10-25-2016

Importance of Forest Structure for Amphibian Occupancy in North-Central Florida: Comparisons of Naturally Regenerated Forests with Planted Pine Stands

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Importance of Forest Structure for Amphibian Occupancy in North-Central Florida:
Comparisons of Naturally Regenerated Forests with Planted Pine Stands

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a concentration in Ecology and Evolution Biology
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Date of Approval:
October 7, 2016

Keywords: silviculture, plantation, enclosure, land use, management

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# TABLE OF CONTENTS

LIST OF TABLES iii  
LIST OF FIGURES iv  
ABSTRACT vi  

INTRODUCTION TO DISSERTATION 1  
LITERATURE CITED 3  

CHAPTER 1: Response of Forest Groundcover Structure to Pine Canopy and Shrub Abundance on Public Lands of North Central Florida 6  
ABSTRACT 6  
INTRODUCTION 7  
METHODS 10  
Vegetation Sampling 10  
Data Analyses 12  
RESULTS 14  
Canopy 14  
Shrubs 15  
Groundcover 16  
Statistical Power 17  
OBVM Relationships 17  
Predictive Models 18  
DISCUSSION 19  
Light Competition 19  
Groundcover 20  
Restoration 21  
Use of OBVM Metrics 26  
LITERATURE CITED 27  

CHAPTER 2: Importance of Forest Groundcover for Predicting Occupancy of Rare and Common Amphibians along a Gradient of Pine Canopy in North Central Florida 46  
ABSTRACT 46  
INTRODUCTION 47  
METHODS 51  
Amphibian and Vegetation Sampling 51  
Data Analyses 54
CHAPTER 3: Landscape Dependent Influence of Forest Structure on Amphibian Desiccation and Movement along A Gradient of Planted Pine Density and Native Groundcover

ABSTRACT 80
INTRODUCTION 81
METHODS 84
Movement Experiments 84
Desiccation Experiments 87
RESULTS 89
Movement 89
Desiccation 91
DISCUSSION 93
Desiccation 94
Forest Floor Soil Moisture 95
Movement 96
Behavior 97
Forest Management 99
LITERATURE CITED 100

APPENDIX A: IRB/IACUC Approval 112
LIST OF TABLES

Table 1.1: Canopy Spearmans Corrs. 37
Table 1.2: Shrub Spearmans Corrs. 38
Table 1.3: Groundcover Spearmans Corrs. 38
Table 1.4: Spearman Corrs. for Wiry Cover. Bold variables selected for regression trees and ZINB model of Wiry Cover 40
Table 1.5: Zero-Inflated Negative Binomial Model of Wiry Cover for Xeric and Mesic Uplands 42
Table 2.1: Means ($\chi$) and standard errors (SE) of vegetation structure at sites occupied and unoccupied by SGCN 73
Table 2.2: Single Season Occupancy Model Comparisons for best three models using OBVM Site Covariates Wiregrass Cover and Pine Basal Area 74
Table 2.3: Parameter Estimates for Species where $\Psi$ was dependent on Wiregrass Cover 75
Table 3.1: Canopy and groundcover metrics at forest types with standard error 106
Table 3.2: Proportion of toads that survived in desiccation enclosures to 24, 48, and 72 hours among forest habitats 110
LIST OF FIGURES

Figure 1.1: Study Site Locations within Historic Longleaf Range 35

Figure 1.2: OBVM Canopy and Shrub w/ 95 % CIs for open canopy mesic (M), xeric (X), and silvicultural mesic (SM) and xeric (SX) sites 36

Figure 1.3: OBVM Ground Cover w/ 95% CIs 37

Figure 1.4: Linear Model with r-squared for OBVM shrub and serenoa metrics 39

Figure 1.5: Power Analysis for mesic (blue) and xeric (red) forest stands and sample size required to differentiate among open canopy and silviculture stands 40

Figure 1.6: Regression tree for longleaf regeneration (LR). LR was found at 77/536 OBVM points 41

Figure 1.7: Regression Tree for Wiry Cover 42

Figure 1.8: Predicted values for wiry cover per m² based on basal area of pine (m² per ha) and mean shrub cover in 4 m² for xeric (A) and mesic (B) sites 43

Figure 1.9: FIA plots used to estimate basal area for Mesic (purple) and Xeric (yellow) landscapes within range Longleaf Pine 43

Figure 1.10: Distribution of Forest Basal Areas (m²/ha) within Longleaf Pine Range for Xeric (A) and Mesic (B) landscapes using FIA Data 44

Figure 1.11: Relationship of Fire Interval at St. Marks and Jennings sites to square root transformed OBVM metrics 45

Figure 2.1: Wiregrass and pine basal area at wetlands occupied by SGCN (gray = present, white=absent) 73

Figure 2.2: Predicted Ψ for L. capito (red) and N. perstriatus (black) using wiregrass cover (+/- 1 SE) 75

Figure 2.3: Predicted Ψ for P. ornata using wiregrass cover (+/- 1 SE) 76

Figure 2.4: CART for L. capito 77

Figure 2.5: CART for N. perstriatus 78
Figure 2.6: CART for P. ornata

Figure 3.1: Design of runway enclosures with shoe boxes (gray squares) and pitfall locations (circles) shown at 10, 20, 30, and 50 m from runway center (x)

Figure 3.2: Individual toads observed per cardinal direction and distance (10 - 50 m) for plantation on mesic soil (SM), sandhill (X), and low wiregrass (LW) forest stands

Figure 3.3: Movement rates (m/d) with 95% CIs among cardinal directions in sandhill (X), low wiregrass (LW), and mesic plantation silviculture (SM) forests

Figure 3.4: Canopy and groundcover metrics at forest types with standard error

Figure 3.5: Soil moisture (+/- 1 SE) within 15 mm at desiccation enclosures by forest type and revisit

Figure 3.6: Proportion of water lost from toads (+/- 1 SE) among forest types

Figure 3.7: Smooth function for proportion of water loss by juvenile toads per soil moisture (VWC)

Figure 3.8: Juvenile toad survival probability predicted by soil moisture (VWC) in 15 mm
ABSTRACT

Once dominant, longleaf pine forests of the southeastern United States have been modified by 97 percent, resulting in several animal species being listed as endangered and threatened. Pine plantation silviculture (tree plantings) now occupies half of the original longleaf range where several animal species of conservation concern have experienced recent local population declines. In North America, the accepted practice of pine plantations is to plant pines densely in rows for wood production. Given that land use is considered a primary local driver for the 30% of amphibian species currently at risk of extinction, and planted pine is predicted to expand coverage by 2020, sustainable land management will require integrating ecological and economic goals, including conservation objectives. To understand how amphibian species characteristic of longleaf pine forest are affected by planted pine forestry, it is necessary to understand how associated shifts in habitat structure associated with aging pine stands influence species composition across a wide geographic area, especially populations of rare species. The purpose of this dissertation is to examine how forest structure (natural regeneration vs plantation) affects amphibian species composition and occupancy of small isolated wetlands embedded within the forest. Particular emphasis is placed on assessing several potential causal mechanisms of regional declines in amphibian species. This study was performed on Florida public forests where active forest management is a potential conservation tool and historic populations of rare amphibians occur. Sites where occupancy was assessed included: Goethe State Forest, Ocala National Forest, Jennings State Forest, Saint Marks National Wildlife Refuge, and Apalachicola National Forest. Withlacoochee State Forest was used for examining potential causal
mechanisms of amphibian declines because of close proximity of reference condition forest to planted pine.

Chapter One of this dissertation uses Objective Based Vegetation Monitoring (OBVM) metrics to quantify differences in forest habitat structure surrounding study sites (among planted pines and naturally regenerated second growth pine) and relationships between vegetation metrics. Increased basal area of planted pine resulted in decreased canopy openness and a significant decrease of ground cover, especially wiregrass. *Serenoa repens* petiole counts and percent cover of woody shrubs also significantly decreased wiregrass cover, and variance partitioning indicated that the effects of woody shrub invasion and pine basal area on wiregrass were independent on public forests. Absence of bare ground because of pine needle litter was a significant predictor of wiregrass absence in a zero-inflated negative binomial model (ZINB). ZINB predictions for wiregrass cover along a gradient of pine basal area and woody shrub abundance demonstrated the importance of habitat management for native groundcover on public forests. OBVM metrics were often positively correlated within the canopy, subcanopy, and groundcover categories.

Chapter Two presents the results of site occupancy modeling comparing the presence of 10 species of amphibians in planted pine and naturally regenerated forest, focusing on the influence of canopy and groundcover habitat structure on occupancy of individual amphibian species. Amphibian species of greatest conservation need (SGCN) in Florida adapted to xeric soils, i.e. the gopher frog (*Lithobates capito*) and striped newt (*Notophthalmus perstriatus*), were found where basal tree areas were below 10.3 m²/ha, and a mesic amphibian SGCN, the ornate chorus frog (*Pseudacris ornata*), was found at pine basal areas up to 13.1 sq. m²/ha. All SGCN were found at sites with average woody shrub cover below four percent, and litter cover below
80 percent. Wiregrass cover was higher than four and six percent per m$^2$ at sites with *L. capito* and *N. perstriatus*, respectively. Of 33 site detections for SGCN, only three sites had < 2.5 m$^2$ wiregrass cover. Only one species, the pinewoods treefrog (*Hyla femoralis*), was more common on planted pine stands, and both *Lithobates sphenocephala* and *L. grylio* had constant probability of occupancy regardless of forest type. Percent wiregrass cover was a significant predictor of occupancy for five species, particularly for State listed species *L. capito* and *N. perstriatus*, suggesting it may be a useful indicator of habitat quality for longleaf-dependent amphibians.

Chapter Three focuses on experimental release of juvenile southern toads into two types of terrestrial enclosures to independently determine if amphibian movement and desiccation is determined by forest management. Movement enclosures consisted of four 50 m x 2.5 m unidirectional runways joined at the center to determine movement rate, distance, and behavior among forests of varying habitat structure. Movement rates were relatively consistent among forest types and positively related to rainfall, which itself did not vary among forests. Canopy closure and ground slope were predictors of behavior as toads move preferentially toward canopy openings and negative slopes, particularly when in planted pine habitat. Ten desiccation enclosures, each 15 cm diameter by 45 cm tall, were used to determine water loss and survival of toads for up to 72 hours. The proportional water loss from toad bodies was significantly related to ambient soil moisture at enclosures, with moisture consistently less at planted pines sites in xeric soil, suggesting a potential source of mortality for species specialized to sandhills. Juvenile survival was particularly low at sandhills planted with pine where dry duff replaced native groundcover and likely prevented successful water conservation behavior.

The results of these studies suggest that land management decisions related to planted pine forests will determine the species composition at embedded isolated wetlands for both rare
and common amphibians. As natural disturbance regimes that limit woody shrub invasion are replaced by plantation silviculture tree plantings that further decrease light transmittance, native groundcover is reduced to greater extent than stands allowed to naturally regenerate following past timber harvest. Current and predicted expansion of pine plantation will particularly limit occupancy for amphibian SGCN endemic to sandhills, where planting dense pines lowers ambient soil moisture and juvenile survival. The vegetation metrics presented will allow land managers to guide forests toward conservation goals, to predict suitability of forests for amphibian species, and enhance success when repatriation efforts are needed. The significant relationship of amphibian occupancy to sensitive herbaceous vegetation (wiregrass) highlights that greater emphasis on forest groundcover is needed where amphibian SGCN occur and that cumulative impacts of forest management on native groundcover should be considered.
INTRODUCTION TO DISSERTATION

More than 30% of all known amphibian species are threatened with extinction, and a number of studies have both documented various anthropogenic activities associated with amphibian declines and provided management strategies to prevent further losses (Lannoo, 2005). Although the physiology and life history of amphibians predisposes them to be among the most vulnerable vertebrates to environmental conditions, there remains significant gaps in understanding their conservation requirements, despite documented declines (Means and Means, 2005). Because abundance of amphibians comprising a single population can vary temporally by orders of magnitude because of stochastic factors (Greenberg and Tanner, 2005), recent studies have focused on loss of populations across landscapes, requiring spatial replication. In addition, experimental studies have begun to investigate potential factors behind declines (Popescu et al. 2011; Todd and Rothermel, 2006), recognizing that multiple factors may lead to extirpation.

Habitat loss and/or modification is considered the most likely cause behind local amphibian declines, particularly for species that require not only small isolated wetlands for breeding, but also relatively larger areas of adjacent terrestrial habitat (Lannoo, 2005). As amphibians disperse into terrestrial habitat to forage and over winter, they are exposed to modified habitat conditions that may not be suitable (Means et al. 1996). Juveniles are especially vulnerable to desiccation and often experience high mortality rates (Popescu et al. 2011); yet how movements are influenced by habitat remains poorly known for most species. Further, amphibians with limited dispersal form populations localized to individual small, geographically isolated wetlands, and may depend on metapopulation movements for long term
persistence (Marsh and Trenham, 2001). Of the 74 amphibian species native to the southeastern US, 1/3 spend part of their life cycle in longleaf pine (*Pinus palustris*) forests, which was the historic habitat matrix with embedded freshwater wetlands (Dodd, 1995; Lannoo, 2005). These forests had an open canopy of longleaf pine and low woody shrub cover due to frequent fire, which reduced light competition and led to abundant herbaceous groundcover of wiregrass (*Aristida* spp.), and many other species (Frost, 2006). Longleaf pine forests have declined dramatically because of fragmentation and replacement by urban and agricultural lands (Means, 1996). Among the longleaf-dependent amphibians, Dodd (1997) noted that several were at risk of decline and 1/3 were ranked G1-G4 by the Nature Conservancy, indicating species ranging from uncommon to critically imperiled (Lannoo, 2005).

One of the most widespread forestry practices is planting pines in dense rows called plantations that now occupy many of the remaining pine forests on both public and private lands where rare amphibian species live. This land use is composed of loblolly pine (*Pinus taeda*) planted in rows and harvested every 15-25 years (Fox et al. 2004). Trees are often densely planted, leading to reduced light for ground cover vegetation in older stands, which is the forest layer most amphibians utilize for forage and cover. Thus, plantations are potentially very different ecologically from open canopy longleaf forest that is rich in herbaceous plant species and invertebrate prey for amphibians (Walker, 1993; Folkerts et al. 1993). Prior to planting, stands are prepared by mechanical disturbance that can compact or remove soil and groundcover. Over time, as pine canopies close, the rich groundcover of longleaf forests is diminished from light competition, which was historically curtailed by frequent fire disturbances (Means and Means, 2005).
The planting of loblolly pine over extensive areas of the longleaf pine range has coincided with recent declines of local amphibian populations, including several species of conservation concern (Means and Means, 2005). Florida is a perfect region for examining forestry impacts as it is home to 53 amphibians, including several endemic or nearly endemic species, and yet has a high proportion of longleaf conversion to commercial forestry (Meshaka and Babbitt, 2005; Kautz, 1993). It is unknown to what extent plantation silviculture limits amphibian occupancy of embedded wetlands compared to similar wetlands in open canopy forests, but observational studies have noted that intensive habitat conversion can lead to exclusion (Means et al. 1996). Moreover, investigation of how vegetation parameters determine amphibian occupancy is needed to supplement an increasing effort on public lands to foster ecologically based forestry decisions. This is particularly important for amphibians of conservation concern whose remaining populations exist primarily on public lands in proximity to planted pines. This dissertation investigated: 1) amphibian occupancy at open canopy forests versus planted pine, 2) which vegetation metrics in forests best predict amphibian occupancy, and 3) whether movement or desiccation is affected by planted pine.

LITERATURE CITED


CHAPTER 1: Response of Forest Groundcover Structure to Pine Canopy and Shrub Abundance on Public Lands of North Central Florida

ABSTRACT

Pine plantation silviculture (tree planting) can modify forest habitat structure on public lands that are home to threatened vertebrate species. Isolated wetlands on public lands across North-Central Florida were selected for study based on > 50% coverage of pine plantation or second growth pine forest within a 200 m buffer upslope from wetland edge, and objective based vegetation metrics (OBVM) were recorded at 10 random points within the buffer. OBVM is a low cost vegetation assessment procedure designed to assess progress towards land management goals on public conservation lands, and includes a suite of canopy, shrub, and groundcover measurements. Bootstrapped confidence intervals and t-tests indicated that basal area of pine, pine tree density, and canopy closure were increased significantly when longleaf was replaced by planted loblolly pine. Although plantation forest on mesic and xeric soils also had increased woody shrub cover and *Soroboa repens* (saw palmetto) petiole counts, neither shrub metric was strongly correlated with pine basal area. Variance partitioning further confirmed that increased shrub cover or *S. repens* petiole counts were independent of pine basal area. Wiregrass cover was significantly reduced in stands of planted pine, regardless of soil type, when compared to nearby open canopy pine forests, likely because of light competition. A zero-inflated negative binomial model (ZINB) selected both pine basal area and *S. repens* petiole counts as significant predictors of wiregrass cover. A binomial component predicting excess zeros for wiregrass cover in the ZINB model using bare ground cover was significant for xeric sites, indicating that plots with no bare soil had nearly zero probability of wiregrass presence. These results are
useful for public land managers to consider thresholds in pine density and woody shrub abundance, which likely act synergistically to reduce historically dominant groundcover, including wiregrass.

