

ECOLOGY OF TWO RARE AMPHIBIANS OF THE GULF COASTAL PLAIN

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ABSTRACT

Globally, amphibian species have been in decline and a wide range of factors have been purported to be driving the decline. The Gulf Coastal Plain of Florida has a high degree of endemism and rarity and the biodiversity in the region includes a diverse suite of amphibian species. Degradation of habitat has been considered by many to be a major part of amphibian declines, however amphibian declines are complex and in many cases multiple factors are occurring in concert. My dissertation research examined aspects of habitat ecology and occupancy for two rare amphibians, Florida Bog Frog (*Rana okaloosae*) (Chapter 1, 2, and 3) and Reticulated Flatwoods Salamander (*Ambystoma bishopi*) (Chapter 5), that are both restricted to the Northern Gulf Coastal Plain. Further, for *R. okaloosae* I examined the influence of a sympatric congener, Bronze Frog (*R. clamitans clamitans*), on microhabitat selection (Chapter 1) and growth of tadpoles (Chapter 4). My overall goal was to be able to elucidate factors that limit the geographic range of *R. okaloosae* and *A. bishopi* and to identify habitat characteristics that managers could maintain or create to conserve or increase populations of these species.

My first chapter examined the microhabitat relationships between *R. okaloosae* and *R. c. clamitans*. *Rana okaloosae* is endemic to northwestern Florida and is sympatric with *R. c. clamitans*, a more common and widely distributed congener. Further, the two species appeared to be syntopic, have overlapping breeding seasons, and are known to hybridize. The objectives of this chapter were to assess the microhabitat selection of both species and to assess differences in microhabitat use of males of both species during the breeding season. My modeling of habitat selection and comparison of variables used by each species suggests that males of these species

select different resources when calling. Therefore, these sympatric ranids select for different resources at a fine scale, however there does appear to be some overlap among some selected habitat characteristics.

In Chapter 2, I assessed the habitat use of *R. okaloosae* at multiple spatial scales. I surveyed for *R. okaloosae* and evaluated habitat characteristics at used sites and sites where I had no detections to develop among- and within-stream habitat models for *R. okaloosae*. *Rana okaloosae* used habitats with high amounts of emergent vegetation at both the among-stream scale and the within-stream scale. Emergent vegetation appears frequently in models of anuran habitat selection, particularly those that occur in fire-dominated landscapes. Further understanding the habitat requirements of *R. okaloosae* will allow land managers to use appropriate management activities (e.g., prescribed fire) that will increase emergent vegetation and potentially restore habitat that may help increase populations of *R. okaloosae*.

In Chapter 3, I conducted aural surveys for *R. okaloosae* at two different spatial scales: *range-wide* and *stream-level* scales to understand how occupancy and colonization of *R. okaloosae* may be influenced by scale. My results suggest that at both spatial scales occupancy of *R. okaloosae* was best described by the presence of mixed forest wetlands at survey sites. At the range-wide scale, colonization and detection were constant across years, however, at the stream-level scale, colonization was predicted by the number of years since last fire and detection was best predicted by the additive combination of relative humidity and temperature. Occupancy of *R. okaloosae* was patchy at the range-wide and at the stream-level scales and colonization was low at both scales, while derived estimates of local extinction were moderately high. While *R. okaloosae* still occur in 3 watersheds where they were initially observed in the

1980's, one of the three watersheds appears to be very isolated and detections there are becoming very infrequent.

In Chapter 4, I experimentally evaluated the effects of *R. c. clamitans* tadpoles on *R. okaloosae* tadpoles. My results suggest that there was limited influence of *R. c. clamitans* on *R. okaloosae*. Conversely, it appeared that *Rana c. clamitans* was more susceptible to intraspecific competition than interspecific competition. The lack of a strong competitive effect of *Rana c. clamitans* on *Rana okaloosae* suggests that competitive interactions among tadpoles may have a limited effect at the densities I examined.

In Chapter 5, our objectives were to evaluate a suite of within-pool factors (i.e., vegetation structure, water level, and an index to presence of fish) that could influence occupancy of breeding wetlands by larval flatwoods salamanders on Eglin Air Force Base in Florida, USA. Site occupancy over a 4 year period was best described by a model that incorporated high herbaceous vegetation cover and open canopy cover. Detection probability was assessed, but it varied among years and was not included in the model. Our study suggests that managing the breeding habitat of flatwoods salamander for open canopies and dense herbaceous vegetation may contribute to this species' recovery.

In conclusion, Chapters 1-3 of my dissertation contribute to a growing understanding about the habitat ecology of *R. okaloosae*. I have evaluated habitat use of *R. okaloosae* at multiple spatial scales. At the finest spatial scale *R. okaloosae* selected for sites that had an abundance of cover probably decreasing their risk of predation (Chapter 1). Similarly, in Chapter 2 at two spatial scales, among and within-streams, *R. okaloosae* selected for emergent vegetation. Finally, at the broadest spatial scale, range-wide, *R. okaloosae* were found to be associated with mixed forest wetlands (Chapter 3). I did not find strong support for competition

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ATTRIBUTION

A brief description of the contributions made by colleagues that assisted with the preparation of one of my dissertation chapters is outlined below.

Chapter 5. Factors related to occupancy of breeding wetlands by flatwoods salamander larvae. Carola A. Haas (Department of Fisheries and Wildlife Sciences, Virginia Tech) assisted with study design and edited drafts of the manuscript. David C. Bishop (Department of Fisheries and Wildlife Sciences, Virginia Tech) currently at Low Country Institute, Okatie, SC, collected data, assisted with study design of sampling protocols, and edited drafts of the manuscript.

