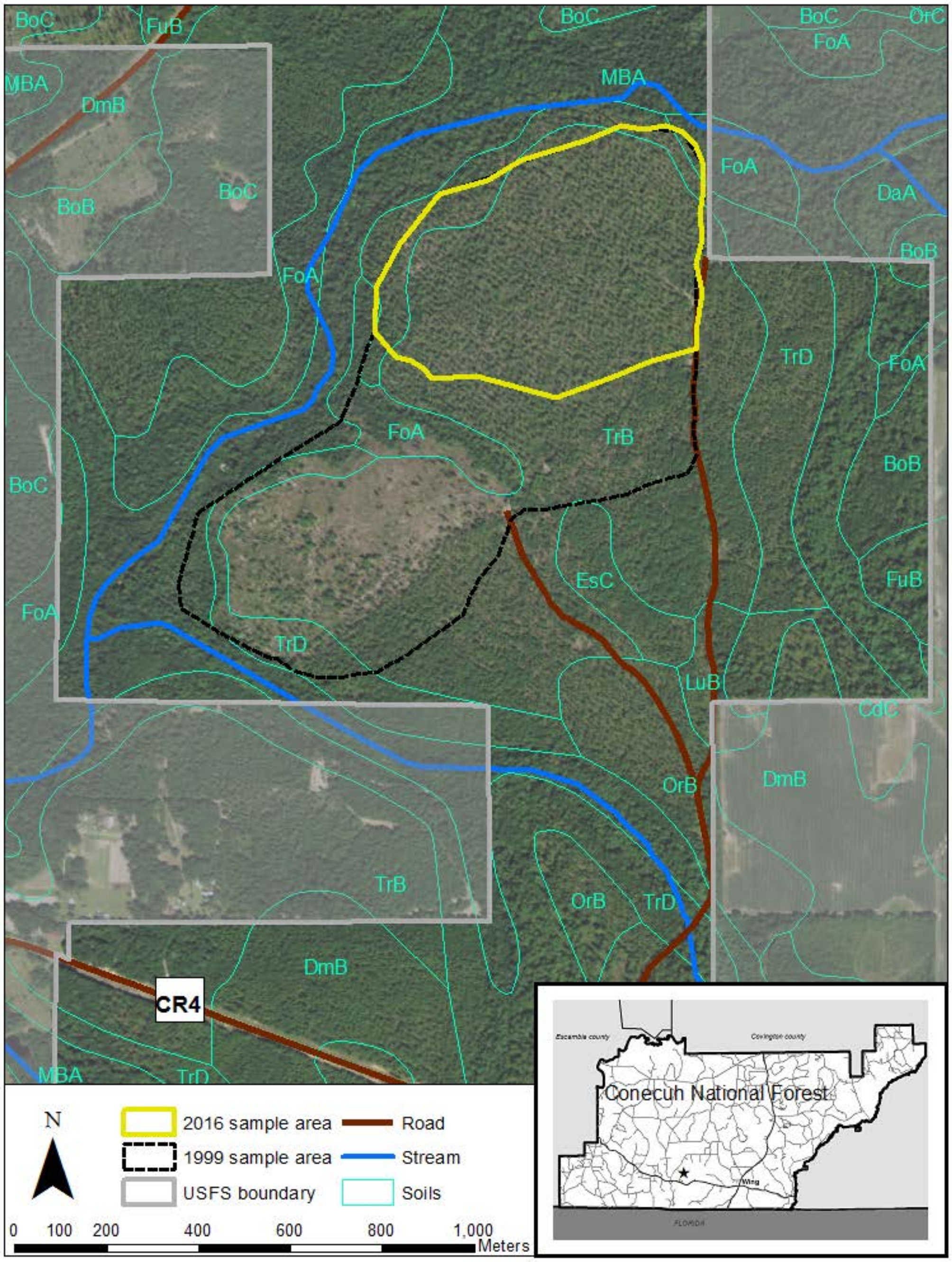

Figure 1. Soils at study site and vicinity in Conecuh National Forest, Covington County Alabama. Tr = Troup loamy sand, Fo = Florala sandy loam, Dm = Dothan and Malbis sandy loams, Or = Orangeburg sandy loam, Lu = Lucy loamy sand, Cd = Cowarts-Dothan complex, Es = Esto sandy loam, Bo = Bonifay loamy fine sand, MB = Muckalee, Bibb, and Osier soils frequently flooded, Da = Dorovan muck. Slope is designated with the suffix A = 0-2% slopes, B = 0-5% slopes, C = 5-8% slopes, and D = 8-15% slopes. Study site is indicated in inset map with a star.