INTRODUCTION

Both stand structure and species distributions in forest ecosystems are influenced by a series of abiotic factors. Forests are complex, dynamic systems prone to disturbances that open canopy gaps, expose bare soil surfaces, and regenerate nutrient stocks for plant utilization. Fire is a key disturbance in many forests worldwide, including North American forests from ponderosa pine and chaparral in the west, boreal forest in the far north, to longleaf pine in the southeast (Keeley et al. 2009). Many forests are subject to relatively new disturbances generated by anthropogenic activities ranging from deforestation to suppression of disturbance regimes, and forest coverages have changed dramatically regionally, with rapid timber cutting of old growth trees in the southern US and subsequent conversion to agriculture on cutover sites prior to the 1950s (Fox et al. 2004). During habitat conversion, embedded wetlands were often earth filled or drained, threatening native animals such as amphibians which require both freshwater wetlands for breeding and adjacent forest (Loveland et al. 2002). While preservation efforts for some old growth areas of the Pacific Northwest were successful, the extent of loss for such forests in the eastern US was far greater, including longleaf pine forests of the Southeast (Lannoo, 2005). To date, approximately 97 percent of old growth longleaf forests have been lost or otherwise degraded by human activities (Frost, 2006), which exceeds proportional losses of temperate or tropical rain forests, and has led to several threatened longleaf animal species (Lannoo, 2005).
The longleaf pine ecosystem historically encompassed a nearly 37 million hectare (ha) continuous area of open canopy woodland forest or savanna of *Pinus palustris* in the southeastern US and possessed an abundant herbaceous layer dominated by wiregrass (*Aristida* spp.) (Frost, 2006; Van Lear et al. 2005). These are among the most biologically diverse forests with up to 42 plant species per 0.25 m² (Walker and Peet, 1983) and over 900 endemic species mostly associated with a rich herbaceous ground layer (Kirkman et al. 2001). Longleaf forests ranged from xeric sandhills to mesic flatwoods, with variation in groundcover composition because of edaphic conditions, disturbance regime, and geographic location (Carr et. al 2009).

Frequent lightning-ignited fires are a key disturbance for reproduction of dominant canopy and understory species by removing litter from the soil surface (Outcalt, 1994), recycling nutrients, such as nitrogen, in mineral poor soils (Carter and Foster, 2004; McKee, 1982), and eliminating woody shrubs that reduce light penetration to the forest floor (Frost, 2006; Van Lear et al. 2005; Noss, 1989). Woody shrubs can resprout following fire suppression or attain fire-resistant stem sizes (Waldrop, 1992; Grady, 2012) and compete for both light and space with herbaceous plant species (Peet, 2006; Van Lear et al. 2005). While the dominant herbaceous groundcover wiregrass can persist vegetatively for decades without fire (Clewell, 1989), increasingly closed canopies via subtle changes in fire regime (woody encroachment) or pine planting practices can result in long term changes in habitat structure that might reduce wiregrass.

Tree plantations, with trees planted in rows to maximize timber production, have replaced a considerable portion of global forests and have had a substantial impact on forest ecosystems. More than 40 percent of global plantations are planted with pine (Brown and Ball, 2007), including the southeastern United States, where it is the most widespread land cover within
public forests that are managed to promote conservation of rare species in the region, and supplies a major economic market globally (Fox et al. 2004). Plantations in the region typically utilize either loblolly (*Pinus taeda*) or slash pines (*P. elliottii*), which are harvested approximately every 25 years for wood products (Crocker, 1987). One consequence of pine plantations is the loss of subcanopy and/or ground cover over time, posing a concern for biodiversity (Hedman et al. 2000; Andreu et al. 2009). Of the 32 million ha of planted pine in the southern US (Fox et al. 2007), loblolly pine covers 14 million ha, occupying nearly half of the original longleaf pine coverage and representing half of the world's industrially managed forest (Fox et al. 2004). Since 1980, pine plantations resulted in a loss of 500,000 ha of wetlands (Loveland et al. 2002), with plantation silviculture comprising 42 percent of pine forest in the SE US in 2002 and projected to increase to 60 percent by 2020 (Wear and Gries, 2002; Wear and Gries, 2012).

While much research has been directed at promoting pine stem production per area (South, 2006), it is relatively unknown how structural variables at the canopy and subcanopy levels influence herbaceous cover, a key factor in animal diversity. Land managers in areas of planted pine are faced with the problem of balancing economic viability with growing interest in restoring habitat for endangered or declining species that rely on the longleaf groundcover for forage and habitat (Engstrom, 1993; James et al. 1997; Aresco and Guyer, 1999). Such information is critical not only for restoration of pine forest on former plantations, but also for conservation of rare species in areas with active silviculture operations. While only 4000 ha of old growth longleaf forest remain (Means, 1996), millions of hectares of former pine plantations are now being replanted with longleaf pine (see Nokuse plantation; Oswalt et al. 2012) as part of a conservation goal to double existing longleaf coverage from 1.5 to 3 million ha (America's
Successful recovery of former forested lands requires understanding how to assess vegetation structure of longleaf forest stands in both degraded and reference condition and how to maintain it sustainably to maximize conservation value and provide realistic restoration goals.

The purpose of this study was to quantify differences in habitat structure between planted pine and open canopy pine forests on public lands spanning a wide geographic area in North Central Florida where declining populations of several threatened vertebrate species have been linked to plantations. Public forests offer an opportunity to record the response of native groundcover vegetation, such as wiregrass, which can be absent or nearly so in plantation sites (see Archer et al. 2009). The relationships among canopy, shrub subcanopy, and groundcover vegetation was examined to provide guidelines supporting ecologically sustainable forests and/or restoration. Another objective was to determine the power of the sampling methodology needed to quantify differences among forest stands to reduce both potential redundancy in measurements and field time for personnel assessing habitat management progress.

METHODS

Vegetation Sampling

The sites used in this study were small (< 2 ha) palustrine wetlands geographically isolated within either open canopy pine habitat or pine plantation silviculture (Cowardin, 1979). The dominant terrestrial habitat was determined using ArcGIS v 9.3 with forest coverage provided by the Florida Natural Areas Inventory, and the St Johns River, Northwest Florida, and Suwannee River Water Management Districts (https://fgdl.org). Plantation silviculture was selected using the Florida Land Use Cover and Forms Classification System (FLUCCS) code 4410, which is considered coniferous plantation. These shapefiles were then converted to raster
coverage with a 10 x 10 m resolution and focal statistics were used to estimate the percent cover of forest types within 300 m of each cell to identify potential study sites with > 50% coverage of mesic flatwoods, xeric sandhills, or planted pine forest.

A random set of potential study sites were selected in each forest type on public lands across North-Central Florida (Figure 1.1) including, in order of increasing latitude (# in parentheses): Goethe State Forest (12), Ocala National Forest (21), Jennings State Forest (15), Saint Marks National Wildlife Refuge (12), and Apalachicola National Forest (22). The number of sites per forest varied based on forest size and ground truthing to accessibility of sites for sampling. Approximately 20 study sites were selected within each of four forest types: open canopy mesic (M), open canopy xeric (X), mesic silviculture (SM) and xeric silviculture (SX) forests. Within a 200 m buffer upslope from the wetland edge of each study site 10 random vegetation sampling points were placed within terrestrial habitat using Hawth's Tools in ArcGIS v 9.3.

Vegetation sampling was conducted at each of 10 random points per site in October 2014 following the OBVM Vegetation Monitoring Standard Operating Procedure (2007). Within 7 m of each point, the number of pine trees > 6 ft. tall were counted and while standing at each point, the basal area (BA) of pine was recorded with a BA factor 10 prism. Canopy cover was also recorded while facing each of four cardinal directions, using a spherical densiometer. A 4 m² vegetation quadrat was placed at a distance of 5 m in compass bearings of 0°, 120°, and 240° from each point. The quadrat was divided into four 1 m² section and marked at 10 cm intervals to estimate percent cover. For each compass direction, within the 4m² quadrat, OBVM (2007) protocols were used to record shrub cover, shrub stem density, max shrub height (< 2” DBH), max shrub DBH, max *Serenoa repens* height, *S. repens* cover, *S. repens* petiole density,
subcanopy density, and weedy species cover. In the lower left 1 m$^2$ section of the quadrat (when facing away from the OBVM point), the percent covers of bare ground, herbaceous species, litter, exotic plants, woody debris, and wiry graminoids were estimated as well as litter depth. The only deviation from OBVM (2007) procedure was that wiry vegetation was held vertically by hand while estimating percent cover. Additionally, shrubs < 91.4 cm in height were not recorded both to speed data acquisition in the field and because these shrubs were likely defoliated in prescribed burns.

Data Analyses

The software R 3.2.1 was used to generate 95% confidence intervals (CIs) for 10,000 bootstrapped replicates around mean values for each OBVM variable, and results were plotted using the ggplots2 package. For OBVM variables at the 4m$^2$ and 1m$^2$ plots, the average value per OVBM point was calculated when comparing among site types. Welch two sample t-tests among site categories were used to determine significance for OBVM variables with non-overlapping CIs. Spearman's correlation coefficients was used among OVBM variables and a power analysis was performed using the pwr package to determine sample size necessary to detect differences among forest stands. For binary response variables, such as longleaf regeneration, a test of equal proportions among site types was used.

Classification and regression tree (CART) analyses in the rpart and tree packages were used to determine the relationship between wiry cover and both shrub and canopy variables. Tree analysis uses recursive partitioning of a response variable by repeated breaking it into homogeneous groups based values of a predictor, wherein each split maximizes the deviance explained until no further subsets can be made. Deviance explained (R-squared) is supplied per group, and tree plots were used to detect threshold values in vegetation structure relevant to
management recommendations. Cross validation was used to determine model fit and tree pruning to prevent spurious groupings. Highly correlated OVBM variables \((r > 0.6)\) were not used in the same regression tree.

For variables best correlated with wiry cover, generalized linear models were used to test relationships among OBVM metrics. Wiregrass percent cover had many zero values, and an initial quasi-poisson model fit indicated overdispersion. In addition, a log transformation could not normalize the data; therefore, a zero-inflated negative binomial (ZINB) model in the pscl package was used to predict wiry cover based on other OBVM variables using the pscl package in R 2.15. Pearson's correlations among OBVM were then used to select explanatory variables most related to wiry cover. CIs were bootstrapped for ZINB parameter estimates in the boot package to determine if CIs overlapped zero. Variance partitioning using the vegan package was performed to confirm variables used in the final model had independent effects on wiregrass cover. Bare ground cover was used as a source of excess zeros in wiry cover based on field observations that plots with no bare ground were most often lacking wiregrass. Plots with zero bare ground were given a 1, while other plots were given a 0 value in the binomial portion of the ZINB model.

Forest Inventory and Analysiss (FIA) data available from the USDA Forest Service were used to categorize forests in the South region by basal area. FIA is a nationwide standardized assessment of forests at plots located every 2428 ha to assess the state of America's forests broadly (www.fia.fs.fed.us). FIA reference data tables from DataMart were used to create points in ArcMap 9.3 and to select by location only FIA plots within the longleaf pine range, using a shapefile provided by the USDA Natural Resources Conservation Service (www.nrcs.usda.gov).
A pie chart was produced using the *plotrix* package in R and tallies of FIA plots per basal area grouping were found for both xeric and mesic landscape settings.

RESULTS

*Canopy*

Pine basal area (BA) was significantly greater at planted pine forests when compared to open canopy forest of similar soil edaphic conditions, and estimated 95% confidence intervals (CIs) generated with 10,000 replications around average BA values were non-overlapping (Figure 1.2). Specifically, differences in BA between mesic (M=12.5, SE=0.63) and planted pine mesic (SM) sites (M=18.7, SE=0.93), and between xeric (M=10.0,SE=0.51) and plantation xeric (SX) sites (M=14.2,SE=0.71) were significant.

A test of equal proportions indicated significantly greater proportion of longleaf pine regeneration (LR) at open canopy pine forest compared to plantation silviculture stands for both xeric (X-squared = 18.8, df = 1, p < 0.001) and mesic forest types (X-squared = 18.6, df = 1, p < 0.001). The proportion of vegetation plots with LR was 21.0 and 26.7 for mesic and xeric uplands, respectively, and was not significantly different (X-squared = 0.66, df = 1, p = 0.4). There was no longleaf regeneration at plantation forests (Figure 1.6).

Pine tree density was similar among mesic (M= 4.4, SE= 0.38), SM (M= 5.6, SE= 0.40), and SX (M= 6.5,SE=0.49) forest types, but was significantly lower at xeric forest (M=3.1, SE= 0.24) than both SX forest (t = -6.28, df = 149, p < 0.001) and mesic forests (t = -2.29, df = 257, p = 0.02). Estimated 95% CIs of pine density at xeric forest did not overlap with CIs of other forest stand types. Pine densities ranged from 0 - 24 stems per OBVM point. Tree canopies were significantly more open for both xeric (t = 3.7, df = 229, p < 0.001) and mesic forest types (t = 4.57, df = 246, p < 0.001) than their respective plantation silviculture counterparts (Figure
Non-pine tree density was generally low among all site types; *Quercus laevis* was typically observed on xeric soils and *Quercus virginiana* on mesic soils. Because of the low occurrences, no comparative analyses were performed.

**Shrubs**

Mean shrub cover (Figure 1.2) was not significantly different ($t = -1.18, df = 290, p = 0.2$) among open canopy mesic ($M=2.65, SE= 0.54$) and xeric sites ($M=3.57, SE= 0.55$). SM forest had significantly higher shrub cover ($M=9.56, SE= 1.37$) than open canopy mesic sites ($t = -4.6, df = 174, p < 0.001$), but comparisons between SX ($M=3.92, SE= 0.63$) and xeric sites were not significant ($t = -0.42, df = 230, p = 0.6$). Shrub cover at mesic and SM sites was predominately gallberry (*Ilex glabra*), which reached a maximum of 55 and 77 percent in $4m^2$, respectively.

Differences in shrub density were also significant between mesic site categories ($t = -4.69, df = 153, p < 0.001$), but comparisons between SX and xeric sites were not significant ($t = -1.29, df = 192, p = 0.2$). Shrubs at SM sites were also significantly taller ($M=21.83, SE=2.0$) compared to mesic open canopy forest ($M=10.1, SE=1.0$), but differences were neither significant between xeric ($M=8.1, SE=13.0$) and mesic open canopy forest ($t = 1.42, df = 288, p = 0.15$) nor SX ($M=11.2, SE=1.37$) and xeric categories ($t = -1.82, df = 198, p = 0.06$). Maximum shrub heights were 51 cm at SM sites. There was no significant difference in maximum shrub diameter at breast height (DBH), percent cover of weedy species, or subcanopy density among study categories.

*Serenoa repens* percent cover was greatest at SX forest ($M=9.88, SE=1.28$) and least at xeric sites ($M=1.98, SE=0.38$), a significant difference ($t = -5.9, df = 117, p < 0.001$). *S. repens* cover was also elevated at SM ($M=7.37, SE=1.0$) compared to open canopy mesic
S. repens petiole counts followed identical trends among forest stand types as S. repens cover, with largest counts at SX sites (M=18.87,SE=1.99) and lowest at xeric forest (M=2.47,SE=0.45). Similar to S. repens cover estimates, petiole counts were elevated more at SM (M=12.04,SE=1.53) than mesic sites (M=10.18,SE=1.32). Mesic sites also had significantly more S. repens petioles per plot than xeric sites (t = 5.5, df = 152, p < 0.001). S. repens petiole counts peaked at 118 per 4m$^2$. S. repens heights at SX sites (M=16.37,SE=1.51) exceeded heights at xeric sites (M= 2.77,SE=0.48) by a factor of seven.

**Groundcover**

Percent bare ground was significantly greater (Figure 1.3) at mesic open canopy pine forest (M=22.4,SE=2.54) than both xeric forest (M=14.6,SE=1.31) and SM forest (M=9.3,SE=1.54). SX site percent bare ground (M=20.3,SE=2.78) did not differ from xeric sites (t = -1.85, df = 145, p = 0.06). Percent cover of wiry graminoids (wiregrass) was greater at open canopy mesic (M=7.2,SE=8.06) and xeric sites (M=4.8,SE=3.83), than SM (M=1.5,SE=2.99) and SX sites (M=0.76,SE=1.24) by a factor of 3-7. Lower average wiregrass cover at plantations relative to open canopy forest was significant for both mesic (t = 7.44, df = 155, p < 0.001) and xeric sites (t = 12.93, df = 230, p < 0.001). Trends in herbaceous cover were comparable to wiregrass, with over twice as much cover at open canopy mesic (M=16.5,SE=1.5) and xeric sites (M=6.8,0.33) than SM (M=6.6,SE=0.82) and SX stands (M=2.8,SE=0.38). Differences in herbaceous cover among open canopy and plantation sites were significant (p < 0.001) for both mesic and xeric comparisons. Litter cover was on average > 60 percent across all site types (Figure 1.3), with mesic open canopy sites having significantly less litter coverage than other habitat types. Litter depth averaged from 2.5-5 cm, with differences being non-significant for
comparisons between open canopy and plantation silviculture stands. Percent cover of woody debris averages were low and averages ranged between 0.47 and 2.03 percent.