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Dedicated in memory of an inspirational friend,

Jacy Kahil Henderson

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PREFACE

The Coastal Plain of the Gulf of Mexico is rich with biological diversity and is considered to have a high degree of rarity and endemism (Chaplin et al. 2000). A primary component of the landscape in this region of the United States is the longleaf pine ecosystem. The longleaf pine ecosystem was a dominant vegetative feature on the landscape prior to European settlement (Noss 1989). In recent history this ecosystem has been in a dramatic decline from an estimated >35 million ha pre-Columbian era to a current estimate of <2 million ha (Engstrom et al. 1996). A diverse suite of amphibians is known to be associated with this ecosystem (Means et al. 2004); therefore the decline of the longleaf pine ecosystem is significant to the conservation of amphibians as well as other taxonomic groups. The longleaf pine ecosystem is well-known to be a fire-maintained system, however fire frequency in wetlands that occur in this system is not well understood (Frost 1995, Kirkman 1995). Further, the response of amphibians to fire and/or fire suppression is not well understood, but it is likely that fire is essential to maintain habitat characteristics that are suitable for many species of amphibians (Russell et al. 1999, Pilliod et al. 2003, Schurbon and Fauth 2003, Bishop and Haas 2005).

Global amphibian declines have been documented and over the past 25 years efforts to understand these declines have increased (Blaustein and Kiesecker 2002, Beebee and Griffiths 2005). Many causes of amphibian declines have been reported and combinations of these different factors are likely impacting different regions of the world. Leading causes include habitat loss and fragmentation, global warming, environmental contamination, increased levels of ultraviolet radiation, disease, and invasive species (Blaustein and Kiesecker 2002). However, loss of habitat is considered a leading factor associated with many of these declines (Blaustein and Kiesecker 2002, Beebee and Griffiths 2005). An increasing concern with amphibian

declines is the recognition that many amphibian species occur in isolated small populations and species that naturally occur as small populations can be particularly susceptible to stochastic events (Caughley 1994, Gaston 1998). Therefore, identifying and monitoring species that occur in small populations is of particular interest for conservation of biodiversity.

My dissertation research involved examining the ecology of two rare amphibians, Florida Bog Frog (*Rana okaloosae*) and Reticulated Flatwoods Salamander (*Ambystoma bishopi*), that are both restricted to the Northern Gulf Coastal Plain. *Rana okaloosae* are endemic to northwestern Florida and the species was discovered and described in 1982 (Moler 1985). *Rana okaloosae* are a small member of the genus *Rana* that have been located in tributary streams associated with only 3 rivers in northwestern Florida making their geographic range very small (Moler 1993, Bishop 2004). Overall, there is a paucity of information related to the habitat associations of this species and how it uses habitat in comparison to a widespread sympatric congener, Bronze Frog (*R. clamitans clamitans*) (Gorman et al. 2009). The region of northwestern Florida where *R. okaloosae* occurs has a high number of perennially wet first order streams that originate from springs (i.e., “steepheads”) (Means 1975) and are located within the coastal plain of the Gulf of Mexico (Means 2000). These drainages may have been vulnerable to isolation by small increases in sea level (Means 2000). It has been hypothesized that the potential isolation of these drainages may have been integral in the differentiation of several new species and may have been a primary factor resulting in the differentiation of *R. okaloosae* from *R. c. clamitans* (Means 2000). In contrast to *R. okaloosae*, *R. c. clamitans* is widely distributed and occurs throughout most of the southeastern United States. *Rana c. clamitans* is closely related to *R. okaloosae* (Austin et al. 2003) and is potentially its principal interspecific competitor (Moler 1992, Bishop 2005).

Ambystoma bishopi was listed as federally endangered in March 2009 (United States Department of the Interior, Fish and Wildlife Service [USFWS] 2009) following a recent discovery that the species *A. cingulatum* represents two distinct species, *A. cingulatum* and *A. bishopi* (Pauly et al. 2007). Pauly et al. (2007) recommended that the line of demarcation between the two species was the Apalachicola River and that *A. cingulatum* occurred to the east of the river and *A. bishopi* to the west. For *A. bishopi*, the loss of habitat is considered a main threat and is thought to be the cause of population declines throughout the species' ranges (Means et al. 1996, Palis 1996, USFWS 1999), which are substantially smaller than thought before the taxonomic split. The loss and deterioration of habitat includes habitat for both the post-larval and larval flatwoods salamanders. Larval salamanders develop in isolated, seasonally flooded wetlands, whereas post-larval *A. bishopi* are fossorial and occur in mesic longleaf pine flatwoods and savannas (Palis 1996). Recently it has been recognized that previously used breeding wetlands are no longer being used. Habitat characteristics thought to be important for larval development, such as herbaceous vegetation, are declining, while woody vegetation is increasing. Further, invasions by fish and changes to hydrology have been purported as possible compounding factors leading to deterioration of breeding wetlands.

I studied characteristics of habitats used by both *R. okaloosae* and *A. bishopi*, and for *R. okaloosae* I also included additional research to help understand possible limits to their geographic range besides habitat. Specifically, I examined the habitat ecology of *R. okaloosae* at multiple spatial scales, microhabitat selection (Chapter 1), macrohabitat selection (Chapter 2), and occupancy of habitat types at the stream-level and range-wide scales (Chapter 3). The objectives of these chapters were to understand the habitat ecology of this rare species, understand potential limitations on the species' geographic range, and to use the results to better

manage and conserve this species. Also, I examined the interspecific interactions between *R. okaloosae* and a sympatric congener, *R. c. clamitans*, using adult males and tadpoles. Specifically, I assessed the microhabitat relationships between male *R. okaloosae* and *R. c. clamitans* (Chapter 1). Further, I assessed the influence of *R. c. clamitans* tadpoles on the growth of *R. okaloosae* tadpoles (Chapter 4). The objectives of these two chapters were to understand the ecological relationships between closely related species, but also to understand the potential influence of a common species on a rare congener. Finally, I examined potential factors that may influence occupancy of breeding wetlands by *A. bishopi* (Chapter 5). The objective of this research was to develop a better understanding of within-pool characteristics, so land managers can direct management actions towards restoring characteristics selected by salamanders.

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STUDY SITE DESCRIPTION

Eglin Air Force Base (Eglin) is located in the counties of Okaloosa, Santa Rosa, and Walton in northwestern Florida. Eglin is a large military installation that spans 187,774 ha (Williams et al. 2006). The topography of the study area is level to rolling with the highest elevation at ~ 75 m and slopes that generally range from 0-30% (Eglin Air Force Base 2002). The Lakeland association covers 78% of Eglin and is mainly fine sands. There are 7 less frequently found soil associations on Eglin and these are: St. Lucie-Paola, Bonifay-Troup-Dothan, Norfolk, Chipley-Foxworth-Albany, Rutledge-Leon, Kingston-Bibb, and Dorovan-Pamlico. The upland habitat of the study area is a longleaf pine (*Pinus palustris*) and turkey oak (*Quercus laevis*) sandhill community that encompasses the majority of Eglin.