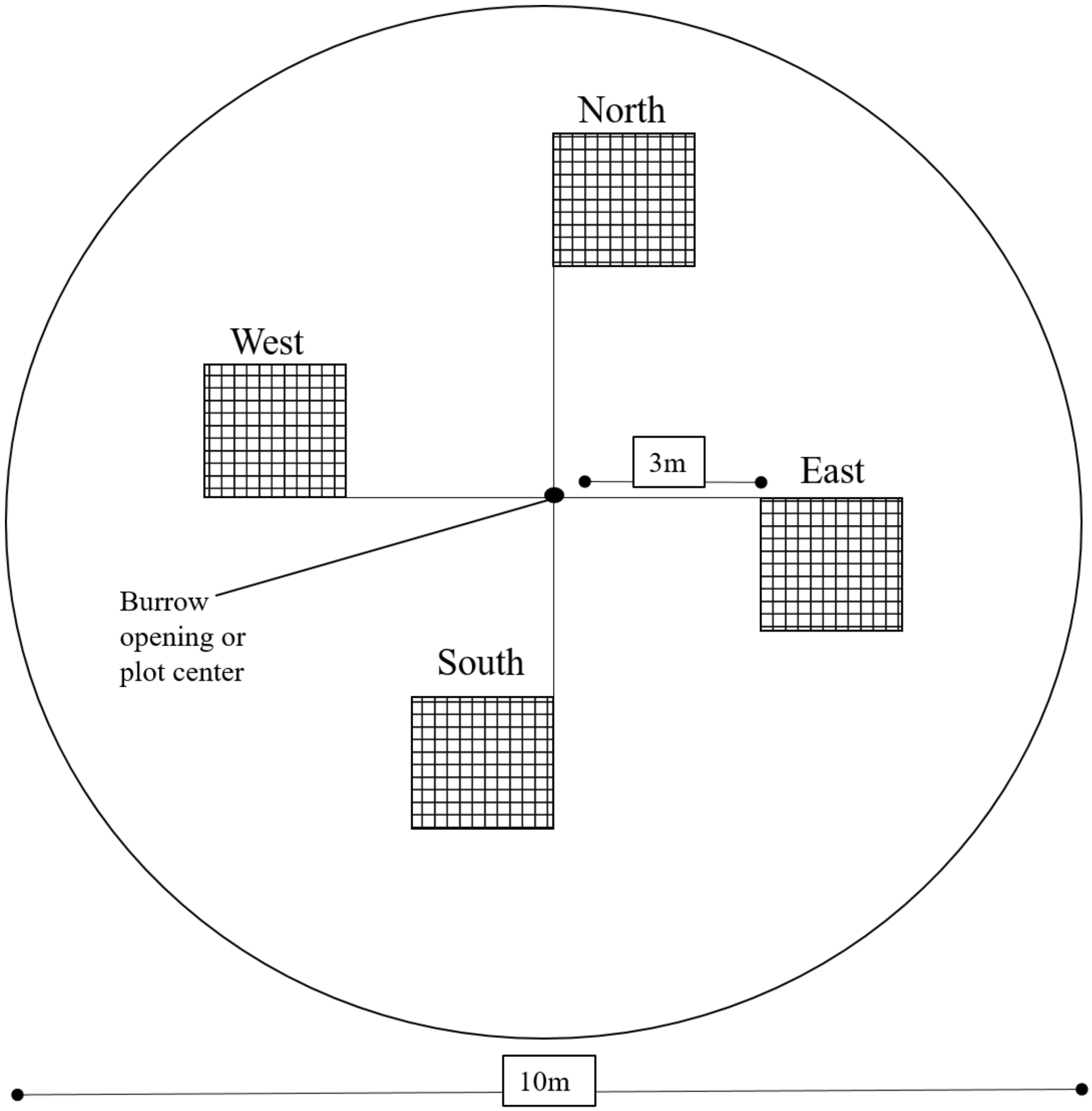

Figure 2. Diagram of vegetation sampling method at burrow openings or control points. Central point represents burrow or random point; squares represent $1m^2$ understory subplots; circle represents 10m diameter plot used for tree and shrub samples.

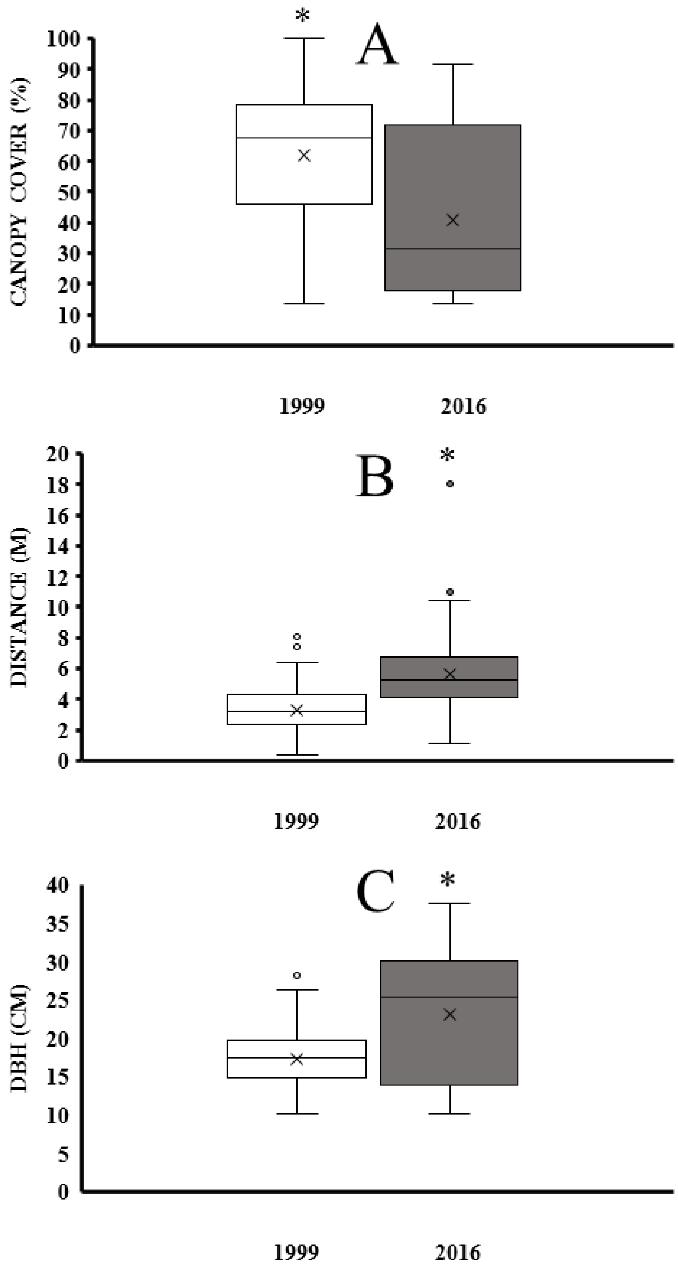

Figure 3. Box and whiskers plot for overstory vegetation at random points in 1999 (open boxes) and 2016 (gray boxes). DBH = diameter at breast height; box = 25th and 75th percentiles, vertical bars = min and max values, x = mean, circles = outliers, horizontal bar = median. See table 1 (A). Nearest three trees to plot center were measured for B and C. Significant differences ($P < 0.05$, one way ANOVA) are indicated by a *.