**Statistical Power**

A t-test power analysis in the package *pwr* was used to determine a sample size for a two-tailed hypothesis test with a power of 0.8 and alpha level of 5 percent for comparing mesic and xeric open canopy OBVM data with their plantation stands. A power of 0.8 is an arbitrary value considered to represent a large statistical effect for ecological data sets. For wiry cover, 23.5 and 13 1m² plots would be required to find significant differences among open canopy and plantation stands for mesic and xeric study groups, respectively. The same analysis was repeated but used the averages of the three wiry cover values taken per OBVM point, and indicated that 18.9 and 8.7 1m² plots were sufficient. For basal area of pine, 30 and 42 OBVM points would be required to differentiate mesic and xeric open canopy stands from plantations stands, respectively, because of larger variance than wiry cover among OBVM points. To verify sample sizes, bootstrapped power estimates were then performed by resampling the data set 1,000 times at varying sample sizes and calculating the proportion of resamples where a t-test failed to detect a significant difference among forest types at that sample size (Figure 1.5).

**OBVM Relationships**

Spearman's Correlation Coefficients (r) were significant but varying in strength for comparisons within canopy, shrub, and groundcover OBVM metrics. As anticipated, pine basal area increased with pine density and was strongly associated (r > 0.60) with less open canopies (Table 1.1); however, non-pine densities were neither associated with planted pine nor canopy closure (p > 0.05). OBVM measures of shrub cover, max shrub height, and shrub DBH were all highly correlated (r > 0.9), as were *S. repens* petiole counts, maximum heights, and cover (Table
1.2). Relationships were well represented by linear models (Figure 1.4). *S. repens* OBVM measures were weakly related with shrub abundance ($r < 0.2$). Within the $1m^2$ plots, litter cover was strongly associated with less bare ground and wiregrass or herbaceous vegetation (Table 1.3), the latter of which were highly correlated ($r > 0.7$). Variance partitioning also suggested basal pine and *S. repens* petiole cover were significantly related to wiry cover ($p < 0.005$) and that the shared variation was low ($< 1\%$), indicating independent effects.

A regression tree for wiregrass cover using default criteria in the *tree* package selected five splits, which we confirmed as optimal using cross validation and pruning wherein additional branches did not improve deviance explained. The regression tree contained divisions for pine basal area, mean petiole cover, and mean shrub cover, with an overall $R^2$ value of 0.3 (Figure 1.7). The first (strongest) division was for petiole mean values, with wiry cover averages of 1.5 at petiole mean $> 8.5$ per $4m^2$. For plots with petiole mean $< 8.5$, the next division was shrub cover, with wiry cover being 1.1 where mean shrub cover exceeded 7.2 per $4m^2$. At plots with $< 8.5$ petiole and $< 7.2$ shrub cover, the basal area of pine largely determined wiry cover, with basal areas $< 5$ producing an average 14 percent wiry cover but representing a low number of plots (11). At these shrub and petiole abundances, pine basal areas $\geq 75$ were far more common (84) and were associated with mean wiry cover of 3.2 percent per m$^2$. At pine basal areas $\leq 75$, wiry cover was 5 and 7 percent per m$^2$ dependent on whether petiole covers were above or below 0.65, respectively.

*Predictive models*

As wiregrass cover was best correlated with *S. repens* petiole count, shrub cover, and pine basal area (Table 1.5), which was supported by tree analysis, these variables were selected for GLM modeling of wiregrass cover. Petiole counts and shrub cover were used in xeric and
mesic site comparisons, respectively, as metrics of woody shrub abundance, based on non-overlapping 95% CIs at those locations (Figure 1.2). In addition to pine basal area, both shrub cover and *S. repens* petiole counts were significant negative predictors of wiregrass cover at xeric and mesic sites (Figure 1.8), respectively, in a zero-inflated negative binomial (ZINB) model. When bare ground was absent, there was a significantly larger probability of zero percent wiregrass cover in the xeric ZINB model. ZINB was chosen over a negative binomial using a Vuong test (p < 0.001), and bootstrapped CIs for ZINB parameter estimates in the *boot* package did not overlap zero. ZINB models predicted as either basal area of planted pine or woody shrub abundance increased, wiregrass cover would decline significantly (Table 1.5). Within the 95% CI for basal pine areas (Figure 1.2), wiregrass cover in the ZINB model was predicted to be < 2 percent per 1 m² when woody shrub and petiole abundances are ≥ bootstrapped averages at SM (M=9.6) and SX (M=18.9) forests, respectively (Figure 1.3).

**DISCUSSION**

**Light Competition**

Forest canopy determines light transmittance into forest ecosystems, and shrubs in the subcanopy further compete for light with groundcover, providing a mechanism for the significant reduction in wiregrass and longleaf regeneration found at planted pine stands (Battaglia et al. 2003; Kirkman et al. 2004; McGuire et al. 2001). For example, Brockway et al. (1998) and Glitzenstein et al. (2005) found that reduction of turkey oak subcanopy significantly increased wiregrass groundcover in both Ocala and St. Marks sandhills of Florida. Harrington (2011) found competitive effects of pine canopy on groundcover independent of shrub cover, and McGuire et al. (2001) found that light in canopy gaps was a primary factor in herbaceous abundance. In addition, Hedman et al. (2000) found significant drops in herbaceous cover when
pine canopies increased by a factor of three where understories were comparable. Plantation silviculture pines in the region are typically planted at 1-3 m intervals along rows spaced 2-6 m apart, corresponding to approximately 34 m²/ha of loblolly pine. Whereas densities up to 46 m²/ha are merchantable, planting above 40 m²/ha provides no significant advantage in lumber yields (PMRC, 1997), and Carr et al. (2009) found a maximum of 39 m²/ha from 271 plots throughout Florida. Planted loblolly pines compete with one another for light at densities of approximately 27.5 m²/ha, which can be alleviated by opening canopies to 16 - 20 m²/ha (Georgia Forestry Commission, 2015). A significant negative relationship between woody shrub abundance and groundcover (Figure 1.8) agrees with Benecke et al. (2015), who found woody shrub dominance related to time since fire. The predominate woody shrubs of the current study sites (I. glabra and S. repens) are both slow growing evergreen species that are natural components of longleaf forest, but increase within four years without fire (Benecke et al. 2015; Brockway and Lewis, 1997; Cronan et al. 2015; Van Lear et al. 2005). Lemon (1943) found I. glabra coverage > 8 percent after eight years of fire suppression, which is similar to observed average shrub cover for SM sites of the current study (Figure 1.2). As pine basal area was not correlated with woody shrub abundances at each OBVM point (Table 1.1), which agrees with Moser and Yu (2003), the negative relationship of both woody evergreen shrubs and pine basal area to native groundcover appears to be independent and synergistic on these public lands (Figure 1.8).

**Groundcover**

The wiregrass A. beyrichiana was the dominant groundcover species across the current study region before human settlement (Van Lear et al. 2005) but has declined with infrequent fire and soil disturbance (Kirkman and Mitchell, 2006; Myers and Ewel, 1990). Given its sensitivity
to disturbance regimes (Clewell, 1989), wiregrass is associated with relatively intact longleaf forest (Kirkman et al. 2013) and is thus an indicator species for longleaf habitats (Mitchell et al. 2015). More broadly, herbaceous cover may include species not characteristic of reference sites (Hedman et al. 2000) that can increase quickly and ephemerally following canopy removal. Woody shrubs, by contrast, have below ground root systems that permit rapid regrowth, making them fairly resistant to disturbance (Olson et al. 1995), whereas wiregrass roots are shallow and seed banks short lived (Coffey and Kirkman, 2006; Mulligan et al. 2002). Wiregrass cover averaged 5 - 7.5 percent per 1m$^2$ in open canopy longleaf forest of the current study (Figure 1.3), and given that a single wiregrass clump typically occupied one percent of the 1 m$^2$ quadrat, these estimates agree with 5 clumps per 1 m$^2$ estimates by U.S. FWS (2014) for open canopy flatwoods and sandhills. Results indicated greater wiregrass cover on mesic than xeric sites (Figure 1.3), which is consistent with Kirkman et al. (2001) and likely reflects soil productivity. Aschenbach et al. (2010) provided short term survival estimates for replanted seedlings of several species and found that wiregrass survival and cover can be significantly reduced due to competition with other species. The current results support their conclusion that overstory thinning and shrub reduction will be necessary for managers seeking to restore longleaf on former pine plantations (Figure 1.8).

**Restoration**

Fire records for the past 20 years from St. Marks National Wildlife Refuge and Jennings State Forest confirm greatest shrub reduction and wiregrass coverage at sites with a fire frequency of 2-3 years (Figure 1.11), whereas shrub abundance can increase as pine stands lack fire for 10-15 years (Andreu et al. 2009). Comparing the observed *S. repens* heights to Foster and Schmalzer (2012), several of the current study sites have lacked fire for over 10 years, longer
than historical fire intervals of 3-10 years (Myers and Ewel, 1990), which complicates shrub removal. For example, a prescribed burn of planted pine in St. Marks National Wildlife Refuge with *I. glabra* of 203 cm height was unable to burn its leaves (personal observation). An open subcanopy can be re-established at sites unburned for < 10 years by introducing fire (Walker and Silletti, 2006) before transition to a mixed hardwood forest (Costanza et al. 2015), while stands unburned longer likely require mechanical or herbicidal treatments (Brockway and Outcalt, 2005; Brockway et al. 1998; Freeman and Jose, 2009) that impact non-target species (Kaeser and Kirkman, 2011). Ideally, fire regimes should be based on historical data and incorporate spatial variation to not eliminate native species (Thaxton and Platt, 2006; Wall et al. 2012); however, woody shrubs *I. glabra* and *S. repens* are unlikely to be extirpated from a stand by fire (Abrahamson, 1984). As both *I. glabra* and *S. repens* resprout rapidly after fire (Abrahamson, 1984), reduction of light and soil moisture competitive effects by opening pine canopies may be nullified by competitive increase in shrubs unless the latter are also addressed (Harrington and Edwards, 1999).

Pine thinning fosters restoration of wiregrass or longleaf seedlings by increasing light to the forest floor (Kirkman et al. 2002; Harrington and Edwards, 1999), and restoration ecologists should set future target conditions for pine basal area guided by data from relic open canopy longleaf forests with abundant groundcover. Similar to Carr et al. (2010) greater pine basal areas were found at mesic flatwoods than xeric sandhills in the current study, reflecting site productivity (Figure 1.2), and basal areas of 10 m²/ha were predicted to permit wiregrass cover > 2/m² where shrub encroachment was low or moderate (Figure 1.8). These canopy densities more closely resemble naturally regenerated forests, permitting more light to the ground and encouraging both wiregrass abundance and longleaf regeneration. The pine basal areas at open
canopy sites in this study averaged 10 and 12.5 m$^2$/ha for xeric and mesic sites, respectively (Figure 1.2), which are below the 13.8 m$^2$/ha recommended to provide abundant wiregrass cover by the U.S. Fish and Wildlife Service (2014). However, only 38 and 26 percent of FIA plots for xeric and mesic soils, respectively, had basal estimates below average values at open canopy sites in the current study (Figure 1.10). Approximately 25 percent of FIA plots (Figure 1.9) had basal areas > 27.5 m$^2$/ha, which study models suggest will support wiregrass coverage below two percent, even where woody shrubs are low (Figure 1.8). FIA data together with results of this study could be used to prioritize stands for restoration and ground truthing used to verify management progress. OBVM plot counts of 10-20 provided appropriate power to differentiate average wiregrass cover, while pine basal area was more variable within site types (Figure 1.5). Multiple measures of *S. repens* or woody shrubs appear redundant (Figure 1.4) and could be ignored to reduce field time for land managers performing their own habitat structure assessments. While growing emphasis in longleaf restoration has been placed on establishment of herbaceous ground cover, management of proper canopy or shrub densities that foster ground layer abundance have been lacking (Kush et al. 1999).

Previous studies have investigated herbaceous response to canopy reduction (Harrington, 2011), for example, Andreu et al. (2009) found thinning allows a subset herbaceous species to return on former closed canopy pine forest (31m$^2$/ha), but they noted a disappearance of wiregrass from the seed bank; therefore, re-establishment of wiregrass on former plantations may require replanting. The results of the current study are useful for determining basal area thresholds for thinning pine basal area to meet groundcover restoration goals (Figure 1.8). Despite an initial significant decline in herbaceous cover because of disruption from heavy machinery, Brockway and Outcalt (2015) found increased wiregrass cover six years after
thinning of a flatwoods pine canopy at Goethe State Forest in Florida from 16m$^2$/ha to 11.5m$^2$/ha and 5.8m$^2$/ha using select harvest (1/3 trees removed) and shelter wood methods (2/3 trees removed), respectively. They noted, however, a larger initial decline in wiregrass when their methods were applied to xeric sandhills. Harrington (2011) followed a pine thinning treatment for 14 years and found that it should be part of a long term commitment, requiring repeat treatments, to restore groundcover given increases in canopy cover from pine crowns and woody shrubs.

Given the absence of longleaf regeneration within planted pines (Figure 1.6), successful restoration of longleaf on former agriculture or cut silviculture lands will depend on large-scale replanting efforts (Bell et al. 1997) at appropriate densities. Replanted longleaf in the early grass stage is vulnerable to light competition (Berrill and Dagley, 2010; Hua et al. 2012) and Glizenstein et al. (2005) found longleaf recruitment was essentially absent when basal areas reached 14 m$^2$/ha at St. Marks National Wildlife Refuge of Florida. Hua et al. (2012) recommended pine basal areas ≤ 9m$^2$/ha for high longleaf seedling survival in North Carolina. No longleaf seedlings were found at any of the 212 planted pine plots of the current study, as (naturally) regenerated longleaf was dependent on canopy and litter cover (Figure 1.6), the latter of which impedes seedling establishment (Facelli and Pickett, 1991). Whether a land manager employs active reintroductions or awaits new propagules to regenerate naturally from local seed banks following canopy and/or litter reduction (the "Field of Dreams" hypothesis; Palmer et al. 1997), will depend on species life history and degree of stand modification. Landscape context must also be considered as Glitzenstein et al. (2005) found that longleaf spend more time in the fire vulnerable juvenile stage in (mesic) flatwoods than sandhills. Addington et al. (2015) found that litter cover was important in determining herbaceous vegetation, which agrees with the
results of the current ZINB models for xeric forests (Table 1.5). Regarding wiregrass, Mulligan et al. (2002) found that depending on degree of pine thinning, replanting 5 plants per m\(^2\) to match natural densities at open canopy forest was not essential for successful reestablishment, and lower densities such as 5 plants per 10 m\(^2\) were sufficient.

Andreu et al. (2009) determined species most likely to regrow naturally at pine plantations with long term canopy closure, and others have found that both wiregrass and longleaf may exist for decades with some degree of mixed canopy closure, suggesting potential resilience for the longleaf ecosystem (Glitzenstein et al. 2005; Clewell, 1989). Hedman et al. (2000) suggested that stands without agricultural planting in the past 50-60 years are more likely to have native longleaf associated species and foster successful restoration. For example, after 65 years of natural re-establishment of less vulnerable species, wiregrass was still nearly absent at a silviculture site situated on agricultural land converted to planted pine (Kirkman et al. 2004). As most public forests in the South were acquired in the 1940s, and have some history of prescribed fire after the 1960s, they are good candidates for restoration. As reviewed by Fill et al. (2015), long term restoration will be best encouraged, where ownership allows, by restoring the functional vegetation-fire feedback mechanism that historically existed.

**Natural Disturbance**

Outcalt (1994) provided land managers in the current study region with guidance for timing of fire application to encourage wiregrass propagation or hardwood reduction (Glitzenstein et al. 1995; Kirkman et al. 2013) as modest shifts in fire frequency influence vegetation structure (Kirkman et al. 2013), emphasizing that periodic assessments by land managers will be necessary on public forests. Fire temperatures correlate with woody species reduction (Glitzenstein et al. 2005), which is why prescribed fires are suggested early in the
growing season, when duff is driest (Ferguson et al. 1998; Hughes et al. 1964). In addition to competition for light from increased canopy, Hiers et al. (2007) noted greater litter accumulation at planted pines can physically reduce herbaceous cover and increase fire temperatures (Ellair and Platt, 2013), leading to mortality of pine seedlings. Given differences in shrub cover, land managers should investigate the effectiveness of any dormant season prescribed fires at plantations, where pyrogenic longleaf litter and wiregrass are absent or reduced. As longleaf pine requires bare ground to establish (Facelli and Pickett, 1991), the current study found no regeneration at plantation silviculture, where litter cover was increased (Figure 1.6).