Currently Eglin is the largest forested military base in the United States. Eglin has been a military base since 1935 and its primary role is research, development and testing of air-delivered weapons (Eglin Air Force Base 2002). The majority of Eglin (155,400 ha), which was formerly the Choctawhatchee National Forest, was ceded to the Department of War in 1940. As a National Forest, this land was integral to the naval stores industry that declined in the 1920's when management shifted to timber production (Stratman and Pelton 2007). Presently Eglin accounts for the largest contiguous tract of longleaf pine under a single ownership and has extensive stands of old growth that account for 50% of the remaining known stands of old growth (Moranz et al. 1998, Eglin Air Force Base 2002).

The study area for Chapters 1-3, focusing on the ecology of *Rana okaloosae*, occurred along numerous small perennial streams. From recent and past survey history (Moler 1985, Moler 1993, Bishop 2004) it appears that *R. okaloosae* occur in 3 populations (Appendix A). Often *R. okaloosae* are associated with a unique habitat type known as steepheads. Steepheads

are small ravines associated with perennially wet first order streams that originate from springs (Means 1975, Schumm et al. 1995). Steepheads can have slopes $\geq 30\%$ and can be as much as 30 m below the surrounding surface habitat (Means 1975, Eglin Air Force Base 2002). The vegetation associated with these steepheads and other wetlands used by *R. okaloosae* are dominated by black titi (*Cliftonia monophylla*), sweetbay magnolia (*Magnolia virginiana*), Atlantic white cedar (*Chamaecyparis thyoides*), swamp titi (*Cyrilla raceformia*), and blackgum (*Nyssa sylvatica*).

The study area for Chapter 5, which described occupancy of *Ambystoma bishopi* larvae, was located in the southern portion of Eglin and occurred in 18 previously occupied breeding wetlands. These sites were wetlands that were occupied by flatwoods salamanders at least once since 1993. Fourteen wetlands were located in the East Bay Flatwoods East in Okaloosa County, 1 wetland was located in the East Bay Flatwoods West in Santa Rosa County, and 3 wetlands were located in Oglesby's Flatwoods in Okaloosae County (Appendix A). Each of these 3 designated flatwoods regions was considered a population for recovery (USFWS 2005). The breeding sites of *A. bishopi* are ephemeral wetlands that fill with water in the fall and winter months. These sites had overstories of longleaf pine, slash pine, pond cypress (*Taxodium ascendens* Brongn.), and blackgum (*Nyssa sylvatica* Marsh.) and have open to dense midstories dominated by myrtle-leaved holly (*Ilex myrtifolia* Walter) and Chapman's St. John's-wort (*Hypericum chapmanii* Chapman).

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CHAPTER 1

Microhabitat relationships between a rare and common species of frog

Abstract: Quantifying habitat selection of related, sympatric species may help elucidate the extent of resource partitioning and competition. Specifically, analyses of microhabitat selection assist researchers and managers in identifying the specific vegetation structures or physical conditions that may be important to persistence of populations or species. The Florida Bog Frog (*Rana okaloosae*) is endemic to northwestern Florida and is sympatric with the more widely distributed Bronze Frog (*Rana clamitans clamitans*). My objective was to determine whether these closely related, sympatric frogs selected different microhabitat characteristics at male calling sites, which in turn may influence successful reproduction (e.g., egg survival and hatching rate) and/or survival. From 2006-2008, I quantified microhabitat characteristics of male calling sites used by both species of frogs in the unique steephead habitat on Eglin Air Force Base, Florida. I created a suite of *a priori* models and used paired logistic regression to assess each species' habitat selection. Further, I compared the habitat characteristics from each species' most highly supported model to directly compare differences in habitat use. Model selection indicated that calling sites for *R. okaloosae* were best described by habitat features consistent with microhabitat cover (i.e., submergent vegetation, emergent vegetation, woody debris, frog-level canopy cover, and distance to cover) ($w_i = 0.85$), whereas *R. c. clamitans* selected sites based on features that may be favorable for female oviposition or egg development (i.e., depth, water movement, depth*water movement interaction) ($w_i = 0.54$). Further, *R. okaloosae* selected sites with 3.7 times more submergent vegetation ($df = 153$, $t = -3.24$, $P = 0.002$) and 1.6 times shallower water depths ($df = 153$, $t = 3.43$, $P < 0.001$) than *R. c. clamitans*. At this fine scale,

these sympatric ranids appear to select microhabitat differently; however, there does appear to be overlap among some selected variables.

Key words: Bronze Frog, Florida, Florida Bog Frog, *Rana clamitans clamitans*, *Rana okaloosae*

INTRODUCTION

Understanding the role of competition in structuring communities is an important aspect of ecology. Directly interpreting competitive interactions between closely related species can be difficult, but generally it is assumed that coexistence of two species with similar ecological niches is the result of resource partitioning (Schoener 1968). Resource partitioning between species can be influenced by multiple factors, including present and historical competitive interactions (Connell 1980), predation, and physiological constraints (Toft 1985). Generally, components of a species' niche are related to habitat, and knowledge of species-specific habitat relationships can also be considered an important component of conservation and management (Hobbs and Hanley 1990, North and Reynolds 1996). In particular, areas where specific activities are carried out, such as feeding or breeding, can offer insight into a species' niche.