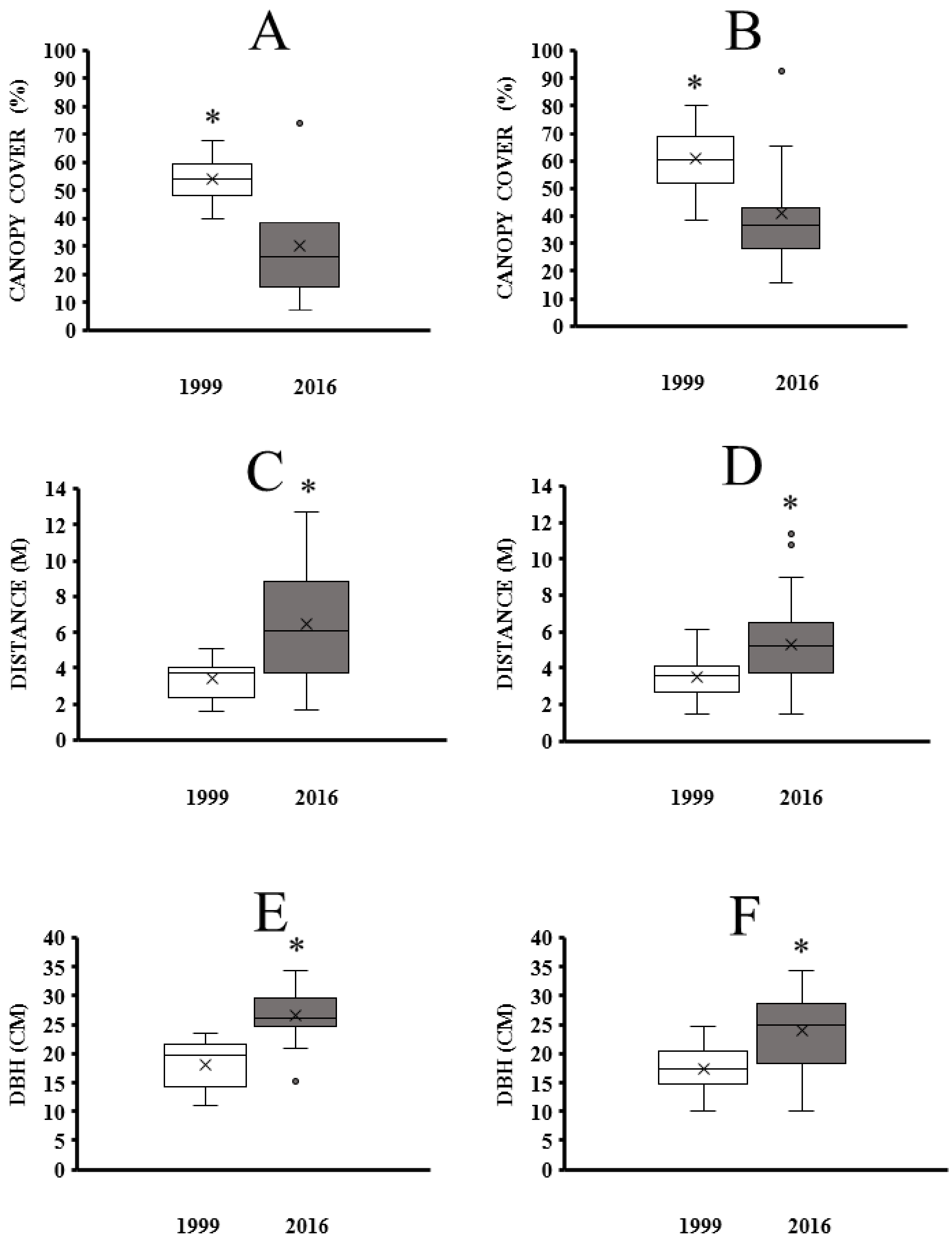

Figure 4. Box and whiskers plot for overstory vegetation at burrows that were active in 1999 (open bars) and remained active in 2016 (gray bars; A,C,E) and burrows that were active in 1999 (open bars) and became abandoned by 2016 (gray bars; B,D,F). DBH = diameter at breast height; box = 25th and 75th percentiles, vertical bars = min and max values, x = mean, circles = outliers, horizontal bar = median. See table 3 (A) and table 6 (B). Nearest three trees to plot center were measured for C-F. Significant differences ($P < 0.05$, t-test) are indicated by a *.