Use of OBVM Metrics

Studies of silviculture practices may be confounded by pre-treatment effects and thus inferences of planted pine impacts in this study focused on comparisons to nearby reference condition forests (Figures 2-3). The low explanatory power of the presented models (Figure 1.8) are attributed to land use legacies (Brudvig et al. 2013), including past agricultural, fire (Figure 1.11), and mechanical methods, as well as variation in shrub abundance within study groups (Figure 1.2). For example, depending on operational use of heavy machinery for logging, varying degrees of soil compaction and disturbance could leave impacts for many years (Hatchel et al. 1970), and in the future land managers should better consider cumulative impacts of forest management. Archer et al. (2009) suggested such impacts are more dramatic on sandy soils, and cited a potential for interactive effects on herbaceous species. Other authors have examined specific silviculture methods and found low intensity roller chopping to reduce woody vegetation (Willcox and Giuliano, 2010) may retain wiregrass cover (Walker et al. 2004). Results of the current study focus on empirical models relating canopy to groundcover (Beckage et al. 2006), demonstrate potential redundancy in OBVM use (Table 1.4), and provide land managers with
practical guidelines toward setting realistic management goals where habitat restoration includes wiregrass on planted pine lands. Explanatory power of presented models are comparable to experimental treatments relating herbaceous to combined pine and shrub densities (R-squared 0.35 in Harrington, 2011), likely since previously mentioned confounding limiting factors on groundcover are present in experimental studies.

In the foreseeable future, area of planted pine will continue to far exceed longleaf pine forests, and on more industrial pine plantations the proposed models suggest groundcover and associated wildlife value will be significantly different than open canopy forests. For areas of longleaf pine, a considerable majority exist with mixed hardwood or partially closed canopies (Costanza et al. 2015), and the return of active habitat management is needed to prevent succession (Fill et al. 2015). Open canopy longleaf forest is associated with richness of plants and endemic vertebrate species including the Gopher tortoise (Yager et al. 2007), a keystone species that provides refuge for threatened species such as the gopher frog (Lithobates capito). Quantifying vegetation structure using relatively simple methods employed in this study can thus provide land managers with assessment of conservation not just for longleaf pine forest, but dependent animal species.

LITERATURE CITED


Figure 1.1 Study Site Locations within Historic Longleaf Range.
Figure 1.2 OBVM Canopy and Shrub w/ 95 % CIs for open canopy mesic (M), xeric (X), and plantations on mesic (SM) and xeric (SX) sites.
Figure 1.3 OBVM Ground Cover w/ 95% CIs.

Table 1.1 Canopy Spearman’s Corrs.

<table>
<thead>
<tr>
<th></th>
<th>Pine Basal Area</th>
<th>Non-pine Density</th>
<th>Pine Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrub Cover</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petiole Count</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-pine Density</td>
<td>-0.121**</td>
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<tr>
<td>Pine Density</td>
<td>0.621***</td>
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</tr>
<tr>
<td>Canopy Openness</td>
<td>-0.640***</td>
<td>-0.033</td>
<td>-0.398***</td>
</tr>
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</table>

*** p < 0.001, ** p < 0.01
Table 1.2 Shrub Spearmans Corrs.

<table>
<thead>
<tr>
<th></th>
<th>SER Petioles</th>
<th>SER Cover</th>
<th>SER height</th>
<th>SH DBH</th>
<th>SH Height</th>
<th>SH Density</th>
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<tbody>
<tr>
<td>S. repens Cover</td>
<td>0.980***</td>
<td></td>
<td></td>
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<tr>
<td>S. repens height</td>
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<td>0.966***</td>
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<tr>
<td>Shrub DBH</td>
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<tr>
<td>Shrub Height</td>
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<td>0.160***</td>
<td>0.925***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrub Density</td>
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<td>0.134**</td>
<td>0.131**</td>
<td>0.889***</td>
<td>0.953***</td>
<td></td>
</tr>
<tr>
<td>Shrub Cover</td>
<td>0.101*</td>
<td>0.124**</td>
<td>0.113**</td>
<td>0.894***</td>
<td>0.946***</td>
<td>0.944***</td>
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*** p < 0.001, ** p< 0.01, * p< 0.05

Table 1.3 Groundcover Spearmans Corrs.

<table>
<thead>
<tr>
<th></th>
<th>Bare Ground</th>
<th>Wiry Cover</th>
<th>Herbaceous Cover</th>
<th>Litter Depth</th>
<th>Litter Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wiry Cover</td>
<td>0.359***</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Herbaceous Cover</td>
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<td>0.752***</td>
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</tr>
<tr>
<td>Litter Depth</td>
<td>-0.572***</td>
<td>-0.244***</td>
<td>-0.245***</td>
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<td></td>
</tr>
<tr>
<td>Litter Cover</td>
<td>-0.865***</td>
<td>-0.541***</td>
<td>-0.682***</td>
<td>0.483***</td>
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<tr>
<td>Woody Cover</td>
<td>-0.008</td>
<td>-0.08</td>
<td>-0.088*</td>
<td>0.067</td>
<td>-0.027</td>
</tr>
</tbody>
</table>

*** p < 0.001, ** p < 0.01, * p < 0.05
Figure 1.4 Linear Model with $r$-squared for OBVM shrub and serenoa metrics.
Figure 1.5 Power Analysis for mesic (blue) and xeric (red) forest stands and sample size required to differentiate among open canopy and silviculture stands.

Table 1.4 Spearman Corrs. for Wiry Cover. Bold variables selected for regression trees and ZINB model of Wiry Cover.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
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<tr>
<td>Pine Basal Area</td>
<td><strong>-0.274</strong>*</td>
</tr>
<tr>
<td>Non-pine Density</td>
<td>-0.065</td>
</tr>
<tr>
<td>Pine Density</td>
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<tr>
<td>Canopy Openness</td>
<td>0.253***</td>
</tr>
<tr>
<td>S. repens Petioles</td>
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</tr>
<tr>
<td>S. repens Cover</td>
<td>-0.359***</td>
</tr>
<tr>
<td>S. repens height</td>
<td>-0.386***</td>
</tr>
<tr>
<td>Shrub DBH</td>
<td>-0.332***</td>
</tr>
<tr>
<td>Shrub Height</td>
<td>-0.321***</td>
</tr>
<tr>
<td>Shrub Density</td>
<td>-0.322***</td>
</tr>
<tr>
<td>Shrub Cover</td>
<td><strong>-0.343</strong>*</td>
</tr>
</tbody>
</table>

*** p < 0.001, ** p< 0.01, * p< 0.05
Figure 1.6 Regression tree for longleaf regeneration (LR). LR was found at 77/536 OBVM points.
Figure 1.7  Regression Tree for Wiry Cover.

Table 1.5 Zero-Inflated Negative Binomial Model of Wiry Cover for Xeric and Mesic Uplands.

<table>
<thead>
<tr>
<th>Xeric</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>z value</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
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<td>-3.65</td>
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<td>0.006</td>
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<td>&lt;.001</td>
</tr>
<tr>
<td>Bare Zero</td>
<td>-1.59</td>
<td>0.416</td>
<td>-3.82</td>
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</table>

<table>
<thead>
<tr>
<th>Mesic</th>
<th>Estimate</th>
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<th>z value</th>
<th>p-value</th>
</tr>
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<tbody>
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<td>0.010</td>
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</tr>
<tr>
<td>Bare Zero</td>
<td>-2.22</td>
<td>1.58</td>
<td>-1.41</td>
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</table>
Figure 1.8 Predicted values for wiry cover per m$^2$ based on basal area of pine (m$^2$ per ha) and mean shrub cover in 4 m$^2$ for xeric (A) and mesic (B) sites.

Figure 1.9 FIA plots used to estimate basal area for Mesic (purple) and Xeric (yellow) landscapes within range Longleaf Pine.
Figure 1.10 Distribution of Forest Basal Areas (m$^2$/ha) within Longleaf Pine Range for Xeric (A) and Mesic (B) landscapes using FIA Data.
Figure 1.11 Relationship of Fire Interval at St. Marks and Jennings sites to square root transformed OBVM metrics.
CHAPTER 2: Importance of Forest Groundcover for Predicting Occupancy of Rare and Common Amphibians along a Gradient of Pine Canopy in North Central Florida

ABSTRACT

The practice of pine tree planting has replaced the majority of historic longleaf pine\textit{(Pinus palustris)} forest, creating a highly modified vegetation structure that persists for decades. \textit{P. palustris} forests are used by threatened amphibian species that breed in embedded wetlands and require adjacent forest as adults. To investigate the relationship between forest management and recent declines of amphibian populations, isolated wetlands were selected on public lands across North-Central Florida with \textgreater{} 50\% coverage of pine plantation or second growth pine forest within a 200 m buffer upslope from wetland edge. Objective based vegetation metrics (OBVM) were recorded at 10 random points within the 200 m buffer, including a suite of canopy, shrub, and groundcover metrics. Amphibian larvae were then sampled 3-4 times at 82 isolated wetlands embedded within either open canopy or planted pine forests, and occupancy analyses using site specific OBVM metrics were performed. Occupancy models with variability by forest type were favored over constant occupancy models for eight species. Wiregrass cover was a significant positive predictor of occupancy for five species, including three species of conservation need (SGCN): the gopher frog (\textit{Lithobates capito}), striped newt (\textit{Notophthalmus perstriatus}), and the ornate chorus frog (\textit{Pseudacris ornata}). Occupancy (\(\Psi\)) for both \textit{L. capito} and \textit{N. perstriatus} was predicted to be \textless{} 50 percent at sites where wiregrass cover was \textless{} 2.5 percent per m\(^2\), which appeared to be a critical value as only three site detections were made below it, all for \textit{L. capito}, possibly due to nearby landscape heterogeneity. Site \(\Psi\) for \textit{Lithobates}}
sphenocephala and Lithobates grylio was independent of forest structure, and Hyla femoralis \( \Psi \) increased on planted pine stands. These results indicate that habitat structure, particularly groundcover, is a strong determinant of the presence and absence of both common and at risk amphibians, and should be a priority for conservation goals or future ecologically based forest certification programs.

INTRODUCTION

Forest habitat complexity is a key determinant of vertebrate communities (August, 1983; Lannoo, 2005) and is increasingly dependent on actions of land managers influencing habitat structure (Coates et al. 2008). The vegetation structure of forests, in turn, can have an impact on local microclimates (Aussenac, 2000) and the distribution of animals sensitive to disturbances in microclimate, such as amphibians (Collins and Storfer, 2003). While at least 30% of amphibian species are declining worldwide, and a number of causes have been implicated, the dominant factor is loss of local habitat (Stuart et al. 2004). Approximately 82 percent of amphibian species require forests (Stuart et al. 2004), which are increasingly degraded to meet global agricultural production including timber. Fortunately, there has been a recent movement in the United States focused on managing forests for ecological function, as documented in the Northwest Forest Plan (NWFP) and Longleaf Stewardship Fund (LSF), which encompass millions of hectares (ha) and direct millions of dollars towards recovery or maintenance of rare forest species (http://www.nfwf.org/longleaf/Pages/home.aspx) and historical habitat structure (Hanson et al. 2012).

Loss or degradation of forest habitat can limit amphibian distributions (Semlitsch, 1998) by reducing refugia from extremes in temperature and dryness (Waldick, 1997), as well as foraging habitat (Semlitsch and Bodie, 2003), the impact of which is dependent on the degree of
habitat modification and species life history (Knutson et al. 1999). Amphibian physiology is particularly susceptible to environmental stress, and local populations at ephemeral wetlands (Sudol et al. 2009) show stochastic natural fluctuations even without human disturbances (Dodd, 1993; Meshaka and Babbitt, 2005; Greenberg, 2001). In regions where wetlands are predominantly small < 2 ha (Hart and Newman, 1995), the bulk of the adult amphibian carrying capacity is particularly dependent on the adjacent forest (Baber, 2001). As adult amphibians largely utilize a few hundred meter radius of habitat surrounding isolated wetlands, it is this forest habitat which is of key concern for local population persistence (Semlitsch, 1998).

Among US forests of relatively high endemism, longleaf pine ecosystems have suffered the greatest proportional loss with the majority of habitats subject to human modified disturbance regimes (Lannoo, 2005). Amphibians endemic to longleaf pine evolved in a once vast, continuous pyrogenic pine savanna, with open canopy and abundant ground cover (Myers and Ewel, 1990; Van Lear et al. 2015). Dominant canopy and groundcover species, such as longleaf pine (Pinus palustris) and pineland threeawn or wiregrass (Aristida spp.), are adapted to fire frequencies that lower competition with woody species capable of encroaching within five years of fire exclusion (Christensen, 1988); therefore, these forests are dependent on active management to maintain habitat structure and ecological function. While wiregrass is a useful indicator species of longleaf habitat (Clewell, 1989), the relationship of longleaf habitat structure and endemic amphibians is poorly known. Old growth longleaf forests have been logged almost completely (Kautz, 1993) and are now either invaded by woody species or have been converted to varying densities of planted pine silviculture (Ware et al. 1993; Lannoo, 2005).
Over half of the 198 amphibian species in the US are found within the longleaf pine range of the Southeast, which has experienced the most dramatic expansion of pine plantation silviculture and associated habitat modification (Ashton and Ashton, 1988, Thomas et al. 2004). Planted pine stands are common globally from boreal (Bergeron and Harvey, 1997) to tropical latitudes (Dawkins and Phillips, 1998), and have reduced habitat complexity (Coates et al. 2008) and animal richness (Loehle et al. 2005). Pine (*Pinus*) tree plantations have replaced over half the region which was historically longleaf pine forest with embedded isolated wetlands (Thomas et al. 2004), including 90 percent replacement of longleaf by commercial forest in Florida (Kautz, 1993). Amphibian populations dependent on isolated wetlands can reach high biomass (Gibbons et al. 2006) and although 74 species disperse from these wetlands to adjacent longleaf pine habitat as juveniles or adults (Dodd, 1992; Dodd, 1995), studies of forest management impacts on amphibians were largely absent until the past decade (Russell et al. 2002b; Lannoo, 2005).

Declines in amphibian abundance following timber harvest have been found in short term studies (< 2 years) mostly involving plethodontid salamanders of mixed hardwood or closed canopy forests (Petranka et al. 1993); however, a meta-analysis found extirpation by timber removal to be unlikely, and counts often increased as forests mature to closed canopies (Tilghman et al. 2012). Moreover, the presence of coarse woody debris (CWD) can ameliorate short term plethodontid declines (deMaynadier and Hunter, 1995; Patrick et al. 2008), suggesting forest floor habitat may moderate declines. Amphibians adapted to open canopy forests also decline with canopy removal (Enge and Marion, 1986) but appear less responsive to CWD manipulation on pine plantations (Owens et al. 2008; Russell et al. 2002b). These differences suggest that amphibians adapted to open canopy cover and low CWD due to frequent fire (Van
Lear and Waldrop, 1994) may utilize soil burrowing behavior (Fritts et al. 2015) or forest floor microhabitats characteristic of mature forests, such as vegetative groundcover (Riedel et al. 2008; Gorman et al. 2009).

As densely planted pine stands age, a closed canopy can develop leading to decreased groundcover (Means and Means 2005; White et al. 1975), and posing a conservation concern for amphibians of longleaf pine (Means et al. 1996). Such stands are considered a serious threat to the gopher frog (*Lithobates capito*) and striped newt (*Notophthalmus perstriatus*), both listed as Near Threatened (NT) by the International Union for Conservation of Nature (IUCN) due to significant declines in local populations (Means and Means, 2005; Hammerson and Jensen, 2004). Both species are considered species of greatest conservation need (SGCN) by the State of Florida (Florida Fish and Wildlife Conservation Commission, 2012), together with three additional species that have also declined or have unknown status: the tiger salamander (*Ambystoma tigrinum*), ornate chorus frog (*Pseudacris ornata*), and flatwoods salamander (*Ambystoma cingulatum*). Densely planted pine monocultures that exclude several amphibians (Means and Means, 2005) hinder best management practices for wildlife (FDACS, 2014).

While it has been suggested that several amphibian species risk extirpation within intensive planted pine (Means and Means 2005), few studies have examined habitat variables such as pine density and/or included study sites with rare species (Enge and Marion, 1986; Russell et al. 2002). Such data gaps make occupancy predictions difficult, especially for less invasive stands of planted pine that are widespread on public lands where remaining SGCN populations persist. Recent efforts to restore longleaf pine forest focus primarily on former plantation silviculture tracts existing in habitats in various levels of recovery. Establishment or augmentation of populations of at risk amphibian species, such as *L. capito* and *N. perstriatus*, is
a high priority conservation goal of the Southeast, and efforts are currently underway for the latter on forest land where planted pine may have contributed to population declines (Means et al. 2001; Means and Means, 2005). The long term success of such efforts will require knowledge of which forests have suitable vegetation structure prior to repatriation of amphibians. Public forest managers will be key arbiters of sustainable forestry practices and amphibian conservation (Meshaka and Babbitt, 2005), which itself will require biologically informed decision making (Hartley, 2002) and knowledge of relationships between the biological and abiotic controlling factors.