Florida Bog Frogs (*Rana okaloosae*) are endemic to northwestern Florida and the species was discovered and described in 1982 (Moler 1985). Because of its relatively recent discovery there is a paucity of information related to habitat associations and other aspects of the species' life history. *Rana okaloosae* is the smallest member of the genus *Rana* and it has been found in relatively few locations in northwestern Florida (Bishop 2004). The portion of Florida where *R. okaloosae* occurs is a system of "steephead" ravines (i.e., perennially wet first order streams that originate from springs [Means 1975]) that are near the coastal plain of the Gulf of Mexico

(Means 2000). These types of drainages are thought to have become isolated by increasing sea levels of as little as 2-5 m (Means 2000). It has been hypothesized that the potential isolation of these drainages may have been integral in the differentiation of several new species and may have been a primary factor resulting in the differentiation of *R. okaloosae* from the Bronze Frog (*Rana clamitans clamitans*) (Means 2000). In contrast to *R. okaloosae*, *R. c. clamitans* is a widely distributed species that occurs throughout most of the southeastern United States. *Rana c. clamitans* is closely related to *R. okaloosae* (Austin et al. 2003) and is potentially their principal interspecific competitor (Moler 1992).

Many members of the genus *Rana* have overlapping geographic ranges. For example, in northwestern Florida, Pig Frog (*R. grylio*), Southern Leopard Frog (*R. sphenoccephalus*), *R. c. clamitans*, and *R. okaloosae* all have overlapping ranges (see Lannoo 2005), and all of these species can occur at the same site and be heard calling on the same night (Gorman, unpublished data). However, these ranids have evolved different strategies for breeding and attracting mates and it is believed that at some scale, whether temporal or spatial, resource partitioning occurs among these species to fill a variety of niches. For example, *R. sphenoccephalus* generally breed from December – March, which only slightly overlaps the breeding season of these other three ranids (see Lannoo 2005). However, *R. sphenoccephalus* may be found at sites with the other three species and aggressive interactions between *R. c. clamitans* and *R. sphenoccephalus* have been reported (Ritchie et al. 2008). *Rana grylio*, *R. c. clamitans*, and *R. okaloosae* are prolonged breeders and primarily breed from March – August (Lamb 1984, Martof 1953, Moler 1992, respectively). *Rana grylio* do not appear to be syntopic with these other species, because they are known to call from deeper water habitats (Lamb 1984) than any of these other species are capable of exploiting. In contrast to these examples of occasional interactions, *R. c. clamitans*

appear to be syntopic with *R. okaloosae* during the breeding season and both species are prolonged breeders with overlapping spring/summer breeding seasons (Gorman et al. 2009). In addition, hybrids between these two species have been reported (Moler 1993, Bishop 2005). Based on nearest neighbor distances and dynamic interactions *R. okaloosae* and *R. c. clamitans* did not appear to spatially exclude each other during the breeding season (Gorman et al. 2009). These two species appeared to have a clumped distribution at breeding sites, which suggested there may be competition for resources that make up suitable calling sites.

My goal was to evaluate the microhabitat selection of male *R. okaloosae* and *R. c. clamitans* and to compare the amount of overlap in microhabitat use between each species when males are selecting calling sites. I hypothesized that *R. okaloosae* would be a habitat obligate compared to *R. c. clamitans* and that I would observe differences in microhabitat selected by the two species. Further, I predicted that the factors that would influence site selection would be those that were more likely to provide protection to calling males from predators or those that would provide good conditions for oviposition. Based on my experience with this system I selected habitat variables accordingly (Table 1.1).

STUDY AREA

The study area is located on Eglin Air Force Base (Eglin) in the counties of Okaloosa and Santa Rosa in northwestern Florida (Figure 1.1). Eglin occurs within the Coastal Plain physiographic province and the overall topography has little relief, but there are steep ravines associated with headwater streams (i.e., steepheads) where slopes can be $\geq 30\%$ (Eglin Air Force Base 2002, Means 1975). The vegetation associated with these steepheads and other wetlands used by *R. okaloosae* are dominated by black titi (*Cliftonia monophylla*), sweetbay magnolia (*Magnolia virginiana*), Atlantic white cedar (*Chamaecyparis thyoides*), swamp titi (*Cyrilla raceformia*), and blackgum (*Nyssa sylvatica*) (Chapter 2). The upland habitat of the study is a

longleaf pine (*Pinus palustris*) and turkey oak (*Quercus laevis*) sandhill community that encompasses the majority of Eglin. I selected 4 sites that were known to have both *Rana okaloosae* and *R. c. clamitans* present (there are no known sites where *R. okaloosae* occur without *R. c. clamitans*). The study sites were located on 3 separate stream drainages (2 sites were located on 1 drainage, but were separated by approximately 1.3 km straight line distance) that occurred throughout the known geographic range of *R. okaloosae*. Two study sites were located at steepheads and 2 sites were located adjacent to the main stream channel in areas more broadly classified as fen habitat.

METHODS

Capture

I captured, marked, and recaptured *Rana okaloosae* and *R. c. clamitans* at night when the frogs were most active from May-August, 2006-2008. During each sampling event I extensively searched each site to capture all calling males of both species. I marked individuals of both species using visible implant alphanumeric (VI alpha) tags (Northwest Marine Technologies, Inc., Olympia, WA) that each have an individual alpha-numeric value. Tags were implanted into the hindlimb of the frog. A blood lancet was used to make a small incision in the skin and then a tag implanter was used to insert the tag between the skin and the adductor muscles (Buchan et al. 2005). VI alpha tags have been successfully used in other members of the family *Ranidae* and are used extensively in *Hyla regilla*, a species as small as *R. okaloosae* (Buchan et al. 2005).

Morphology

I determined the sex of each individual primarily by its calling status and secondarily for *R. okaloosae* by examining the presence or absence of vocal air sacs (Bishop 2005) and for *R. c. clamitans* by assessing the size of its tympanum (Martof 1956). I voided the bladder and

weighed each animal to the nearest 0.2 g using a 20 g Pesola spring scale and measured the snout-vent length to the nearest 0.1 mm using dial calipers. I compared body mass and snout vent length using independent samples t-tests.

Microhabitat Selection

Capture locations were marked and georeferenced with Asset Surveyor TDC2 GPS unit (Trimble Navigation Limited, Sunnyvale, CA) with sub-meter resolution. Microhabitat variables were measured at the first location where the individuals were captured and at a paired random location (Compton et al. 2002). Paired locations were chosen by randomly selecting a distance between 1-5 meters from the used location of the frog at a random azimuth and were restricted to the wetland area.