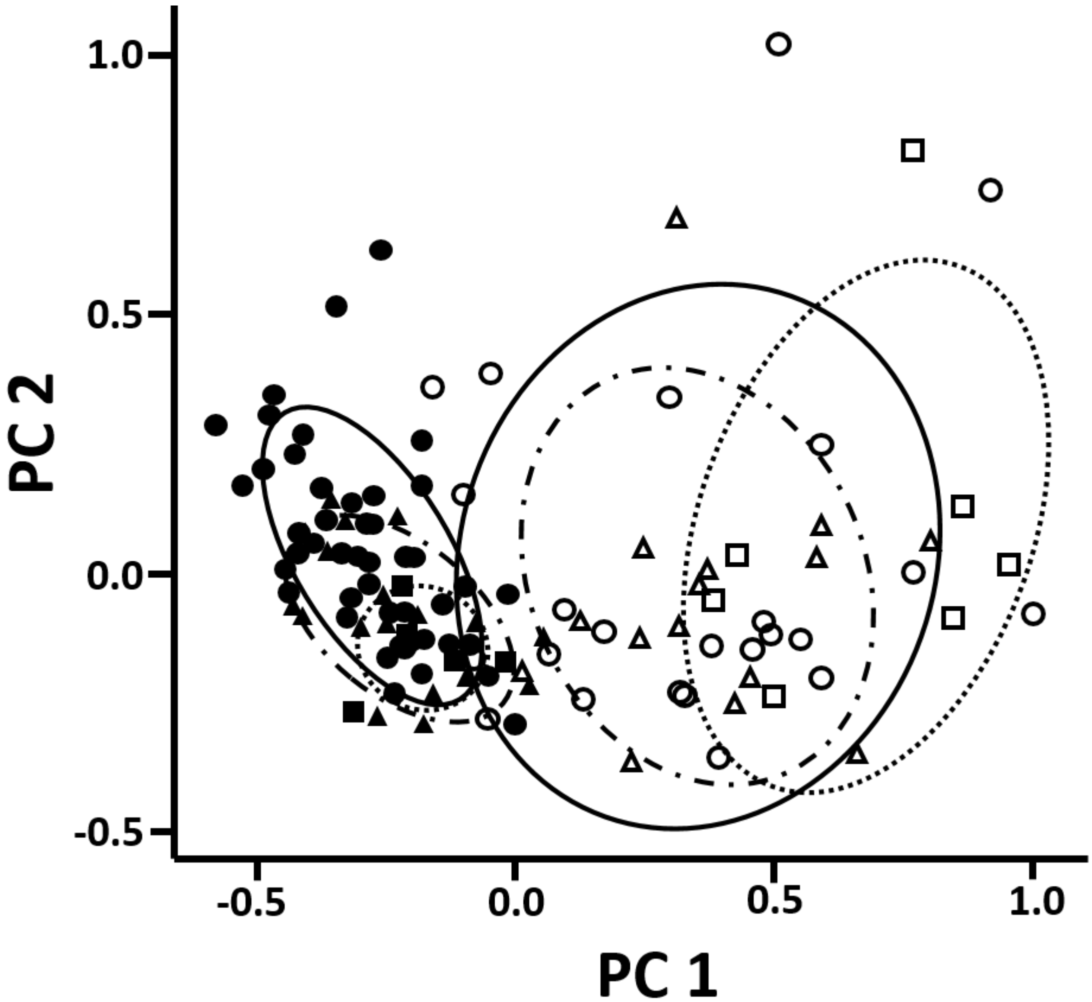

Figure 5. PCA of 1999 and 2016 understory, midstory, and canopy vegetation for study site. Solid black circles are for 1999 random locations; open circles are for 2016 random locations; solid black squares are for vegetation at 1999 active burrows that remained active in 2016; open squares are for vegetation at 2016 active burrows that were active in 1999; solid black triangles are for vegetation at 1999 active burrows that were abandoned in 2016; open triangles are for 2016 abandoned burrows that were active in 1999. Solid ellipses are for random locations in both sample years; dotted ellipses are for active burrows in both years; and dashed ellipses are for abandoned burrows in both years.

Chapter 3

Inferring Changes in Gopher Tortoise Population Dynamics by Re-evaluating Burrow Dispersion and Size-Class Distribution After 17 Years

Introduction

Gopher tortoise (*Gopherus polyphemus*) populations throughout their range have continued to decline despite conservation efforts for more than the past thirty years (Auffenberg and Franz 1982, McCoy et al. 2006). In Alabama, between 1.5% and 6.5% of the historical population remains (Guyer et al. 2011). Long-term reassessment of persisting populations is the only way to evaluate changes in demography and evaluate habitat management implications (Tuberville et al. 2014). In addition, continuous monitoring of at-risk populations may be the only method that can detect subtle responses to both positive or negative changes in habitat (Gitzen et al. 2016).

Few previous long-term studies focus on population dynamics of gopher tortoises. Diemer et al. (2012) retrapped tortoises at a northern peninsula Florida site approximately 28 years after their study began. They recaptured 8% of the tortoises originally trapped at this commercial timber production site with a complicated yet typical management history. Even after extensive data collection acquired from trapping, radio telemetry and burrow surveys, questions about the dynamics of this population remain. Another study compiled tortoise capture and burrow data to create 5-11 year datasets from 3 sites (Tuberville et al. 2014). Size frequency distributions at these sites varied and was likely due to differences in site history and management of vegetation conditions. At an Everglades site, the southernmost tip of the gopher tortoise range, transects were surveyed for burrows in 1979, and repeated in 1990 and 2001 (Waddle et al. 2006). Active burrows declined at this site 76% over 22 years. A south-central

Florida site was surveyed 17 years after the reintroduction of fire (Ashton et al. 2008). This study conducted burrow surveys to estimate tortoise density, in addition to measuring habitat structure by evaluating bare ground and canopy cover. At this site burrows were found at the highest densities in frequently burned habitat with reduced canopy cover and more extensive bare ground, however, this study did not measure change over time.