The objective of this study was to compare occupancy of amphibian species breeding in isolated wetlands embedded within open canopy reference longleaf pine stands and those planted for pine ≥ 15 years ago. The association between amphibian occupancy and habitat characteristics is essential to understanding how pine plantations act to restrict distributions of rare amphibians (Means, 1996), and habitat structure shifts over the life of a pine plantation are likely to pose an evolving matrix of impediments for amphibian populations. The relationship between forest structure and amphibian occupancy was evaluated using the objective based vegetation management (OBVM) procedure, a set of metrics that public land managers can use to assess how management decisions influence habitat structure. Predictions of SGCN within pine forests of varying pine density and groundcover are presented to supplement future ecologically based forestry guidelines.

METHODS

*Amphibian and Vegetation Sampling*

In this study, sites were small (< 2 ha) palustrine wetlands (Cowardin, 1979) geographically isolated within either open canopy pine habitat or pine plantation silviculture
determined using ArcGIS v 9.3. Potential study sites were categorized based on > 50% coverage of mesic flatwoods, xeric sandhills, or planted pine forest (≥ 15 years old) within 200 m of the wetland-terrestrial edge. A total of 82 wetlands were selected in five publicly owned forests (see Figure 1.1 in Chapter One) across North-Central Florida including, in order of increasing latitude: Goethe State Forest (12), Ocala National Forest (21), Jennings State Forest (15), Saint Marks National Wildlife Refuge (12), and Apalachicola National Forest (22). These public forests were selected for having wetlands with documented populations of SGCN in close proximity to wetlands on stands of planted pine, in order to minimize differences in precipitation when comparing occupancy at naturally regenerated pine forest to adjacent planted pine stands.

Approximately 20 study sites were selected within each of four forest types: open canopy mesic (M), open canopy xeric (X), mesic plantation (SM) and xeric plantation (SX) forests. The winter of 2013-2014 was particularly wet, and all study sites held sufficient standing water for amphibian sampling. At each of the five public forests, several study sites within open canopy forest were known to have been occupied historically by SGCN, but had not been surveyed in several years, while other sites either had never been surveyed or did not have known populations.

Rainfall in Florida can vary dramatically and suddenly, signaling synchronous breeding migrations for several anuran species and necessitating efficient timing of sampling methods (Dodd, 2010). Florida spans a large latitudinal gradient, and many anuran species in Central Florida breed as isolated wetlands fill with convective summer rain, while several anurans and particularly salamanders in North Florida time their reproductive migrations to winter cold fronts (Lannoo, 2005). In 2014, each site was visited three times to sample amphibian larvae during both winter (January - March) and summer (May - August) seasons to characterize the
amphibian communities of each isolated wetland, and sites in Jennings SF were sampled an additional time in July, for a total of four visits. All study sites held sufficient water in both seasons to support amphibian larvae. During each visit, a Ward's 12" diameter D-frame dip net with a 1,000 micrometer mesh was used to sample amphibian larvae. Both the number of 1-m net sweeps performed and water level at the deepest point of the wetland were recorded.

Monitoring biological diversity is best performed using standardized methodology to collect quantitative data regarding the distribution of imperiled species (Heyer et al. 1994). Methods that account for detectability of different species are now favored in the scientific literature, and detectability was accounted for in this study (MacKenzie et al. 2003). Site occupancy (Ψ) provides a probability estimate that breeding sites beyond those sampled during the study will contain a given amphibian species, and has become a preferred tool for monitoring amphibian distributions given populations are the currency of declines, but population size itself is highly variable (Greenberg and Tanner, 2005). Amphibians, despite their abundance and ecological importance, are often difficult to detect due to behavioral avoidance of diurnal moisture extremes and diverse life histories (Heyer et al. 1994). For example, Ambystomids (mole salamanders) and toads (Bufonids, Gastrophyrynge, Scaphiopus) found in this study are fossorial most of the year, and inconspicuous until sufficient precipitation occurs during the breeding season for emergence and movement in terrestrial habitat (Lannoo, 2005). While adults are sparsely distributed under cover in terrestrial habitats, during breeding seasons, amphibian larvae are concentrated in wetlands for weeks or months (Heyer et al. 1994) increasing detection. Thus, this study employed dip netting larvae, across both winter and summer breeding seasons, as an efficient method for sampling over methods that target adults, such as drift fences that can
interfere with breeding migrations and can cause mortality of adult females potentially carrying thousands of eggs (Dodd, 2010).

The objective based vegetation monitoring procedure (OBVM, 2007) was used in October 2014 to determine forest habitat structure at 10 random points surrounding each wetland within 200 m from the wetland edge. Vegetation metrics included pine tree counts within 7 m, pine basal area (BA) using a factor 10 prism, canopy cover using a spherical densiometer. A 4 m² vegetation quadrat was placed 5 m from each point in compass bearings of 0°, 120°, and 240° to record subcanopy metrics (shrub cover, shrub stem density, max shrub height (< 2” DBH), max shrub DBH, max Serenoa repens height, S. repens cover, S. repens petiole density, subcanopy density, and weedy species cover). The lower left 1 m² section of the quadrat (when facing away from the OBVM point), was used to record the percent covers of bare ground, herbaceous species, litter, exotic plants, woody debris, and wiregrass were estimated as well as litter depth. The only deviation from OBVM (2007) procedure was that wiregrass vegetation was held vertically by hand while estimating percent cover. Additionally, shrubs < 91.4 cm in height were not recorded both to speed data acquisition in the field and because these shrubs were likely defoliated in prescribed burns.

Data Analyses

Single season occupancy models (MacKenzie et al. 2003) were performed in PRESENCE 7.8 and the R package unmarked for each species with > 5 detections. In PRESENCE, occupancy (Ψ) was first modeled as a function of a categorical covariate for site type (Ψ_site_type), with 4 levels representing the previously defined categories of predominant pine forest type in 200 m ("M", "X", "SM", and "SX") to determine variation in Ψ among natural and forests planted with pine ≥ 15 years ago. Model Ψ_site_type was compared by species to a model of constant
occupancy ($\Psi$) using Akaike Information Criteria (AIC). For amphibians where $\Psi$ was dependent on site type, AIC was then used to select among models forcing $\Psi$ as a linear function of site specific mean values for OBVM listed in Table 2.1. PRESENCE was also used to estimate a homogeneous detection probability during the study. The empirical Bayes estimate of the proportion of sites occupied was found using unmarked, which represents the proportion of the finite samples occupied. In addition, $\Psi$ estimates were calculated using unmarked for species where wiregrass cover was favored ($\Psi$ (wiregrass)), which represents the probability of occupancy as a linear function of wiregrass cover for an infinite list of hypothetical sites. Finally, $\Psi$(wiregrass) in unmarked were used to predict $\Psi$ along a range of wiregrass cover and a chi-squared goodness of fit test with 1,000 bootstraps was performed to assess model fit to observed data.

Classification and regression tree (CART) analyses in the rpart and tree packages were used to determine the relationship between presence of three amphibian species of greatest conservation need (SGCN) and OBVM variables. Tree analysis is a recursive partitioning method whereby a response variable is repeatedly split into homogeneous groups to maximize deviance explained in the response based on values of a predictor. Deviance explained (R-squared) is supplied per group and tree plots are then used to detect threshold values in vegetation structure relevant to management recommendations. Cross validation and pruning were used to determine model fit and avoid splits that do not reduce error.

RESULTS

A total of 14 anuran (frog) species were detected by dip net during the study, which included the oak toad (Bufo quercicus), Florida cricket frog (Acris gryllus dorsalis), eastern narrow mouth toad (Gastrophyne carolinensis), squirrel treefrog (Hyla squirella), barking
treefrog (*Hyla gratiosa*), pinewoods treefrog (*Hyla femoralis*), little grass frog (*Pseudacris ocularis*), spring peeper (*Pseudacris crucifer*), ornate chorus frog (*Pseudacris ornata*), gopher frog (*Lithobates capito*), bullfrog (*Lithobates catesbiana*), pig frog (*Lithobates grylio*), southern leopard frog (*Lithobates sphenoecephala*), and eastern spadefoot toad (*Scaphiopus holbrooki*). Other species detected included the mole salamander (*Ambystoma talploideum*), dwarf salamander (*Eurycea quadridigitata*), striped newt (*Notophthalmus perstriatus*), eastern newt (*Notophthalmus viridescens*), and southern dwarf siren (*Pseudobranchus axanthus*). During this study, all wetlands held water in winter 2014 and retained water into the following summer sampling period, permitting up to four sampling occasions for species with long larval periods.

In total over 12,200 one meter, net sweeps were conducted (χ = 85 per visit), and the number of unique visits where detection occurred varied by species: *L. sphenoecephala* (82), *H. femoralis* (44), *A. gryllus dorsalis* (29), *L. capito* (29), *H. gratiosa* (25), *N. perstriatus* (16), *L. grylio* (15), *E. quadridigitata* (14), *P. ornata* (12), *P. ocularis* (7), *Bufo* spp. (4), *A. talpoideum* (4), *N. viridescens* (4), and *P. axanthus* (3). All obligate isolated wetland species were captured except flatwoods and tiger salamanders. Three of the five SGCN were found, including: *L. capito*, *N. perstriatus*, and *P. ornata*.

Vegetation structure at sites occupied by SGCN were characterized by decreased pine basal area and woody shrub cover, with greater wiregrass cover than at unoccupied sites (Table 2.1). Xeric specialized species (*L. capito* and *N. perstriatus*) were found at basal areas below 10 m²/ha, and *P. ornata* was found at pine basal areas up to 13 m²/ha (Figure 2.1). All SGCN were found at sites with average woody shrub cover below four percent and less than 80 percent litter cover. Wiregrass cover was greater than four and six percent at sites with *L. capito* and *N. perstriatus*, respectively (Table 2.1). Of 33 site detections for SGCN, only three sites had
detections < 2.5 m² wiregrass cover, and all were for *L. capito* (Figure 2.1). Wiregrass cover at mesic sites was greater than xeric forests, and averaged over nine percent at sites occupied by *P. ornata* (Table 2.1).

Occupancy varied by site type (*Ψ*(site type)) for *L. capito, N. perstriatus, P. ornata, P. ocularis, H. gratiosa, H. femoralis, E. quadridigitata,* and *A. gryllus dorsalis* (ΔAIC > 2), whereas for *L. sphenocephala* and *L. grylio*, occupancy was constant across sites (*Ψ(·)*) (Table 2.2). Detection during the study varied from 0.34 to 0.65, depending on species (Table 2.3); with further variation dependent on visit date for *L. capito* (0.39-0.77), *N. perstriatus* (0.09-0.47), *L. sphenocephala* (0.35-0.78), and *A. gryllus dorsalis* (0.27-0.61). Detection probability was constant for *E. quadridigitata* (0.3, SE = 0.08), *H. gratiosa* (0.63, SE = 0.1), *P. ocularis* (0.08, SE=0.03), *P. ornata* (0.37, SE = 0.19), *L. grylio* (0.19, SE = 0.12), and *H. femoralis* (0.78, SE=0.07).

Site occupancy models for all obligate isolated wetland species captured during the study were improved by incorporating OBVM site specific covariates for vegetation structure (ΔAIC > 2). For xeric adapted SGCN (*L. capito, and N. perstriatus*), *Ψ* models were highly improved (ΔAIC > 10) by incorporating average wiregrass cover within 200 m of wetland breeding sites (Table 2.2). Occupancy models for three other longleaf specialists, including *P. ornata* (ΔAIC > 6), *E. quadridigitata* (ΔAIC > 4) and *P. ocularis* (ΔAIC > 5) were also improved by incorporating wiregrass cover versus *Ψ*(site type). Model selection favored survey specific detection (*p(s)*) during the study for *L. capito, N. perstriatus, L. sphenocephala,* and *A. dorsalis* (ΔAIC < 2); therefore, survey specific covariates (# net sweeps and water depth) were included for these species before presenting *Ψ* parameter estimates. *L. capito* detection was negatively related to water level and number of sweeps; however, more sweeps provided a small advantage.
in detection of *N. perstriatus*. After considering both detection (survey specific) and OBVM (site specific) covariates, parameter estimates were determined for the model that best fit observed data per species using AIC values (Table 2.2). Wiregrass cover was a significant positive predictor of Ψ for all obligate isolated wetland amphibians in this study, and for both *P. ocularis* and *E. quadridigitata* (Table 2.2).

Both *L. capito* and *N. perstriatus* displayed Ψ of 0.07 for the study region, and Ψ was 0.22 for *P. ornata* (Table 2.2). Naïve Ψ is defined as the number of sites with a species present divided by the total number of sites surveyed. The greatest difference between naïve and estimated Ψ values for the best fitting site covariate model (Ψ (wiregrass)) in this study was for *L. capito* and *N. perstriatus* (Table 2.2). Predicted Ψ for *L. capito, N. perstriatus,* and *P. ornata* was > 50 percent when wiregrass cover was approximately two, six, and eight percent per 1m², respectively (Figures 2.2-2.3), and this was further supported by the classification and regression tree analyses (CART). Presence of *L. capito* was highest when wiregrass cover was greater than one percent and pine basal area < 13.8 m²/ha, respectively (Figure 2.4). CART for *N. perstriatus* included at division at 3 percent wiregrass cover, below which the species was not found (Figure 2.5). A CART analysis for *P. ornata* indicated the species can be found at sites with low wiregrass, however, occurrence was < 50 percent where wiregrass was < 3.3 percent per m² (Figure 2.6).

**DISCUSSION**

*Sampling Methodology*

In the current study, detection probabilities for SGCN were moderate to high (Table 2.3) and ranged from 0.34 (0.08) to 0.60 (0.09), indicating that multiple visits using standardized methodology was an effective sampling strategy. Detection increased at lower water levels for
L. capito and increased with number of net sweeps for N. perstriatus. These results agree with field observations that larvae concentrate as wetlands become shallow, reducing water volume for tadpole evasion of nets and allowing more captures per effort. For N. perstriatus, and similar species with small larvae typically at low densities, increased sweep effort will be required for detection. That detection rates for SGCN in this study were comparable to several more common amphibians (Table 2.3) suggests the site selection methods were effective at incorporating open canopy pine forests with active SGCN populations. Previously unknown populations of L. capito were found in both Ocala and Goethe forests, and all species in this study were captured multiple times at the same site, further suggesting that the dip net effort (χ = 85 1m sweeps per visit) used was effective for the entire amphibian community; therefore, given moderate to high detection rates for all species it is reasonable to conclude that differences in Ψ among site types in this study are largely associated with site specific vegetation structure.

Study Sites (OBVM Among Forest Types)

Life histories of all obligate and most opportunistic breeders of isolated wetlands require terrestrial forest as juveniles or adults for foraging and shelter, and adjacent forest within approximately 300 m of the wetland edge appear key for sustaining local populations (Semlitsch and Bodie, 2003). Objective based vegetation monitoring metrics at four previously defined categories of predominantly pine forest in 200 m ("M", "X", "SM", and "SX) were effective at quantifying differences in habitat structure for amphibian occupancy models with site specific covariates (Ψ(site type)). Significant differences in canopy, shrub, and groundcover vegetation found within mesic (M vs. SM) and xeric (X vs. SX) forest types are presented in Chapter One of this dissertation, and include increased pine basal area, increased woody shrub cover (Ilex glabra at SM and Serenoa repens at SX), and decreased wiregrass cover at planted pine (SM, SX)
relative to open canopy pine forest (M, X). Presence of amphibian species varied with vegetation structure surrounding isolated wetlands (Table 2.1). Among SGCN, *N. perstriatus* was most specific in distribution, requiring sites with both low basal area and high wiregrass cover, followed by *L. capito* which was occasionally found at lower wiregrass coverages than *N. perstriatus*, while *P. ornata* was more frequent at wetlands surrounded by planted pine (Figure 2.1). Bayes estimates, representing proportion of finite study sites occupied for SGCN, supported trends in presence by being lower for *N. perstriatus* than *L. capito*, and ranging from 0.17 (.12) to 0.33 (.18) for *P. ornata* (Table 2.3). These results agree with greater recent declines in *N. perstriatus* populations near planted pine (Means and Means, 2005) than the other two SGCNs, as *N. perstriatus* is currently being considered for endangered status (USFWS, 2011). Naïve Ψ estimates were higher than Bayes Ψ for SGCN, likely because of the inclusion of several historic breeding sites, and emphasizes that naïve Ψ values are subject to study site selection (Table 2.3). Selection of public forests with coverage of sandhill and mesic flatwoods using Florida Natural Areas Inventory Data (http://www.fnai.org/gisdata.cfm) and recent (post 1990) detections of SGCN (Krysko et al. 2011), together with ground truthing to verify open canopy conditions, was an effective strategy for targeting SGCN.