I measured a suite of habitat variables that included water depth, pH, water temperature, water movement, distance to cover, basal area, percentage of canopy cover at frog-level (i.e., a measure of ground-level horizontal cover), percentage of canopy cover at 1.5 m, percent of submergent vegetation, percent of emergent vegetation (i.e., a measure of ground-level vertical cover), and percent of woody debris. I measured water depth using a metric ruler. I measured pH and water temperature using an Extech ExStik II meter that simultaneously records these parameters. Water movement (i.e., visible movement of water) was recorded as a binary variable (i.e., movement or no movement). Distance to cover was measured to the closest discernible point where cover was available that would conceal above and ≥ 3 sides of an individual (e.g., undercut bank or hummock). I measured basal area using a Jim-Gem Cruz-All (English basal area factor of 5) at both the used and random locations and recorded the number of trees that contributed to the basal area. I measured the percent of canopy cover using a spherical densiometer at the level of the frog and at 1.5 m above the ground. I visually estimated the

percentage of emergent (e.g., *Sphagnum* spp. and *Carex* spp.) and submergent vegetation (e.g., algal mats and submerged *Carex* spp.) and the percentage of woody debris within a 0.5 m X 0.2 m rectangular plot at the used and random location using the Daubenmire (1959) cover class scale.

With these data, I used a paired logistic regression approach to estimate habitat selection of *R. okaloosae* and *R. c. clamitans* at the microhabitat scale (Hosmer and Lemeshow 1989, Compton et al. 2002). This logistic regression technique uses the difference between the used site and a paired random site to create a vector of the differences for all the independent variables and the dependent variable. The logistic procedure is then performed on these vectors so that models do not include an intercept term. I used an information-theoretic approach and Akaike's Information Criteria (AIC) corrected for small samples sizes (AIC_c) to examine the relative strength of *a priori* models. I considered the model with the lowest AIC_c to be the model with the best balance between statistical parsimony and goodness of fit for the empirical data (Burnham and Anderson 2002). Further, I considered models with ΔAIC_c values ≤ 2.0 as equally supported by the data (Burnham and Anderson 2002) and models that were >2.0 and <4.0 to have some support.

Microhabitat Overlap

I qualitatively compared the top models for *R. okaloosae* and *R. c. clamitans* to evaluate the similarity between the habitat selection of calling sites between the 2 species. Further, I compared the means of the habitat variables present in the most highly supported *R. okaloosae* and *R. c. clamitans* models of habitat selection with a Hotelling's T^2 . If a difference between the two species was detected with the omnibus Hotelling's T^2 then I continued with a series of univariate t-tests. Finally, I used frequency distribution plots of habitat variables that were

shown to be important from my modeling for *R. okaloosae* and *R. c. clamitans* to graphically compare the habitat niche of each species. For all analyses I used SAS 9.2 (SAS Institute Inc., Cary, NC) and I set alpha equal to 0.05.

RESULTS

I captured 99 male *Rana okaloosae* and 56 male *R. c. clamitans* at 4 study sites on Eglin Air Force Base from 2006-2008 (Table 1.2). *Rana c. clamitans* had a mean mass of 22.9 g (SE=1.0, Range=10.2 – 48.4) and mean snout vent length of 59.1 mm (SE=1.0, Range=36.8 – 80.0). *Rana okaloosae* had a mean mass of 6.3 g (SE=0.2, Range=1.9 – 13.8) and mean snout vent length of 39.7 mm (SE=0.4, Range=26.6 – 49.8). *Rana c. clamitans* were 3.6 times larger than *R. okaloosae* in mass ($t = -16.59, P < 0.001$) and had 1.5 times larger snout vent lengths ($t = -19.04, P < 0.001$; Figure 1.2).

Microhabitat Selection

My modeling suggested that male *R. okaloosae* select a suite of habitat characteristics describing high levels of cover that are consistent with reducing predation risk (Table 1.3). The most highly supported model out of the 10 *a priori* models was a model that included distance to cover, % cover of woody debris, % cover of emergent vegetation, % cover of submergent vegetation, and frog-level canopy ($AIC_c = 110.56, w_i = 0.85$; Table 1.3). *Rana okaloosae* used sites that had a distance to cover that was 2.3 times closer to cover than random. Odds ratios of coefficients predicted a 4.2% decrease in the chance of a site being used with every 1 cm in increase from cover, a 2.5% increase in use of a site for every 1% increase in woody debris, a 5.3% increase in use for every 1% increase in submergent vegetation, and a 1.7% increase in use of a site for every 1% increase in emergent vegetation (only marginally significant). Frog-level canopy was not significantly different between random and used sites (Table 1.4). When

considering mean values for used versus random sites, used sites had 1.4 times more percent woody debris, 1.5 times more percent emergent vegetation, and 1.6 times more percent submergent vegetation. Frog-level canopy was similar between used and random sites (Table 1.5). The second most supported model was $> 2.0 \Delta AIC_c$ from the most highly supported model. This model included depth, water movement, the interaction of depth and water movement, emergent vegetation, submergent vegetation, and distance to cover ($AIC_c = 114.11$, $\Delta AIC_c = 3.55$, $w_i = 0.14$; Table 1.3).

For *R. c. clamitans*, modeling suggested that males select sites that were more attractive for female oviposition (Table 1.6). The most highly supported model out of the 10 *a priori* models was a model that included water movement, water depth, and the interaction of these two characteristics ($AIC_c = 74.82$, $w_i = 0.54$; Table 1.6). The odds ratio of the coefficient for the interaction of water depth and water movement predicted a 2.5% increase in use of a site when there is a decrease in water movement and a decrease in depth. Individually water depth and water movement were not significant (Table 1.7). The mean values of sites used by *Rana c. clamitans* were 1.5 times less likely to have water movement than random sites and had 1.1 times shallower water depth than random (Table 1.5). The second-most supported model was slightly $> 2.0 \Delta AIC_c$ from the most highly supported model. This model included depth, water movement, the interaction of depth and water movement, emergent vegetation, submergent vegetation, and distance to cover ($AIC_c = 76.91$, $\Delta AIC_c = 2.09$, $w_i = 0.19$; Table 1.6).

Microhabitat overlap

I detected differences between the microhabitat characteristics used by *R. okaloosae* and *R. c. clamitans* (Wilks $\lambda = 0.845$, $df = 147$, $T^2 = 124.22$, $P < 0.001$) based on the variables that were the best predictors of *R. okaloosae* and *R. c. clamitans* calling sites (Table 1.3, Table 1.6).