Gopher tortoises are fossorial, spending the majority of their day inside their burrows (Douglass and Layne 1978, McCoy et al. 2006). The conspicuous burrows, with their big sandy aprons, are more easily studied than the tortoises themselves and are frequently used as a surrogate for estimating tortoise density (Guyer et al. 2012) and tortoise body size distributions (Nomani et al, 2008). While often studied, gopher tortoise burrow use is complicated. They each use multiple burrows. Males have been shown to use an average of 10 burrows, while females at the same site in Georgia used about half that number (Eubanks et al. 2003). Gopher tortoises will share use of burrows, and these behaviors are dependent on soil and habitat type (Castellón et al. 2012). Surveys of burrow density and burrow occupancy can be used to create tortoise-to-burrow correction factors and estimate population density (Auffenberg and Franz 1982). Correction factors are site-specific and can vary by year (Diemer et al. 2012). Much of gopher tortoise ecology is density-dependent (Guyer et al. 2012) and thus when burrow density is used in place of capture data, care must be taken to use the best correction factor available.

The purpose of this study is to use surveys of gopher tortoise burrows to estimate changes in body size distribution, density and dispersion after 17 years at a gopher tortoise site in southern Alabama. This information, in combination with the results of my vegetation structure study presented in Chapter 2, will provide a guide for further study at the site as well as additional information for managers of similar sites within the geographic range of gopher tortoises.

Methods

Burrow survey- The same sandhill site in Conecuh National Forest described in Chapter 2 was used for this study (see Chapter 2 for site description and site history). The same 23.11 hectare polygon that was used to limit the 2016 vegetation sampling area also served as the boundary of a complete burrow survey conducted during September and October 2016 (see Chapter 2 Figure 1). A group of 10 volunteers walked transects in tight formation across the entire site until no new burrow was found. When an active or inactive burrow was found, coordinates of the location were taken, status was assessed, and width of each burrow opening was measured 0.5m inside of the burrow opening, following McCoy et al. (2006) and Smith et al. (2009). Burrows assigned an active status had fresh tracks or scat on the burrow apron, while inactive burrows retained a half-moon shape of a tortoise carapace but lacked these recent signs of use (Mushinsky and McCoy 1994, Waddle et al. 2006). Abandoned or filled burrows were not recorded for this study. Burrow size classes were assigned utilizing the same categories as in Nomani et al. (2008): juvenile (<14cm wide), sub-adult (14-23 cm wide), adult (>23cm). I remeasured the approximate size of the original 1999 study site using ArcMap 10.4.1 (ESRI, Redlands, CA).

Statistical Analysis- Between the years 1999 and 2001 the widths of active burrows were measured at the same site (see Guyer et al. 2012). I categorized burrow width data from 2016 into 50 mm bins in order to compare my data with these previously published data. For analysis, I omitted the largest two bins of Guyer et al. (2012) because there were only two burrows within them in 2016 and none in the earlier sampling. To account for low expected cell values, I used a Fisher Exact Probability Test (R Version 3.3.2, www.r-project.org, accessed 1 February 2017) to test for differences in the size distributions for 1999 and 2016 data.

Spatial Analysis- Coordinates collected during the burrow survey were used for spatial analysis. Inactive and active burrows were combined because, over an annual cycle of activity, these burrows are likely to be occupied by at least one tortoise (Aresco and Guyer 1999a). Average Nearest Neighbor Analysis was performed using Spatial Statistics Tools in ArcToolbox in ArcMap 10.4.1; these data were used to determine whether the dispersion pattern of active/inactive burrows at the site has changed from that reported by Waddle (2000). In this analysis, the observed-to-expected nearest neighbor distance ratio was calculated, with a positive Z score combined with a significant p-value indicating an over-dispersed pattern and a negative Z score and significant p-value indicating a clumped dispersion; non-significant p values indicated a random dispersion.

The same burrow coordinates, along with associated burrow width measurements, were used for an Incremental Spatial Autocorrelation (Morans I) analysis using Spatial Statistics in ArcToolbox in ArcMap 10.4.1 to determine whether burrow size was correlated as distance between burrows changed. I utilized 10 distance bands. Two burrows with no associated width measurement were omitted from this analysis

Results

Burrow survey and statistical analysis- A total of 135 active and inactive burrows were located. Of these, 108 were classified as active, while 27 were inactive. In 1999, 38 active/inactive burrows were documented in the same sample area that I used (Waddle 2000).

Burrow widths revealed that, in 2016, there were 73 adult-sized burrows, 13 subadult burrows, and 47 juvenile burrows (Figure 1). Complete burrow width data from Waddle (2000) were not available for direct comparison, however data collected between 1999-2001 at the same site and presented in Guyer et al. (2012) were used as a comparison to 2016 burrow widths (Figure 2). The distribution of burrow sizes changed significantly between the two sample periods (P = <0.000). Because there was an increase in smaller burrows while adults burrow numbers remained relatively constant, the distribution in 2016 was bimodal.

Spatial Analysis- Average Nearest Neighbor analysis of all active/inactive burrows found at the study site indicates that the burrows were spatially clustered (*Z score* = -3.28, P = 0.001), as opposed to randomly spaced or evenly dispersed (Table 1). The observed mean distance between all burrows was 17.53 meters. Spatial Autocorrelation Analysis indicated that neighboring burrows were increasingly of similar sizes at distances of 0-60 meters, were of similar sizes between 60 and 90 m, and were decreasingly of similar size at distances greater than 90 m (*Z score* = 4.58, $P < 0.0001$, Moran's Index=0.369; (Figure 3).

Discussion

Since 1999, there has been a dramatic increase in active and inactive burrows at my study site in the Conecuh National Forest. To explain this increase, either the number of tortoises increased at the site or each tortoise created and used more burrows. This increase in active/inactive burrows is contrary to results of one of the only other similar long-term studies (McCoy et al. 2006). At nine of 10 protected Florida sites a decrease in total combined active and inactive burrows was found after ten years (McCoy et al. 2006). However, like those declining Florida sites, the ratio of active-to-inactive burrows at CNF also decreased over time.