**Site Occupancy**

Occupancies (Ψ) for amphibians considered obligate breeders in isolated wetlands in this study (Sudol et al. 2009), were dependent on adjacent forest structure within 200 m (Ψ site type), whereas Ψ for several species that breed opportunistically, such as the *L. grylio* and *L. sphenocephala*, were constant (Ψ) across sites (Table 2.2). *L. grylio* and *L. sphenocephala* are habitat generalists, utilizing wetland habitats as adults, and have long distance dispersal (Wright and Wright, 1949). Similarly, *E. quadridigitata* utilizes habitat within wetlands as an adult
(Petranka, 1998), potentially making it less vulnerable than other longleaf specialists and SGCN in this study (Table 2.3). Among potential OBVM metrics (Table 2.1), wiregrass cover was the most significant predictor of $\Psi$ for five species (Table 2.3), particularly obligates of isolated wetlands or so called longleaf specialists, including all SGCN (Lannoo, 2005). Wiregrass cover at sites occupied by SGCN was typically higher than at unoccupied sites by a factor of three (Table 2.1), and CART analysis suggested it was a major indicator of site quality for occupancy (Figures 2.4-2.6). Site occupancy estimates of SGCN for model $\Psi$(wiregrass) were greater for $P. ornata$ than either $L. capito$ or $N. perstriatus$, and indicated that as low as seven percent of isolated wetlands in the study region would have sufficient wiregrass cover to support $L. capito$ or $N. perstriatus$. These results are consistent with Brown and Means (1984) who found that $P. ornata$ has experienced less range wide extirpations than $L. capito$ or $N. perstriatus$, and that $P. ornata$ is considered tolerant of agriculture and a range of soil edaphic conditions. Lower naïve $\Psi$ estimates in this study and more pronounced recent declines for $N. perstriatus$ than $L. capito$ (Table 2.3), could potentially be due to lower desiccation tolerance in salamanders (Rohr and Madison, 2003; Duellman and Trueb, 1986), which can influence landscape movements and population persistence (Harper et al. 2008). $N. perstriatus$ appears more vulnerable to terrestrial habitat degradation than $L. capito$ (Means and Means, 2005), which could account for lower $\Psi$ predictions at intermediate wiregrass coverages (Figure 2.2). While $\Psi$(wiregrass) was lowest for $L. capito$ and $N. perstriatus$, estimates for two more common species ($P. ocularis$ and $E. quadridigitata$) were comparable to $P. ornata$, suggesting the importance of sensitive groundcover species in the distribution of amphibians considered longleaf pine dependent (Lannoo, 2005) but currently not listed as a conservation priority.
Wiregrass is an herbaceous groundcover species sensitive to forest disturbance regimes and therefore is likely a significant predictor of amphibian occupancy because it integrates long term management decisions and physical disturbances, including independent negative effects of pine canopy and shrub cover (Moser and Yu, 2003). The OBVM vegetation metrics employed here are valuable tools now being incorporated into forest habitat management plans (U.S. Fish and Wildlife Service, 2013). Results of this study are useful for public land managers toward selecting techniques which maintain forests with wiregrass cover (Table 2.1), particularly for stands within dispersal distance of extant SGCN populations.

Landscape Heterogeneity

Although occupancy for SGCN was near zero in the absence of wiregrass, three study sites with wiregrass < 2.5 m² supported breeding for *L. capito* (Figure 2.1). In Ocala National Forest *L. capito* was found at one wetland with thick encroachment of hardwood oaks (*Quercus*) where just beyond 200 m, an open canopy pine stand with increased wiregrass cover may have provided suitable habitat or dispersal source. Further, one previously unknown *L. capito* population in Goethe State Forest was largely within a densely planted pine sandhill but had several hectares of fire maintained wiregrass cover > 2.5 m² on one side of the wetland. These observations suggest that impacts of planted pine on amphibians may be dependent on landscape heterogeneity, a previously known determinant of species composition (Werner et al. 2007), although Homyack et al. (2016) found little impact of landscape metrics in silviculture habitat for several common herpetofauna. Greenberg (2001) found woody shrub invasion alone did not exclude *L. capito* relative to open canopy habitat but noted sites were < 30 m apart. Proximity between wetlands is known to influence herpetofaunal occupancy rates (Attum et al. 2008) and strength of landscape relationships to occupancy or community composition may depend on
retained landscape complexity at plantations (Fox et al. 2004; Loehle et al. 2005). For example, embedded wetlands themselves can sustain populations of some amphibians within plantations (Russell et al. 2002a), particularly for generalist species such as *L. sphenocephala* and *L. grylio*. Lack of regulation has lead to few habitat patches at private timber monocultures, and future studies should investigate the conservation role of retained forest in plantation landscapes (Homyack et al. 2014).

Replicate sites with active populations of rare amphibians appear to be a component thus far lacking in experimental studies (Enge and Marion, 1986; Russell et al. 2002), with reference or pre-treatment conditions typically harvested plantations where groundcover was previously absent. While observational studies have found that *E. quadridigitata*, *L. capito*, and *N. perstriatus* were less likely to occupy wetlands in densely planted plantations of sand pine (*Pinus clausa*), and cited differences among forest floor microhabitats (Means and Means, 2005), forest floor microclimate tolerances of amphibians in this study needs further investigation. Densely planted pines can modify soil edaphic conditions, altering behavior and desiccation risk (Moseley et al. 2004), which may reduce fitness for species such as *N. perstriatus* and *L. capito* that are known to move long distances into pine forest.

*Forest Management*

Proper forest management is key to successful conservation of *N. perstriatus*, which spends most of its life in pine forest (Johnson, 2002), and unlike *L. capito* (Greenberg, 2001) extirpations of local populations appear to occur with modest shifts in vegetation structure (Franz and Smith, 1999). Further, this study found amphibian species more common at longleaf pine-wiregrass communities were also dependent on groundcover (Table 2.3). Woody shrub abundance was elevated at sites unoccupied by SGCN (Table 2.1), and likely indicates decreased
fire disturbance, a determining factor of shrub cover (Van Lear et al. 2005). Lack of prescribed fire is a condition for which the majority of planted pine acres appear prone (Wade et al. 2000) and which may act synergistic with planted pine canopy closure on wiregrass cover, particularly when bare ground is eliminated (see Chapter One). This dual threat should be avoided, particularly near sites with rare species endemic to longleaf-wiregrass ecosystems, given that occupancy rates are currently low (Table 2.3), and the few sites with remaining populations should be used as models to guide habitat management toward similar conservation attributes. As planted pine forests continue to occupy more of the landscape with pine canopies which are less open, isolated wetlands on these lands will provide habitat value for at least some amphibians (Table 2.2; Russell et al. 2002); however, greater emphasis on maintaining groundcover will be necessary where conservation of amphibians and other endemic species is a concern (James et al. 1997).

The tradeoff between canopy and/or woody shrub density and abundance of shade intolerant species at odds between purely economic and ecological based forestry (Hartley, 2002) was a significant determinant of several amphibian species in this study, and likely a major factor behind previously documented extirpations at industrial plantations (Means, 1996). Until the relationship between sensitive herbaceous species cover and occupancy of rare amphibians is better understood, land managers should follow practices which do not eliminate sensitive groundcover, particularly in planted pine uplands within dispersal distance of rare amphibians. Wiregrass can be reintroduced to forests through replanting or seeding (Seamon et al. 1989), and *N. perstriatus* have recently been repatriated at isolated wetlands of Apalachicola National Forest (Means et al. 2011) following severe declines attributed in part to intensive planted pine practices (Means and Means 2005). Results of the current study indicate that planted pine forests
pose a considerable challenge for conservation of rare amphibians in the southeastern US given long term differences in forest structure (Table 2.1). Planted pine is projected to increase in the South (Wear and Greis, 2002) and future incentives to provide conservation value for amphibians will benefit from quantitative studies between vegetation and amphibian occupancy to set practical and cost effective goals for groundcover restoration. The importance of disturbance of sensitive herbaceous groundcover for amphibians in *Pinus* plantations is likely not unique to the current study region (Morneault et al. 2004), but only recently have studies investigated the effect of repeated cycles of mechanical disturbance or harvest on amphibian species (Homyack and Haas, 2013), and more work is needed to prevent passing thresholds in groundcover found in this study if conservation is a priority.

LITERATURE CITED


Figure 2.1 Wiregrass and pine basal area at wetlands occupied by SGCN (gray = present, white=absent).

Table 2.1 Means (χ) and standard errors (SE) of vegetation structure at sites occupied and unoccupied by SGCN.

<table>
<thead>
<tr>
<th>Wiregrass cover (%)</th>
<th>χ occupied</th>
<th>SE</th>
<th>χ unoccupied</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. capito</td>
<td>4.3</td>
<td>0.3</td>
<td>1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>N. perstriatus</td>
<td>6.3</td>
<td>0.6</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>P. ornata</td>
<td>9.4</td>
<td>1.1</td>
<td>3.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Basal area pine (m²/ha)</th>
<th>χ occupied</th>
<th>SE</th>
<th>χ unoccupied</th>
<th>SE</th>
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<tr>
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<td>0.6</td>
<td>13.6</td>
<td>0.6</td>
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<td>N. perstriatus</td>
<td>9.9</td>
<td>0.9</td>
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</tr>
<tr>
<td>P. ornata</td>
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<td>16.3</td>
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</table>

<table>
<thead>
<tr>
<th>Woody shrub cover (%)</th>
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<th>SE</th>
<th>χ unoccupied</th>
<th>SE</th>
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<td>P. ornata</td>
<td>2.4</td>
<td>0.44</td>
<td>5.4</td>
<td>0.98</td>
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<table>
<thead>
<tr>
<th>Litter cover (%)</th>
<th>χ occupied</th>
<th>SE</th>
<th>χ unoccupied</th>
<th>SE</th>
</tr>
</thead>
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<tr>
<td>L. capito</td>
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<td>2</td>
<td>80.6</td>
<td>2.1</td>
</tr>
<tr>
<td>N. perstriatus</td>
<td>75.9</td>
<td>3</td>
<td>79.7</td>
<td>1.6</td>
</tr>
<tr>
<td>P. ornata</td>
<td>56.9</td>
<td>5</td>
<td>74.5</td>
<td>1.9</td>
</tr>
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</table>
Table 2.2 Single Season Occupancy Model Comparisons for best three models using OBVM Site Covariates Wiregrass Cover and Pine Basal Area.

<table>
<thead>
<tr>
<th>Species</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>no.Par.</th>
<th>Species</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>no.Par.</th>
</tr>
</thead>
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<tr>
<td>L. capito</td>
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<td></td>
<td></td>
<td>N. perstriatus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>psi(wiregrass),p(s)</td>
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<td></td>
<td>90.2</td>
<td>4</td>
<td>0.49</td>
<td>3</td>
<td>psi(sitetype),p(s)</td>
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<td>psi(sitetype),p(s)</td>
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<td>1</td>
<td>10.16</td>
<td>9</td>
<td>psi(.),p(s)</td>
<td>3</td>
<td>13.9</td>
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<tr>
<td>P. ornata</td>
<td></td>
<td></td>
<td></td>
<td>P. ocularis</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>psi(wiregrass),p(.)</td>
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<td>5</td>
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<td>psi(wiregrass),p(.)</td>
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<td></td>
<td>69.8</td>
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<td>psi(sitetype),p(.)</td>
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<td>psi(.),p(s)</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>psi(sitetype),p(.)</td>
<td>56.3</td>
<td>0</td>
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<td>psi(wiregrass),p(.)</td>
<td>71.5</td>
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<td>81.9</td>
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<td>3</td>
<td>psi(.),p(.)</td>
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<tr>
<td>psi(basal),p(.)</td>
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<td>8</td>
<td>29.58</td>
<td>3</td>
<td>psi(.),p(s)</td>
<td>8</td>
<td>4.28</td>
</tr>
<tr>
<td>A. dorsalis</td>
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<td></td>
<td></td>
<td>H. femoralis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>psi(sitetype),p(s)</td>
<td>107.</td>
<td>4</td>
<td>0</td>
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<td>psi(sitetype),p(.)</td>
<td>103.</td>
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<td></td>
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<td>4</td>
<td>psi(.),p(.)</td>
<td>4</td>
<td>9.03</td>
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<tr>
<td>psi(basal),p(s)</td>
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<td>3</td>
<td>15.9</td>
<td>4</td>
<td>psi(.),p(s)</td>
<td>7</td>
<td>10.4</td>
</tr>
<tr>
<td>L. grylio</td>
<td></td>
<td></td>
<td></td>
<td>L. sphenoecephala</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>psi(wiregrass),p(s)</td>
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<td>psi(.),p(s)</td>
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<tr>
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<td>psi(sitetype),p(s)</td>
<td>6</td>
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</tbody>
</table>
Table 2.3 Parameter Estimates for Species where $\Psi$ was dependent on Wiregrass Cover.

<table>
<thead>
<tr>
<th>Species</th>
<th>Detection p. (SE)</th>
<th>$\Psi$ (SE)</th>
<th>Naïve $\Psi$</th>
<th>GOF p-value</th>
<th>$\Psi_{\text{wiregrass}}$ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. capito</td>
<td>.60 (.09)</td>
<td>.193 (.06)</td>
<td>.36</td>
<td>.5</td>
<td>.07 (.36)</td>
</tr>
<tr>
<td>N. perstriatus</td>
<td>.34 (0.08)</td>
<td>.17 (.12)</td>
<td>.18</td>
<td>.37</td>
<td>.07 (.38)</td>
</tr>
<tr>
<td>P. ornata</td>
<td>.37 (.19)</td>
<td>.33 (.18)</td>
<td>.40</td>
<td>.55</td>
<td>.22 (.11)</td>
</tr>
<tr>
<td>P. ocularis</td>
<td>.65 (.09)</td>
<td>.21 (.08)</td>
<td>.33</td>
<td>.47</td>
<td>.10 (.07)</td>
</tr>
<tr>
<td>E. quadridigitata</td>
<td>.30 (.08)</td>
<td>.41 (.23)</td>
<td>.25</td>
<td>.44</td>
<td>.22 (.11)</td>
</tr>
</tbody>
</table>

Figure 2.2 Predicted $\Psi$ for L. capito (red) and N. perstriatus (black) using wiregrass cover (+/- 1 SE).
Figure 2.3 Predicted $\Psi$ for P. ornata using wiregrass cover (+/- 1 SE).
Figure 2.4 CART for L. capito.
Figure 2.5 CART for N. perstriatus.
Figure 2.6 CART for P. ornata.
CHAPTER 3: Landscape Dependent Influence of Forest Structure on Amphibian Desiccation and Movement along A Gradient of Planted Pine Density and Native Groundcover

ABSTRACT

Habitat modification can contribute to amphibian population declines by increasing mortality and altering movement into upland forest from wetland breeding sites. To investigate the influence of late succession pine plantation silviculture on both potential causal mechanisms behind amphibian occupancy, juvenile southern toads were released into three terrestrial enclosures along a gradient of pine canopy and wiregrass groundcover. Each enclosure consisted of four 50 m x 2.5 m unidirectional runways facing each cardinal direction and joined at the center. Toads were batch marked and photographed for individual identification during each revisit to determine movement rate, orientation, and reversals within runways. Individual toads were also released into ten 15-cm diameter enclosures at four locations varying in planted pine density and wiregrass cover to compare desiccation rates up to 72 hrs. Average movement rate of toads was approximately 6 m/day, and did not vary significantly among runways given comparable rainfall. The majority of toads were captured at 50 m pitfalls indicating movement was directed with few reversals. Toad orientation within runways was negatively predicted by slope and pine basal area, with differences most pronounced for densely planted pine. Juvenile southern toads experienced greater desiccation at pine plantation silviculture on xeric soils, where average soil moisture was up to 46 percent less than at nearby xeric sandhills due to dry needle duff. Proportion of water lost over time and toad mortality rates were significantly dependent on soil moisture, and toad survival rates at plantation forest on xeric soils were < 50
percent after 72 hours. Forest floor microhabitats including native groundcover should be considered critical for amphibian conservation, especially in late successional plantation silviculture on xeric soils.

INTRODUCTION

Amphibians are considered useful indicators of habitat quality because their permeable skin increases susceptibility to desiccation (Jorgensen, 1997) and, as poikilotherms, their metabolic cost is correlated with environmental temperature. The breeding sites for many amphibians include small (< 2 ha) wetlands geographically isolated within forested uplands; therefore, long term persistence of local amphibian populations depends on adjacent forest both to find refuge and food resources, and move between wetland breeding sites (Dodd, 1996). Amphibians exposed to temperature or moisture stress in disturbed terrestrial habitat may either emigrate, retreat to suitable microhabitats, or ultimately perish, all of which can lead to reduced captures (deMaynadier and Hunter, 1995). As amphibian populations can vary stochastically in undisturbed habitat (Greenberg and Tanner, 2005), recent studies have focused less on abundance and more on occupancy, behavior, and especially vital rates (Todd and Rothermel, 2006).

Habitat degradation is a major cause of amphibian declines (Delis et al. 1996), and given profound loss of wetlands and native forest extent in the east US (Lannoo, 2005), proper management of remaining forests is critical to conservation. Modification of terrestrial habitats by forestry practices can decrease amphibian abundance by impacts on temperature, moisture, and habitat structure (Herbeck and Larson, 1999). For example, short term (≤ 2 years) studies of plethodontid salamanders adapted to mixed or closed canopies have found declines following timber harvest (deMaynadier and Hunter, 1995; Herbeck and Larson, 1999; Petranka et al. 1994)
associated with increased temperature and reduced moisture in soils (Gieger, 1965). Reduced canopy forces these species to seek moist microhabitats to avoid desiccation (Ross et al. 2000; Jaeger, 1980), which can increase their energy cost (Homyack et al. 2011), and limit activity, foraging, and/or overall species distribution (Spotila, 1972). However, effects of canopy removal alone may not cause extirpation of forest stands (Tilghman et al. 2012) and can be ameliorated by appropriate ground microhabitats, suggesting that the latter may be key to survival for juvenile amphibians (Earl and Semlitsch, 2015).