Subsequent univariate tests of habitat characteristics determined that *R. okaloosae* selected sites with 3.7 times more submergent vegetation ($df = 153, t = -3.24, P = 0.002$) and 1.6 times shallower water depths ($df = 153, t = 3.43, P < 0.001$) than *R. c. clamitans* (Figure 1.3, Table 1.5). I did not detect a difference between distance from cover ($df = 153, t = -1.77, P = 0.078$), percent of woody debris ($df = 153, t = -1.65, P = 0.101$), water movement ($df = 153, t = 1.25, P = 0.213$), frog-level canopy cover ($df = 153, t = 1.44, P = 0.152$), or percent of emergent vegetation ($df = 153, t = -1.17, P = 0.242$) between the two species (Figure 1.3, Table 1.5). Distributions of the variables used to describe the calling locations used by male *R. okaloosae* and male *R. c. clamitans* highlight areas of overlap and areas of difference between the variables that each species selected (Figure 1.4).

DISCUSSION

My results suggest that male *R. okaloosae* and *R. c. clamitans* are selecting somewhat different microhabitat characteristics when choosing a calling site. *Rana okaloosae* selected for a suite of habitat characteristics associated with increased cover, suggesting they are selecting calling sites that decrease predation risk. Further, *R. okaloosae* selected for complex habitat features within a site at a higher frequency than they were available, which may be an indication that this species is an obligate user of particular microhabitats compared to *R. c. clamitans*. In contrast, *R. c. clamitans* selected for still, shallow water, which suggests they select sites that may be attractive to females for oviposition of eggs. In addition to the modeling of habitat selection, direct comparison of the variables that were selected by each species suggests that *R. okaloosae* and *R. c. clamitans* do not select for the same microhabitat characteristics. However, there is overlap in the habitat characteristics that are used by these two sympatric species (Figure 1.4).

At broader spatial scales my surveys indicate vast overlap in sites that are used by these two species (Gorman, unpublished data), but at fine spatial scales there is some separation in habitat characteristics selected. Therefore, these results are an example of the importance of choosing scale appropriately (Wiens 1989). Conversely, it is possible that because I chose my sites based on the presence of *R. okaloosae* I am seeing limited evidence of competition for resources. Sites where *R. okaloosae* do not occur may reflect past competition for resources. Therefore, it is possible that the limited geographic range of *R. okaloosae* can be explained by competitive exclusion by *R. c. clamitans* from sites that would be marginal for *R. okaloosae* (e.g., deeper water and lower amounts of cover). However, it is equally possible that *R. okaloosae* are so specialized in exploiting a specific habitat type that they view adjacent deeper water habitats as barriers to dispersal and will not attempt to exploit these habitats, as has been hypothesized for other species that occur in similar habitat types (e.g., Means 1975). This seems likely because steephead habitat is a rare habitat type that does not occur much beyond a few counties in the northwestern panhandle of Florida (Florida Fish and Wildlife Conservation Commission 2005). Thus, specialization in exploiting this habitat type would leave *R. okaloosae* little ability to expand its geographic range.

Theoretically, the expectation is that when a species has a large geographic range, it is more likely to be a habitat generalist (Brown 1984). In the northern part of the *R. clamitans* geographic range (i.e., New Jersey) another subspecies, the Northern Green Frog (*R. c. melanota*), and Carpenter Frogs (*R. virgatipes*) are syntopic, have overlapping breeding seasons, and are capable of breeding in the same wetlands (Given 1990). Additionally, Given (1990) reported that these two species selected for similar calling sites that occurred on the edge of wetlands. As *R. okaloosae* has a more restricted distribution than *R. c. clamitans*, similarly, *R.*

virgatipes has a more restricted distribution than *R. c. melanota*. However, *R. virgatipes* still appears to be able to coexist with *R. c. melanota*, despite some evidence that *R. c. melanota* occasionally do displace *R. virgatipes*. In some instances *R. c. melanota* forced calling males of *R. virgatipes* to move their calling position by up to 11 m (Given 1990).

The large differences in mass and snout vent length between *R. okaloosae* and *R. c. clamitans* (Figure 1.2) could be good predictors that these two species are not directly competing for resources. Differences in size could suggest that these two species partition other resources beyond calling sites, such as prey items. Further, there are other clear morphological differences between these two species. For example, *R. okaloosae* have reduced webbing and long toes (Moler 1985), which is likely an adaptation that resulted from the species exclusive use of shallow water habitats. While shallow water was not present in the most highly supported model, this only suggests that within a site where *R. okaloosae* breed there are no differences between water depths at used and random sites. Further, there do appear to be differences in habitat selection within a site between *R. okaloosae* and *R. c. clamitans*. Water depth was one of the habitat characteristics where there were differences, but there were other characteristics with overlap and the two species do interbreed (Moler 1993, Bishop 2005). It is possible that following the divergence between the 2 species, *R. c. clamitans* recolonized areas where they had been excluded due to the higher sea-levels and subsequently came in contact with *R. okaloosae*. *Rana c. clamitans* are known to be capable of moving large distances (Lamoureux and Madison 1999) and may be more capable of dispersing to new habitats than *R. okaloosae*. Also, it has been hypothesized that hybridization occurs more frequently when one species in the interaction is a recent invader of the habitat and/or one species in the interaction is rare (Randler 2002, Riley et al. 2003). Currently, research is being directed towards understanding the conservation

genetics of these two species and how hybridization is impacting populations of *R. okaloosae* (J. Austin, University of Florida, Pers. Comm.).

While it appears there are differences in the selection of habitat characteristics between adult males when selecting calling sites, it is not clear that all life stages of these species exhibit partitioning of resources. Female *R. c. clamitans* are capable of producing up to 20 times the number of eggs that *R. okaloosae* females produce (Wells 1976, Bishop 2005). Experimental evidence suggests that there is some level of asymmetric competition between tadpoles of these species (Chapter 4). Growth of *Rana c. clamitans* tadpoles were lower in intraspecific aquaria as compared to mixed species treatments, whereas growth of *R. okaloosae* were lower in mixed species treatments as opposed to intraspecific treatments, but only 1 treatment was significant (Chapter 4). However, little is known about the habitat use or selection of tadpoles of either species on my study area.