Assuming a burrow-to-tortoise conversion rate of 2.5 burrows per tortoise per year (or a burrow occupancy rate of 40%; Guyer et al. 2012) and no change in this rate over time, approximately 15.2 tortoises utilized the 38 active burrows in the area in 1999 while 54 tortoises utilized 135 active burrows in the same area in 2016, 29.2 of which can be assumed to be adults based on burrow width categories used by Nomani et al. (2008). In 1999, 37 tortoises were captured by Waddle (2000) across a larger area that included my study site (54.42 ha). The 23.11 ha 2016 sample area is approximately 42.5% of the 1999 site. If density was consistent across the entire area sampled in 1999, then approximately 15.7 adult tortoises were captured by Waddle (2000) in the area that I resampled. Because capture efforts rarely are efficient enough to capture all individuals in a population, this represents the minimum number known to be present on the site. This estimate is very close to the one estimated from the burrow-to-tortoise conversion factor and indicates that the correction factor was an accurate predictor of tortoise abundance in 1999. However, 26 tortoises were trapped within my study area by Goessling in 2016 (unpublished data); 17 juveniles, 5 adult females and 4 adult males. Tortoises were classified as adults if they had carapace lengths of at least 180mm (Goessling, pers. comm.) based on Aresco and Guyer (1999b). These counts represent the minimum number known to present in 2016, values far below the 54 individuals estimated from the burrow-to-tortoise conversion factor. Because these values are so divergent, it is unclear whether increased numbers of burrows resulted from increased numbers of tortoises or increased burrowing activity of resident tortoises. If abundance in 2016 is closer to the values from Goessling, then a burrow correction factor of 5.2 is more appropriate for the site in 2016. Nevertheless, these estimates of abundance indicate that adult tortoise density was approximately 0.51 adult tortoises/ha in 1999 (11.9 adult tortoises / 23.11 ha; Guyer et al. 2012) and might have been as low as 0.39 adult tortoises per hectare (9 adult tortoises / 23.11 ha) or as high as 1.25 adult tortoises/ ha (29 adult tortoises / 23.11 ha) in 2016. These density estimates are at or above the 0.4 adult tortoise/hectare minimum recommended in the minimum viable population (MVP) guidelines for gopher tortoises (Styrsky et al. 2010).

The density of active/inactive burrows has increased from 1.64 burrows per hectare in 1999 to 5.84 burrows per hectare in 2016. The new burrow density is much greater than the 1.64-3.2 burrows per hectare reported for the Wade Tract in southwestern Georgia, which is typically described as high quality gopher tortoise habitat, and the 2.4 burrows per hectare in open pine habitats of Green Grove, a second high-quality site in southwestern Georgia (Waddle 2000, Hermann et al. 2002, Guyer et al. 2012). In populations declining due to a reduction in habitat quality, tortoises are thought to aggregate in the best remaining habitat, achieving unusually high tortoise and burrow densities at those sites (McCoy et al. 2006). This process likely affected my study site. An adjacent area immediately to the south (included in the 1999 study), which had been a slash pine (*Pinus elliotti)* plantation since the 1970s, was clear cut and planted in longleaf between 2011 and 2013, creating additional open habitat that historically was occupied by tortoises (Steve Johnson, USFS pers. comm, Waddle 2000). If tortoises dispersed from my sample area onto this additional area, then this might have yielded a small number of resident tortoises that remained on my study site, where loss of quality forage over time required extensive construction of new burrows as individuals attempted to find dwindling food resources (Chapter 2) and mating opportunities (Guyer et al. 2012).

Size data collected from tortoises trapped between 1992 and 2003 at six CNF sites (including my site) presented in Tuberville et al. (2014) indicate that CNF tortoises were unimodal in their size distribution, rather than displaying the bimodal size distribution of sites they classified as high quality. Using the 2016 burrow width data as a surrogate for tortoise size (Guyer et al. 2012), 44 burrows on my study site were between 50 and 150 mm and 62 burrows were greater than 250mm, a biomodal distribution. Although the 2016 burrow survey was exhaustive, it is likely that the true count of juvenile burrows is considerably higher because detectability of small burrows is typically very low (Tuberville et al. 2014). The presence of burrows in the smaller size classes is evidence of sustained recruitment at this site. I used data collected from 1999 through 2001 across the same site sampled by Waddle (2000) to evaluate the tortoise size distribution at that time. I omitted 77 burrows because their status (active, inactive, or abandoned) was unknown. A comparison of this burrow size distribution shows that cohorts of tortoises from 1999 have graduated into larger size classes by 2016 and that smaller burrows have become a much more prominent component of the overall distribution, contrary to findings at Florida protected sites which saw an increase in larger burrow sizes with no increase in the smaller sizes over 10 years (McCoy et al. 2006). There is also a consistent peak of active adult-sized burrows on the CNF, indicating high retention of adult tortoises (Guyer et al. 2012). The spike in juvenile-sized burrows represents sustained successful reproduction (Tuberville et al. 2014).

An increase in larger-sized burrows at this site is interesting because adult gopher tortoises at CNF are smaller than adult tortoises at other sites across the range of the species (Aresco and Guyer 1999b, Tuberville et al. 2014). This has been attributed to a lack of forage to sustain growth through subadulthood due to the unnatural conditions created in a pine plantation system in addition to a history of human predation of adults at the site (Aresco and Guyer 1999b, Waddle 2000). While I suspect stand thinning did yield improved forage immediately after tree removal, this action did not lead to a long-term increase in forage plants because of an inappropriate fire regime (Chapter 2).