While several amphibians of conservation concern endemic to historically open pine canopies in the eastern US have experienced declining populations near plantation silviculture, investigations of behavior and vital rates as stands mature into dense canopies with modified forest floor microhabitats are lacking. Forests managed for rows of planted pines (plantation) develop a closed canopy that often persists for ≥ 15 years leading to thick needle litter replacing herbaceous groundcover, shifting forest floor physiogamy away from conditions of historically open canopy forests. As pines compete for canopy space and light reaching the forest floor is reduced, herbaceous species are lost, and accumulation of pine litter modifies ground substrate. In turn, loss of suitable microhabitat could result in reduced amphibian fitness, and ultimately survival. While declining local amphibian populations are associated with intensive pine plantation silviculture (Means, 1996), experimental investigations of potential causal mechanisms are lacking, particularly comparisons of fitness at planted pine to open canopy forests in reference condition (FNAI, 2009).

Dispersal of juveniles between wetlands is influenced by habitat structure (Harpole and Haas, 1999; Gibbons et al. 2006; Gardner et al. 2007) and is a critical factor for rescuing local populations experiencing declines and allowing sites to be recolonized following extirpation
Although the majority of movement between populations is attributed to juveniles, smaller body size makes them more vulnerable to desiccation than adults (Rittenhouse et al. 2008). Recent experimental studies reported significant differences in movement and/or orientation of juveniles associated with forest clearcutting (deMaynadier and Hunter 1995; Todd and Rothermel, 2006; Semlitsch et al. 2008), resulting in decreased amphibian richness, abundance, and behavioral avoidance. Such studies are relatively recent, and few have incorporated native groundcover characteristic of historic forests which may ameliorate temperature or moisture stress for juvenile amphibians as they move among forest floor habitats (Heatwole, 1960). Differences in the movement patterns of individuals could be useful to identify causal mechanisms behind observed variation in occupancy rates of large scale studies with replicated breeding sites among habitat types.

This study investigated two potential mechanisms behind local population declines of amphibians at planted pine forests. Movement behavior was examined in directional runway enclosures at xeric sandhills with moderate pine density and low wiregrass groundcover (LW), xeric sandhills in reference condition (X) with abundant wiregrass and an open pine canopy, and densely planted pine on mesic soil with thick needle litter covering the forest floor (SM). Relative rates of desiccation were also compared within known fate enclosures at LW, S, and SM sites, plus an additional location of planted pine on xeric soil (SX). Southern toads (Anaxyrus terrestris) were selected for this study because they are reported to be less sensitive to desiccation associated with canopy removal than other amphibians due to lower skin permeability (Duellman and Trueb, 1986) and have previously been used to assess silviculture impacts (Homyack et al. 2013; Todd and Rothermel, 2006). Fritts et al. (2015) found differences in use of coarse woody ground debris by southern toads in dry versus wet periods, indicating the
importance of forest floor environments in fitness conserving behavior. Southern toads also utilize several microhabitats, including live vegetation, for diurnal temperature refugia, suggesting that despite being habitat generalists (Hanlin et al. 2000), they respond similarly to most amphibians by seeking microclimates that prevent high physiological cost (Jaeger, 1980).

METHODS

This study was conducted at Citrus Tract of the Withlacoochee State Forest (WSF) in Citrus County, Florida. The WSF is an approximately 64,600 ha tract managed for multiple uses including timber harvest, wildlife habitat, outdoor recreation, and ecological restoration. The majority of the area is second growth, sandhill habitat interspersed with mesic flatwoods and planted pines. The experimental movement enclosures were located along a gradient of forestry management from reference sandhill with open canopy pine and high wiregrass to densely planted loblolly pine stands with heavy pine litter accumulation.

Movement Experiments

Three experimental movement enclosures were built in May and June 2015, one each at three habitat types: densely planted loblolly pine (SM), open canopy pine with low wiregrass/shrub cover (LW), and open canopy pine in reference condition with high wiregrass cover (X). Each enclosure was made of silt fence stapled to wooden stakes and consisted of four 50 m x 2.5 m runways, each facing one of four cardinal directions and joined at the center (Figure 3.1). Runway walls were 0.45 m tall and buried 30 cm into the soil. Wood baffles (2.5 cm wide) were placed on top of walls to prevent toads escaping. In each runway, pitfalls were placed at 10 m, 20 m, and 30 m intervals behind interior walls angled at 45° to capture only toads that reversed direction toward the release point (Figure 3.1). Pitfalls were also placed at 50 m in each direction to capture individuals traveling the entire runway distance. At the center of each
10 - 30 m interior wall, a 3 L plastic shoe box with sides removed was placed to further encourage unidirectional movement (gray boxes in Figure 3.1). Wooden covers were placed 5 cm above pitfalls and a rain gauge was installed adjacent to each runway enclosure. An Arduino Uno recorded air temperature and humidity among habitat types using a DHT-11 sensor accurate to +/- 0.5 °C. Vegetation structure was assessed within runways using the Objective Based Vegetation Monitoring procedure (OBVM, 2007) in the center of each direction at 10, 20, 30, and 50 meters distance from the center junction of all four runways. Ground slope from common starting point of runways was measured in each runway to a distance of 10 m, and between 10-20 m, 20-30 m, and 30-50 m using mason string, a line level, and wooden stakes.

Two pairs of southern toads in amplexus were collected on 16 July 2015 at an isolated wetland in Chassahowitzka, Florida, approximately 8 km from WSF study sites. Fertilized egg masses were placed into plastic bins and upon hatching tadpoles were placed into wading pools under partial shade. Tadpoles were fed rabbit chow until metamorphosis and southern toad metamorphs began emerging up the sides of wading pools on 6 August, after which they were collected daily for one week and placed onto moist paper towels inside 15 L plastic bins maintained at 25 C. Juveniles were fed fruit flies every other day until release into runway enclosures.

Juvenile toads were randomly divided into 8 batches, each given a unique identification by clipping one toe on a front leg and consisting of 40-50 juveniles released simultaneously per runway. The first batch of toads was released on 10 August in the center of each runway, and subsequent releases were made on the same night for all runways as individuals from the previous batch had reached 50 m. Runways were revisited daily where possible until 19 September, and individuals captured in pitfalls were identified to batch, weighed to 0.1 g, and
released from enclosures. All toads observed moving in enclosures were also identified to batch, weighed, and their individual body patterns photographed, then released into enclosures in the direction of travel. Dispersal orientation was inferred from which cardinal direction an individual was captured or observed after passing a 10 m interior wall. Using 4-5 photographs per individual per encounter, unique toads were identified by body pigment patterns to estimate movement rate and total distance traveled per runway and direction.

For analyses of movement behavior and success in runways, models were built with one data point per unique individual toad, as identified with photography of body patterns, and therefore, repeated measures analyses were avoided. A generalized linear mixed effects (GLME) model in the R package lme4 was used to compare differences in the proportion of unique individuals observed in runways. The explanatory variables habitat and maximum distance traveled were treated as fixed effects. Batch ID was used as a random effect to allow for correlation in behavior for toads released simultaneously. Batch was used as a random variable because its variation was not of direct interest; toad initial masses were comparable, and all individuals per batch were released within an hour at all habitats, leading to potential correlation of behavior within batch. A binomial distribution was used to model the proportion of toads traveling a given runway direction, using the number individuals released per batch as a weight factor. The proportion of individuals in pitfalls at 10 - 30 m was interpreted as the number of toads that changed their initial direction to return to the runway center. To examine whether the proportion of individual toads was directional within runways, the fixed effect of runway ID, a unique value per cardinal direction, was assessed per habitat. During analyses, AIC criteria was used to select among competing models and chi-squared test of nested models used to drop non-significant terms.
Movement rates were estimated as the quotient of distance traveled prior to the first observation of an individual (m) over time (d) since batch release (m/d). The first observation was used because the majority of toad movement was directional, occurring between release and the first observation. Differences in movement rate among habitats were assessed using Wilcoxon-Mann-Whitney rank sum tests. Capture histories were created for individual toads and MARK software was used to determine differences in recapture rates among habitats using Cormack-Jolly-Seber closed population models. Mean movement rates of individuals per revisit were compared to precipitation collected per visit at each runway using beta regression to determine environmental control on movement timing and intensity.

For directional movement, log-likelihood ratio G tests were used to compare observed frequencies of individual toads in each cardinal direction to determine if they were significantly different than equal (25 percent). For runways where toads preferentially selected cardinal directions, a binomial regression was run using OBVM metrics and slope as explanatory variables to determine why directions had significantly greater proportions of individual toads.

Desiccation Experiments

To investigate amphibian desiccation rates among habitat types, 40 enclosures were created, each made of 25 x 45 cm rolls of size 1 mm mesh fiberglass screen rolled into a cylinder around 0.6 cm mesh poultry wire to provide rigidity. Soil disturbance was minimized by installing each enclosure by hand using a spade to a depth of 6 cm. This design allowed light and wind penetration. Ten enclosures were spaced > 3 m apart in each of four forest stands on WSF: planted pine on mesic soil (SM), planted pine on xeric soil (SX), low ground/shrub cover sandhill (LW), and sandhill in reference condition (X) as defined by the Florida Natural Areas Inventory (FNAI, 2009).
Juvenile southern toads collected from the same mating pair used for runway experiments were randomly assigned to one of four habitats, and placed on moist towels four hours prior to release to fully hydrate. Toads were not fed for 24 hrs prior to release, then were individually weighed to 0.001 g and placed into enclosures. During each trial (release), each enclosure was visited three times at approximately 24 hour intervals in the same order of release to keep exposure times comparable among habitats. For each revisit, toads were reweighed and replaced in enclosures. Air temperature, humidity, and soil moisture were recorded adjacent to each enclosure on each visit. Soil moisture was measured using a Vegetronix VH400 sensor, and meteorological data were taken with a DHT-22 and Arduino Uno. The VH400 is a high frequency dielectric sensor that has an output voltage of 0-3 VDC dependent on moisture of soil with an accuracy of 2 percent. The VH400 connected to an Arduino inputs an analog signal that ranges from 0-614 and voltage curves available from Vegetronix were used to convert analog voltage to volumetric water content (VWC). The DHT-22 sensor measures humidity and temperature with 2-5 and 0.5 percent accuracy, respectively. In total, six consecutive trials (each 3 visits) at each of four habitat types were conducted.

Differences in abiotic factors (air temperature, humidity, and soil moisture) at desiccation enclosures were evaluated using an ANOVA. The proportion of water lost by toads per hour of exposure and among forest habitats were analyzed using the betareg package in R. Lack of independence for toad mass over time was assessed with an autocorrelation function. Where non-linear effects were detected in exploratory data analysis, an additive model in the mgcv package was used to confirm significance and assigned a unique ID per frog to account for temporal correlation in additive modeling. A likelihood ratio test was used to determine the overall significance of forest type on proportion water loss.
RESULTS

Movement

Altogether, 1,110 toads were released into runway enclosures, from which 383 marked individuals, identified from photos of body patterns, were recaptured either in pitfalls or while moving along runways. The number of marked individuals encountered was greatest in LW habitat (129), followed by SM (115) and X forest (96). The proportion of toads reversing direction, as indicated by pitfall captures, was higher for X (31) and LW (44) xeric forests than for SM (24) habitats, but differences were not significant among habitats (p > 0.05).

The distance toads moved varied significantly within each runway, with more toads captured at 10 m than 20 m (z = -4.54, SE = 0.18, p < 0.001) or 30 m (z = -5.0, SE 0.19, p <0.001), and still more observations made at 50 m than 10 m (z = 3.33, SE = 0.13, p < 0.001). The proportion of toads recaptured while moving in runways did not vary by habitat ($\chi^2 = 3.40$, df = 2, p = 0.18) and there was no interaction between habitat and distance (LRT = 4.11, df = 6, p = 0.66). At least one individual reached 50 m in all cardinal directions of all three runways and the proportion of toads reaching 50 m did not vary among habitats (LRT = 5.10, df = 2, p = 0.079). The number of toads observed varied by runway direction (LRT = 51.70, df = 3, p < 0.001), and was habitat specific among cardinal directions (N,S,E,W), particularly for SM forest (Figure 3.2).

Movement rate of individual juvenile toads in runways ranged from 0 - 25 m/day, and was significantly different among habitats using a Kruskall Wallis test ($\chi^2 = 17.19$, df = 2, p < 0.001). LW habitat had higher movement rates than both X (W = 4467, p < 0.001) and SM habitats (W = 9314, p < 0.001); however, maximum rates were 20-25 m/d for all habitats. Further, movement rates were relatively consistent among cardinal directions within habitats.
(Figure 3.3) and were approximately 6 m/d for all habitats, despite differences in habitat and microclimate.

There were significant differences in vegetation and slope both within and among runways, which influenced movement behavior of juvenile toads. SM forest had greater pine basal areas (Table 3.1) than either X or LW sites. The LW runway had two directions (E,S) within open canopy condition and wiregrass cover 1-6 percent m$^2$, and two directions (W,N) with denser pine and litter accumulation (65-95 percent m$^2$), with the runway center positioned along the ‘interface’. Groundcover and pine basal areas were consistent within X and SM forests (Table 3.1). Similarly, ground slope was dependent on runway direction for all forest types (p < 0.01). Southern toads selected cardinal directions non-randomly in all habitats, with the greatest degree of directionality for SM forest (G = 67.8, df = 3, p < 0.001), followed by LW (G = 57.3, df = 3, p < 0.001), and X habitat (G = 11.5, df = 3, p < 0.008). Movement was most directional in SM forest, where 68 percent of toad observations were in the south facing runway (Figure 3.2). Using significant canopy and groundcover differences among habitats (Table 3.1) to predict toad orientation, the main factor for variation in toad observations among directions was slope. The proportion of toads per runway direction/distance was negatively predicted by slope ($z = -5.50$, SE = 0.17, $p < 0.001$) and pine basal area ($z = -3.49$, SE = 0.002, $p < 0.001$). There was also a significant interaction between slope and basal area ($z = 2.29$, SE = 0.003, $p = 0.022$). This trend held for the proportion of toads captured in pitfalls. Southern toads oriented away from runway directions of higher pine basal area than the release point, and selected runway directions down slope of the release point (Figure 3.4).
Runway specific vegetation structure contributed to differences in microclimate despite forests being < 8 km apart. X and LW sites did not differ in air temperature or humidity (p > 0.05); however, denser canopies at SM forest contributed to lower air temperature and higher humidity than both X or LW (p < 0.001). Instantaneous measurements at each habitat using DHT-11 sensors indicated that significant air temperature and humidity differences (ANOVA) were evident until approximately 9.5 hours after sunrise, after which trends were non-significant among forest types.

Microclimate and rainfall differences among runways during batch releases influenced the timing of amphibian movements but did not inhibit dispersal. Movement rates were positively related to precipitation (z = 2.78, SE = 0.77, p = 0.006), which itself did not vary among runways (χ² = 0.75, df = 2, p = 0.69). Cormack-Jolly-Seber model selection in MARK did not favor a model with batch specific survival rates versus a model of constant survival (Δ AIC < 2). Recapture rates within runways were higher in SM (0.26, SE = 0.04) than X (0.18, SE = 0.03) or LW (0.12, SE = 0.03) forest types, with at least one toad found during all visits for all runways. Toads were observed to exhibit water conservation behavior on xeric sands (X and LW) by burrowing into the soil and positioning their permeable ventral surfaces toward the ground.

Desiccation

A total of 240 juvenile toads were observed for up to 72 hours within desiccation enclosures. Forest habitat included the three locations used for terrestrial runways (SM, X, and LW) plus a plantation silviculture stand in WSF located on xeric soils (SX). Vegetation structure varied among the four forest types, with groundcover at both SM and SX sites lacking native herbaceous species and greater pine basal areas than X and LW forests (Table 3.1).
Vegetation structure and landscape setting (soil edaphic conditions) influenced microhabitat at the forest floor. Average air temperature and humidity at desiccation enclosures were strongly negatively correlated (-0.86); air temperature did not vary significantly among forest habitats (F = 1.75, p = 0.16), but humidity was significantly different (F = 3.88, p = 0.009), particularly between SM and SX conditions. Soil moisture varied significantly by habitat (F = 12.5, df = 3, p < 0.001), being consistently lowest at SX enclosures (Figure 3.8). Variation in soil moisture during the study (Figure 3.5) was mediated by rainfall, with average soil volumetric water content at 15 mm being up to 46 percent less at SX (M 1.95, SE = 0.20) than for X enclosures (M 2.85, SE = 0.07) following several days without recent precipitation. Total rainfall did not vary significantly among forests during desiccation trials (F = 0.45, df = 20, p = 0.72).