CONCLUSIONS

Rana okaloosae is a rare species of conservation concern that primarily occurs in a region where the suitable habitat type used by the species is also rare (Means 1975). Therefore, conservation of *R. okaloosae* should focus on maintaining breeding habitats of high quality that can support all the species' life stages. High quality breeding habitats should have an abundance of cover (e.g., woody debris and emergent and submergent vegetation; Figure 1.3) and should have shallow but constant water levels that will likely provide the best attributes for successful reproduction and survival of tadpoles. Further, patches of suitable habitat may need to be in close proximity, because populations appear to be displaying metapopulation dynamics (Chapter 3) and *R. okaloosae* may have limited dispersal capabilities. Habitat characteristics used by *R. okaloosae* may be a result of fire in both the uplands and occasionally in the wetlands. For

example, submergent vegetation (e.g., algal mats) may increase following upland fires, because runoff may have higher nutrient concentrations (pers. obs.). Also, fire may play a role in increasing other forms of cover and may increase hydroperiod by decreasing the amount of woody vegetation in the surrounding area. In addition to high quality breeding habitat, many anurans also require high quality terrestrial habitat that surrounds breeding sites (Semlitsch 2002). Terrestrial habitat can be important as refugia, for feeding, or to facilitate movement among breeding sites (Semlitsch 2002). However, data on non-breeding habitat use and frequency of terrestrial habitat use of *R. okaloosae* are limited. While a single long distance movement by an adult male of 130m has been observed, mean minimum daily distances moved are only 1.8 m during the breeding season (Gorman et al. 2009).

It appears that there may be limited direct influence of male *R. c. clamitans* on male *R. okaloosae* during the breeding season. While past and current researchers (Bishop 2005, pers. obs.) have not observed aggressive interactions between these two species, future research should specifically focus on how the males of these species interact. It may be important to investigate how females of both species are selecting males of both species and oviposition sites. For example, female *R. okaloosae* frequently oviposit directly at male *R. okaloosae* calling sites (Bishop 2005, Gorman, unpublished data), so determining whether females are selecting for site quality or male quality may elucidate mechanisms that are facilitating hybridization between these two species.

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Table 1.1. *A priori* models developed to predict whether (1) male frogs would select sites that were suitable for female oviposition, or (2) males would select sites that would provide cover from predators, or (3) males would select a combination of these predictions. Models were ordered by prediction and in order of increasing complexity. This model set was used for the analyses of both *Rana okaloosae* and *R. c. clamitans* microhabitat data from 4 sites on Eglin Air Force Base, 2006-2008.

Model Name	Model Variables
<i>(1) Models associated with the potential for female oviposition</i>	
Depth	Depth
Depth/Water movement	Depth, water movement, depth*water movement
Thermoregulation	Water temperature, canopy, water temperature*canopy
Water	Depth, water movement, depth*water movement, water temperature, pH, water temperature*pH
Water/Emergent	Depth, emergent, water movement, water temperature, pH, water temperature*pH
<i>(2) Models associated with the potential of increased cover from predators</i>	
Macrohabitat cover	Canopy, Basal area, Basal area*canopy
Microhabitat cover	Distance to cover, Woody debris, emergent, submergent, frog-level canopy

(3) Models with components of both decreased predation risk and female oviposition

Microhabitat cover/Water	Depth, water movement, depth*water movement, emergent, submergent, distance to cover
Water/Distance to cover	Depth, water movement, depth*water movement, water temperature, pH, water temperature*pH, distance to cover
Global	Depth, water movement, depth*water movement, water temperature, pH, water temperature*pH, emergent, submergent, Woody debris, distance to cover, frog-level canopy, Basal area, canopy cover, Basal area*canopy

Table 1.2. Number of captures and the percentage of captures of *Rana okaloosae* and *R. c. clamitans* at 4 study sites on Eglin Air Force Base, 2006-2008.

Site	<i>R. okaloosae</i>	<i>R. c. clamitans</i>	<i>R. okaloosae</i> (%)	<i>R. c. clamitans</i> (%)
Weaver	18	19	18.2	33.9
East Bay	23	8	23.2	14.3
Live Oak Trib.	44	21	44.4	37.5
Live Oak	14	8	14.1	14.3
Total	99	56	100.0	100.0

Table 1.3. Paired logistic regression modeling results of microhabitat selection of male *Rana okaloosae* (n = 99) at 4 sites on Eglin Air Force Base, Florida, 2006-2008 (k = # of parameters, AIC_c = second-order Akaike's Information Criteria [i.e., for small sample sizes], ΔAIC_c = the change in AIC_c , and w_i = the relative amount of support for the model). Variables included in each model are listed in Table 1.1.

Model	k	AIC_c	ΔAIC_c	w_i
Microhabitat Cover	5	110.56	0.00	0.85
Microhabitat Cover/Water	6	114.11	3.55	0.14
Global	14	125.65	15.08	0.00
Water/Emergent	8	131.20	20.64	0.00
Water/Distance to Cover	6	131.50	20.93	0.00
Depth/Water movement	3	133.20	22.63	0.00
Macrohabitat Cover	3	133.36	22.80	0.00
Water	6	137.72	27.15	0.00
Depth	1	138.13	27.57	0.00
Thermoregulation	3	139.41	28.85	0.00

Table 1.4. Parameter coefficients, odds ratios, and 95% confidence intervals for the most highly supported model of microhabitat selection of male *Rana okaloosae* at 4 sites on Eglin Air Force Base, Florida, 2006-2008.

Parameter	Coefficient	Estimate	Odds ratio	
			Low	High
Distance to cover	-0.043	0.958	0.929	0.988
Woody debris	0.025	1.025	1.002	1.048
Emergent vegetation	0.017	1.017	0.999	1.034
Submergent vegetation	0.052	1.053	1.022	1.086
Frog-level canopy	-0.003	0.997	0.971	1.024

Table 1.5. Mean habitat characteristics and standard errors (SE) at used calling sites of male *Rana okaloosae* (RO) (n = 99) and male *R. c. clamitans* (RC) (n = 56) and respective paired random sites at 4 study sites on Eglin Air Force Base, Florida, 2006-2008.