The distribution of active burrows has changed over the sampling period from random in 1999 to clustered in 2016. Interpretation of burrow dispersion is difficult because this variable is known to range from random to clustered at the two highest-quality sites that have been examined [Wade Tract (Waddle 2000) and Green Grove (Boglioli et al. 2000), respectively]. Nevertheless, clustered patterns may reflect increased patchiness as habitat recovers from stand thinning. However, in areas with an open canopy and lush understory maintained by frequent fire, an over-dispersed distribution would be expected (Boglioli et al. 2000). It would be informative to know the spatial distribution of the burrows shortly following the 2007 thinning, when the canopy had recently been opened and the hardwood midstory had not yet invaded. The gradual closing of the midstory in large patches may be driving tortoises to cluster in the most open remaining areas. Clustering of active burrows was further autocorrelated with burrow width. Adult burrows are clustered with other adult burrows and juvenile burrows are clustered with other juvenile burrows, which may indicate social structure on this site associated with tortoise size (Guyer et al. 2014; Tuberville et al. 2008). Alternatively, these clusters may indicate that the same individual is utilizing burrows that are grouped spatially to provide cover on long foraging trips. Tortoises typically share burrows and male tortoises have been documented to use an average of 10 burrows while females use an average of 5.2 at high-quality sites (Eubanks et al. 2003). It is likely that tortoises at CNF are utilizing burrows at an even higher rate.

Calculations provided above suggest that gopher tortoises at my study site meet some aspects of an MVP (at least .4 tortoises/ha) but not others (at least 250 adults on at least 100 hectares; McCoy and Mushinksy 2007, Styrsky et al. 2010). Adjoining my study site to the south is an area with known active tortoise burrows that was included in the 1999 study. This area, combined with my study area, is about 78 hectares in size and is dominated by priority tortoise soils (Guyer et al. 2011; calculated in ArcMap10.4.1). Projecting from my density estimates, this area might contain as few as 30.4 tortoises and as many as 97.5. This conforms to a support population under current conservation guidelines generated by The Gopher Tortoise Council (2013). However, a total of approximately 159 contiguous hectares of priority and suitable tortoise soils exist in this area, inclusive of the combined study sites and may contain 199 adults, using the same density projection. Data on habitat quality and population structure suggest a population that would increase, possibly to the level of an MPV, with additional management focused on reducing midstory hardwoods and increasing understory cover.

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Table 1. Average Nearest Neighbor analysis results. 1999 results from Waddle (2000) include the entire 54.42 hectare 1999 study site, while 2016 results are limited to the 23.11 hectare site.

	N	Density	Index/Ratio	Z-score	P-value	Dispersion
1999 Active Burrows	61	1.12/ ha	0.98	-0.32	NA	Random
2016 Active Burrows	135	5.84/ ha	0.85	-3.27	0.001064	Clustered