Juvenile southern toads experienced water loss in all forest habitats, and beta regression results indicated differences in proportion of body mass lost among forest habitats (z = -3.9, SE = 0.076, p < 0.001). Toads had greatest body water loss on average in SX (M 0.69, SE = 0.051) forests, followed by X (M 0.60, SE = 0.057), LW (M 0.56, SE = 0.056), and SM stands (M 0.32, SE = 0.045). Water loss increased with time spent in enclosures (Figure 3.6), and was greater at 72 hr (z = 4.8, SE = 0.12, p < 0.001) and 48 hr (z = 6.6, SE = 0.11, p < 0.001) than 24 hours. A linear model of proportion of water loss by toads did not meet assumptions of homogeneity, and moreover, a significant smoothing term indicated that the relationship between proportion water loss and soil moisture was non-linear. Water loss was auto-correlated at a lag of one revisit, and a log likelihood ratio test of models with correlation for unique toad ID by visit was favored by AIC over a model without correlation (L. ratio = 27.8, p < 0.001). Estimated correlation of residuals separated by one visit was 0.68, confirming results of the auto-correlation function, that
toads with high water loss at revisit one were more likely to have high water lost at revisit two.

A smoothing function for soil moisture was a significant predictor of the proportion of water loss with correlation by toad ID per visit (edf = 5.1, F = 14.7, p < 0.001). Desiccation of juvenile toads increased dramatically below 3.9 volumetric water content (VWC) at 15 mm (Figure 3.7), which was typical at SX forest except when precipitation was > 4.6 cm (Trial 4 in Figure 3.5).

Toad survival was dependent on forest type ($\chi^2 = 15.9$, p = 0.001) and was lowest at SX forest (M 0.43, SE = 0.092), followed by X (M 0.45, SE = 0.12), LW (M 0.54, SE = 0.094), and SM (M 0.75, SE = 0.14). The proportion of toads alive decreased progressively over time (Table 3.2), and only 2 of 60 individuals in SX forest survived to 72 hours. Soil moisture was a significant predictor of toad survival ($z = 5.0$, SE = 0.006, p < 0.001), and survival was predicted to be < 50 percent (within 24 hrs) when soil moisture was < 3.9 VWC (Figure 3.8). Soil moisture was consistently below 3.9 VWC at SX sites except following precipitation (Figure 3.5).

DISCUSSION

Previous studies have documented low survival for juvenile amphibians in terrestrial habitat, for example, Rothermel (2004) found < 15% of toads and salamanders were recaptured after moving 50 m through forest. The current investigation of juvenile southern toads found 13% recapture rates at 50 m within runways, indicating high mortality, which was confirmed using small scale (known fate) enclosures (Table 3.2). Overall survival of individual toads to 72 hours at X forest was higher in runways (28.4%, SE 2.5%) than desiccation enclosures (15.0 %, SE 5.0%), suggesting the ability to behaviorally select among forest floor microhabitats in runways reduces mortality, further supporting previous findings (Rittenhouse et al. 2008). While
a subset of toads within runways may have retreated to suitable cover, avoiding recapture, photographic identification of individuals indicated toads remained surface active weeks after release. For example, two individuals from the first batch (released August 10) were recaptured moving in runways until the end of the study (September 19), which agrees with Todd and Rothermel (2006) that southern toads are useful for landscape studies. Further, MARK analyses did not indicate significant differences in survival among toad batches (Δ AIC< 2).

Desiccation

Because juvenile survival is a critical factor regulating amphibian population size (Biek et al. 2002), any habitat modification that limits survival in the first few weeks after metamorphosis (Harper and Semlitsch, 2007) is a potential causal factor behind decreased amphibian abundances (deMaynadier and Hunter, 1995). In the current study, juvenile toad desiccation in plantation silviculture was greatest at xeric uplands (SX), with differences increasing in significance with time of exposure (Figure 3.6), providing a potential landscape dependent mechanism behind variance in amphibian occupancy. Previous studies found that opening canopies by timber harvest increases water loss (Semlitsch et al. 2009), with negative effects mediated by ground microhabitats (Rittenhouse et al. 2008), the latter of which are reduced as plantation silviculture stands age and canopies close (Table 3.1). As Semlitsch et al. (2015) found that forestry treatments were not a predictor of anuran survival 4-6 years after harvest, and instead noted microclimate associated with microhabitat availability was important, it is likely that diurnal refugia including woody debris, live vegetation, and burrowing behavior moderated juvenile survival among forest stands in the current study (Fritts et al. 2015).

Survival of juvenile toads over 72 hours at SX forest was 3 percent, and lower than nearby X forest by a factor of 5-6 (Table 3.2). Non-significant differences in survival among other forest
types (X, SM, LW) agrees with previous studies of late succession planted pine (Popescu et al. 2011; Semlitsch et al. 2015), and indicates that open (X) or partially open canopy forest (LW) with native groundcover provides sufficient microhabitats for diurnal refuge (Fritts et al. 2015). Decreased pine basal area was associated with higher wiregrass (Table 3.1) and other herbaceous species (Chapter One), among which toads could seek suitable microclimates for temperature and moisture.

**Forest Floor Soil Moisture**

Small scale enclosure studies have documented dry, high temperature conditions increase amphibian desiccation (Todd and Rothermel, 2006), with rates determined by variation in forest floor microhabitat, including soil moisture (Rittenhouse et al. 2008). In the current study, soil moisture predicted juvenile amphibian desiccation (Figure 3.7), with moisture lower at SX than nearby X forest by up to 46 percent (Figure 3.5) because of several millimeters of dry needle and fine woody debris (duff) on the ground surface at SX forest. Juvenile toads display a burrowing behavior to avoid water loss (Lannoo, 2005), and position thin moist skin of the posterior ventral surface towards the cool and moist soil (Pough et al. 1983). Toads were observed burrowing in all forest types, but such activities did not prevent desiccation where duff produced by plantation silviculture occurred on xeric soils and soil volumetric water content was below the threshold for 50 percent juvenile survival to 24 hrs (Figure 3.8), except during rainfall (Figure 3.5). Despite southern toads being considered a habitat generalist, including tolerance of agricultural settings associated in part with water storage in a bladder (Jorgensen, 1997), modified ground litter at xeric plantation sites (SX) of the current study increased juvenile mortality to 97 percent at 72 hours. Light duff at this habitat type appeared to prevent accumulation of dew moisture into the soil, likely increasing loss to evaporation. Results suggest that juvenile amphibians in planted
pines on xeric, well drained soils, will be highly vulnerable to drought conditions. Given juvenile survival was consistently lowest at planted pine on xeric soil (SX) relative to mesic soils (SM), despite similarity of habitat structure, results of the current study agree with Semlitsch et al. (2009) that an inability to evacuate habitats that increase desiccation lead to increased mortality.

Movement

Juvenile southern toads in this study were able to move through forests ranging from closed canopy pine plantation to open canopy longleaf forest, with success dependent more on precipitation than forest condition. These results agree with Graeter et al. (2008) that southern toad movement distance did not significantly differ with habitat condition. Similarly, Popescu et al. (2011) found comparable movement between clearcuts and a dense 11 year old pine plantation. While the proportion of toads released and later observed in the current study was low for all habitats, those individuals that survived to disperse moved an average of 6 m/d (Figure 3.3) and up to 20 m/d regardless of forest type; therefore, thick needle cover at planted pine habitat was not a physical barrier to juvenile movement as at least one individual made it 50 m in all directions of each runway.

A key positive determinant of movement rate was rainfall, which did not vary significantly among runways. Previous studies have documented similar effects of precipitation on amphibian dispersal (Rothermel, 2004; Semlitsch et al. 2009), which may be species specific given differences in water conservation behavior (Graeter et al. 2008; Rittenhouse et al. 2008). For example, during dry periods, salamanders will behaviorally avoid desiccation at the ground surface, but will leave cover following rainfall to migrate and forage (Jaeger, 1980). High movement rates in this study may be influenced by the fact that juvenile toads are also active
diurnally (Lannoo, 2005) and precipitation occurred in five of six trials; therefore, habitat permeability differences among forest types could be more pronounced during dry periods, particularly drought.

Differences in timing of movement may be due to moisture and cover availability among runways, but did not lead to significant differences in permeability at 50 m. Moselely et al. (2004) found removal of pine litter evoked higher salamander movement and use of other ground refuge, and Semlictsch et al. (2008) found lower evacuation when suitable ground refuge was available. The current results agree in that juvenile toads at both X and LW sites had greater initial movement post-release, while movements were delayed at the SM runway, where litter cover and soil moisture were greatest (Figure 3.5). Microclimate of all forest types were comparable at night, suggesting a period when constraints on movement would be low (Rohr and Madison, 2003), which may account for non-significant differences in rates among runways. For example, Fritts et al. (2015) found proportional nocturnal use of woody debris by southern toads was dependent on temperature only when rainfall was absent.

**Behavior**

Juvenile toads showed directed movement, with orientation dependent on physical setting within runways, which agrees with Popescu et al. (2011) and Rittenhouse et al. (2009) that habitat permeability is dependent on behavioral responses to forest structure. Juvenile toads avoided elevated slopes and sought lower basal area forest (Figure 3.4), with a significant interaction between the two, suggesting that behavior integrates multiple factors. Amphibians behaviorally seek microhabitats limiting water loss, including ground layer cover (Patrick et al. 2006), and southern toads in this study moved toward open canopies, which at the study sites encouraged a variety of native ground cover plants. For example, Roznik and Johnson (2009)
found juvenile gopher frogs (*Lithobates capito*), a xeric endemic species, migrated preferentially from wetlands in directions of high wiregrass cover. The current findings are consistent with Graeter et al. (2008), who found that southern toad movement is largely directed toward canopy openings. Orientation for juvenile toads appears to involve short distance cues (Rothermel, 2004) and Fritts et al. (2015) found toad orientation away from ground debris during nocturnal hours, which may partially explain apparent avoidance of closed canopies in the current study (Figure 3.4). It is not suggested that reduced litter of canopy gaps increased movement given that the degree of directionality was greatest in the SM runway (Figure 3.2), where litter cover was uniform. Most reversal behavior was within 10 m and was comparable among habitats, suggesting juveniles have largely directed movement after initial orientation, which agrees with Popescu et al. (2011) and Patrick et al. (2008).

Previous studies have found juveniles move downhill in response to dryness (Rohr and Madison, 2003) that southern toad movement increased in partial canopy harvest versus control Semlitsch et al. (2009). Popescu et al. (2011) used habitat edges to elicit amphibian movements in runways and in the current study movement rates increased in LW habitat in response to a habitat edge (S and E directions, Figure 3.4); therefore, movement is increased where juveniles move both down slope and away from planted pine. The importance of slope for behavior of juvenile southern toads in the first weeks following metamorphosis may explain why orientation can vary between random and directional (Rothermel and Semlitsch, 2002). Slope aspect may also partially explain both significant variation in behavior or local abundance despite non-lethal temperature exposure in treatments (Popescu et al. 2011), and why juvenile toads do not consistently orient toward mature forest (Rothermel, 2004).
Forest Management

Given recent studies have began recommending maintenance of suitable forest floor microhabitat at harvested stands for amphibian persistence (Earl and Semlitsch, 2015), it is suggested that this recommendation be extended for late succession plantation silviculture, particularly on xeric soils. Current conclusions also agree with Earl and Semlitsch (2015) that retention of microhabitat (i.e. soil moisture) could be more significant than forestry treatments. Despite significant differences in groundcover between X and LW sites, retention of approximately 50% of reference condition groundcover estimates at the latter permitted comparable juvenile survival and movement rates. Future studies should investigate the implications of greater directionality of juvenile amphibian movement with canopy closure or ground aspect. For amphibian species that move primarily during major rainfall events, such as spadefoot toads (Todd et al. 2009), or habitat generalists like the southern toad, the effect of forest structure on movement will likely be less than other species. For example, Veysey et al. (2009) found rain allows salamanders, which typically avoid open habitat, to enter and move across clearcuts. If amphibians move during wet conditions and/or at night when conditions are comparable among forests, then availability of suitable refuge during daylight would be key to reduce desiccation and forestry practices should consider impacts not just on canopy but ground cover. Future studies should investigate microhabitat use by species of conservation need where forests are planted with pine, including temporal scales that include when species are most exposed to dry conditions. Given observed reduced fitness and survival of juvenile amphibians in the current study, it is recommended that silviculture practices that eliminate native
groundcover be avoided, particularly surrounding breeding wetlands for endemic xeric amphibians.

LITERATURE CITED


Figure 3.1 Design of runway enclosures with shoe boxes (gray squares) and pitfall locations (circles) shown at 10, 20, 30, and 50 m from runway center (x).
Figure 3.2 Individual toads observed per cardinal direction and distance (10 - 50 m) for plantation on mesic soil (SM), sandhill (X), and low wiregrass (LW) forest stands.
Figure 3.3 Movement rates (m/d) with 95% CIs among cardinal directions in sandhill (X), low wiregrass (LW), and mesic plantation silviculture (SM) forests.

Table 3.1 Canopy and groundcover metrics at forest types with standard error.

<table>
<thead>
<tr>
<th>Forest Type</th>
<th>Pine Basal (m²/ha)</th>
<th>Wiry cover /m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference sandhill (X)</td>
<td>3.8 (0.52)</td>
<td>4.5 (0.55)</td>
</tr>
<tr>
<td>Low wiregrass sandhill(LW)</td>
<td>12.4 (1.71)</td>
<td>1.9 (0.36)</td>
</tr>
<tr>
<td>Plantation mesic (SM)</td>
<td>20.5 (1.48)</td>
<td>0</td>
</tr>
<tr>
<td>Plantation xeric (SX)</td>
<td>15.6 (1.98)</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 3.4 Slope (cm/m) and Basal Area Pine (m$^2$/ha) per runway with toad counts per distance and direction.
Figure 3.5 Soil moisture (+/- 1 SE) within 15 mm at desiccation enclosures by forest type and revisit.
Figure 3.6 Proportion of water lost from toads (+/- 1 SE) among forest types.
Figure 3.7 Smooth function for proportion of water loss by juvenile toads per soil moisture (VWC).

Table 3.2 Proportion of toads that survived in desiccation enclosures to 24, 48, and 72 hours among forest habitats.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>24 hr Survival</th>
<th>48 hr Survival</th>
<th>72 hr Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>SX</td>
<td>0.40 (0.06)</td>
<td>0.17 (0.04)</td>
<td>0.03 (0.02)</td>
</tr>
<tr>
<td>X</td>
<td>0.43 (0.07)</td>
<td>0.17 (0.05)</td>
<td>0.15 (0.05)</td>
</tr>
<tr>
<td>LW</td>
<td>0.51 (0.05)</td>
<td>0.29 (0.07)</td>
<td>0.20 (0.05)</td>
</tr>
<tr>
<td>SM</td>
<td>0.81 (0.05)</td>
<td>0.54 (0.07)</td>
<td>0.36 (0.06)</td>
</tr>
</tbody>
</table>
Figure 3.8 Juvenile toad survival probability predicted by soil moisture (VWC) in 15 mm.
APPENDIX A: IRB/IACUC Approval

MEMORANDUM

TO: Thomas Crisman,
FROM: Institutional Animal Care & Use Committee Division of Research Integrity & Compliance
DATE: 11/16/2012

PROJECT TITLE: AMPHIBIAN COMPOSITION OF ISOLATED WETLANDS IN NORTH CENTRAL FLORIDA RELATED TO UPLAND CONVERSION TO PINE SILVICULTURE

FUNDING SOURCE: USF department, institute, center, etc.

IACUC PROTOCOL #: W IS0000009

PROTOCOL STATUS: APPROVED

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC APPROVED your request to use the following animals in your protocol for a one-year period beginning 11/16/2012:

- Frogs (Anurans) (Variable in wild setting) 1200
- Salamanders (Caudates) (Variable in wild setting) 1200
- Oak Toad (Frog) (Juvenile < 1 year age, sex variable, weight < 30 grams) 200
- Salamanders (Caudates) (Juvenile < 1 year age, sex variable, weight < 40 grams) 200

Please take note of the following:

- IACUC approval is granted for a one-year period at the end of which, an annual renewal form must be submitted for years two (2) and three (3) of the protocol through the eIACUC system. After three years, all continuing studies must be completely re-described in a new electronic application and submitted to IACUC for review.

- All Comparative Medicine pre-performance safety and logistic meetings must occur prior to implementation of this protocol. Please contact the program coordinator at compmed@research.usf.edu to schedule a pre-performance meeting.

- All modifications to the IACUC-Approved Protocol must be approved by the IACUC prior to initiating the
modification. Modifications can be submitted to the IACUC for review and approval as an Amendment or Procedural Change through the eIACUC system. These changes must be within the scope of the original research hypothesis, involve the original species and justified in writing. Any change in the IACUC-approved protocol that does not meet the latter definition is considered a major protocol change and requires the submission of a new application.

- All costs invoiced to a grant account must be allocable to the purpose of the grant. Costs allocable to one protocol may not be shifted to another in order to meet deficiencies caused by overruns, or for other reasons convenience. Rotation of charges among protocols by month without establishing that the rotation schedule credibly reflects the relative benefit to each protocol is unacceptable.
- The PI must assist the IACUC with tracking wild animal field research activities. The PI must report episodes of wild animal use, the approximate range of taxa, and the approximate numbers of animals encountered or used at intervals appropriate to the study but at least once a year.