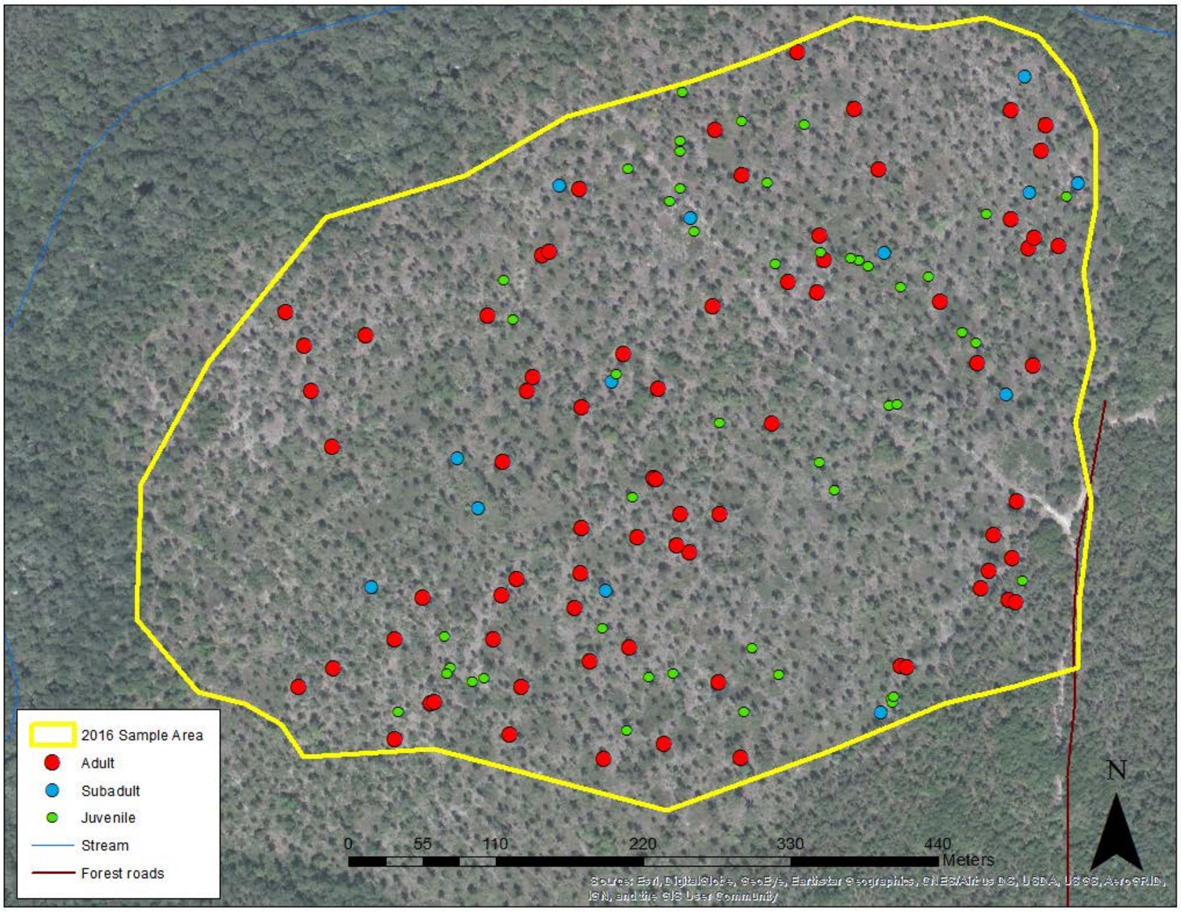

Figure 1. Map of 2016 study site showing location of adult (red) subadult (blue), and juvenile (green) burrows. Blue line shows location of Blackwater Creek.

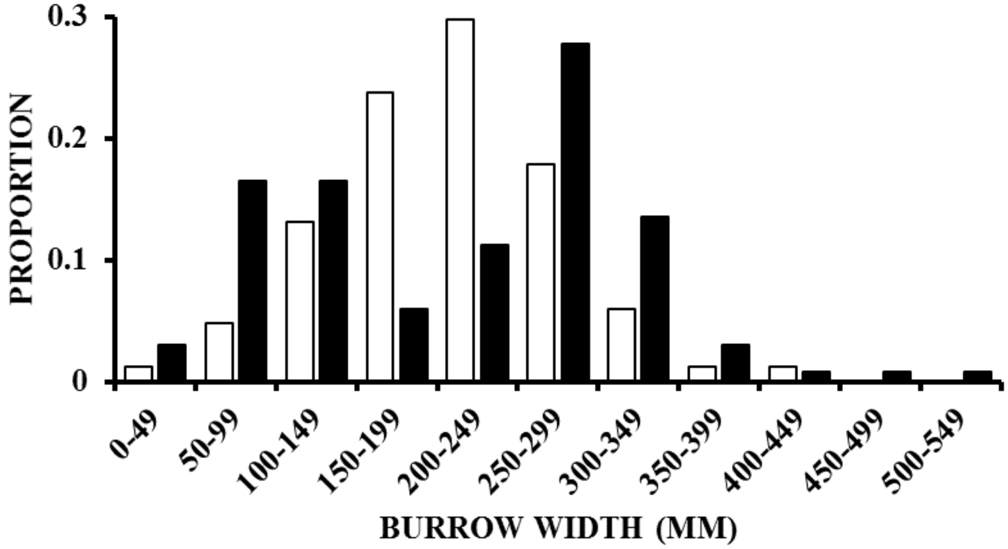

Figure 2. Comparison of distribution of burrow widths. Open bars 1999-2001 burrows; closed bars 2016 burrows

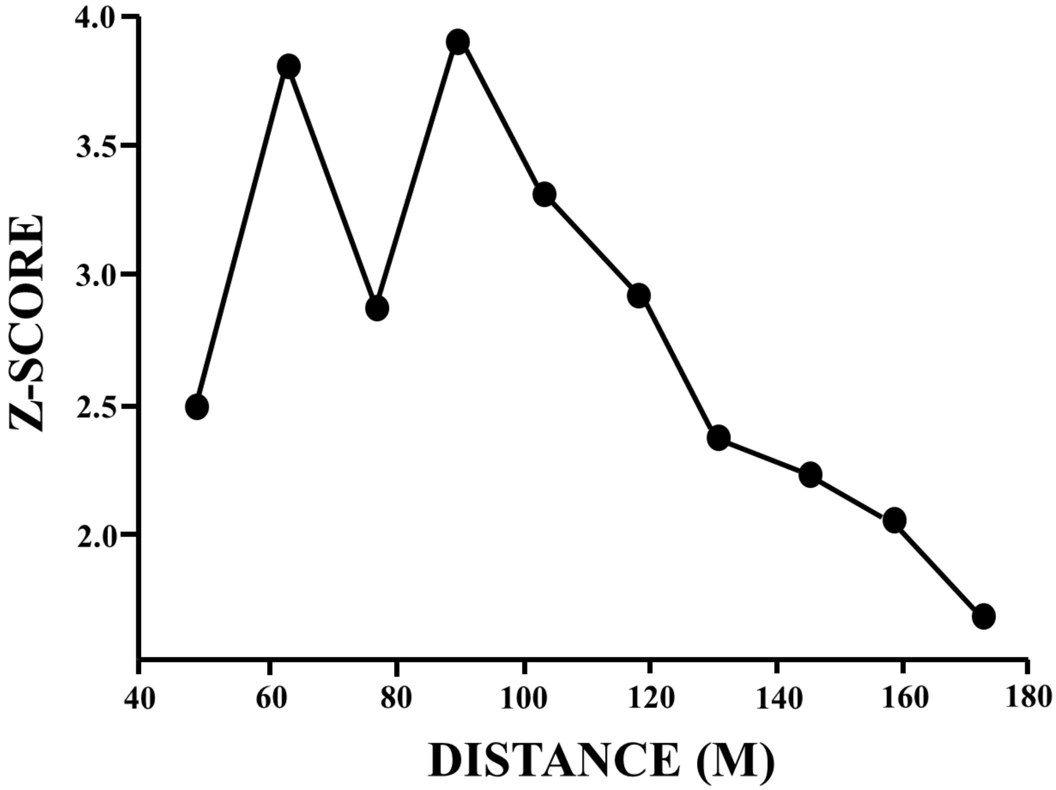

Figure 3. Spatial Autocorrelation of burrow width on inter-burrow distance for 2016 active/inactive burrows.