Habitat Variables	RO Used		RO Random		RC Used		RC Random	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Depth (cm)	3.1	0.3	3.5	0.3	4.8	0.5	5.2	0.7
Basal Area (m ² /ha)	9.9	0.6	10.4	0.6	9.1	0.7	9.1	0.7
Water movement (%)	15.2	3.6	30.3	4.6	23.2	5.7	35.7	6.5
Distance to cover (cm)	10.1	1.4	23.5	3.6	6.3	1.4	12.9	2.8
Canopy cover (%)	62.0	2.2	60.4	2.4	66.8	3.3	62.7	3.9
Frog-level canopy (%)	73.7	1.9	72.0	2.3	78.3	2.5	72.0	3.5
Emergent (%)	32.2	2.8	22.1	2.8	26.7	3.8	21.0	3.7
Submergent (%)	18.0	2.9	11.3	2.0	4.8	1.6	4.6	1.3
Woody debris (%)	19.1	2.0	13.8	1.6	13.3	3.1	12.0	1.8
Water temperature (C)	25.4	0.2	25.5	0.2	24.8	0.3	25.0	0.3
pH	5.19	0.03	5.19	0.03	5.32	0.02	5.32	0.02

Table 1.6. Paired logistic regression modeling results of microhabitat selection of male *Rana c. clamitans* (n = 56) at 4 sites on Eglin Air Force Base, Florida, 2006-2008 (k = # of parameters, AIC_c = second-order Akaike's Information Criteria [i.e., for small sample sizes], ΔAIC_c = the change in AIC_c , and w_i = the relative amount of support for the model). Variables included in each model are listed in Table 1.1.

Model	k	AIC_c	ΔAIC_c	w_i
Depth/Water movement	3	74.82	0.00	0.54
Microhabitat Cover/Water	6	76.91	2.09	0.19
Water/Distance to Cover	8	79.19	4.38	0.06
Thermoregulation	3	79.33	4.52	0.06
Depth	1	79.46	4.64	0.05
Microhabitat Cover	5	79.87	5.05	0.04
Water	6	80.42	5.60	0.03
Macrohabitat Cover	3	81.83	7.01	0.02
Water/Emergent	6	86.42	11.60	0.00
Global	14	96.81	21.99	0.00

Table 1.7. Parameter coefficients, odds ratios, and 95% confidence intervals for the most highly supported model of microhabitat selection of male *Rana c. clamitans* at 4 sites on Eglin Air Force Base, Florida, 2006-2008.

Parameter	Coefficient	Estimate	Odds Ratio	
			Low	High
Depth	0.108	1.114	0.943	1.354
Water movement	-0.507	0.951	0.860	1.045
Depth*Water movement	0.253	1.026	1.006	1.057

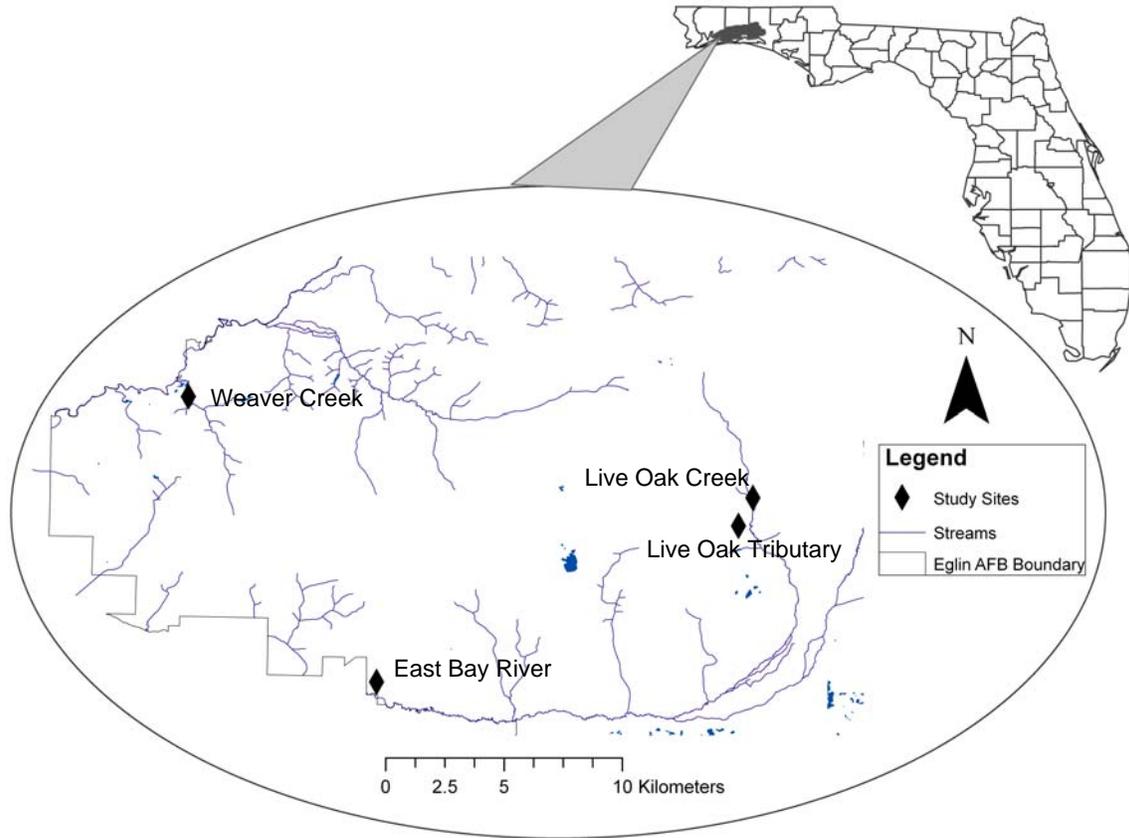


Figure 1.1. Location of four study sites (diamonds) where I captured and marked *Rana okaloosae* and *R. c. clamitans* and sampled used and random habitat characteristics, Eglin Air Force Base, Florida, 2006-2008.

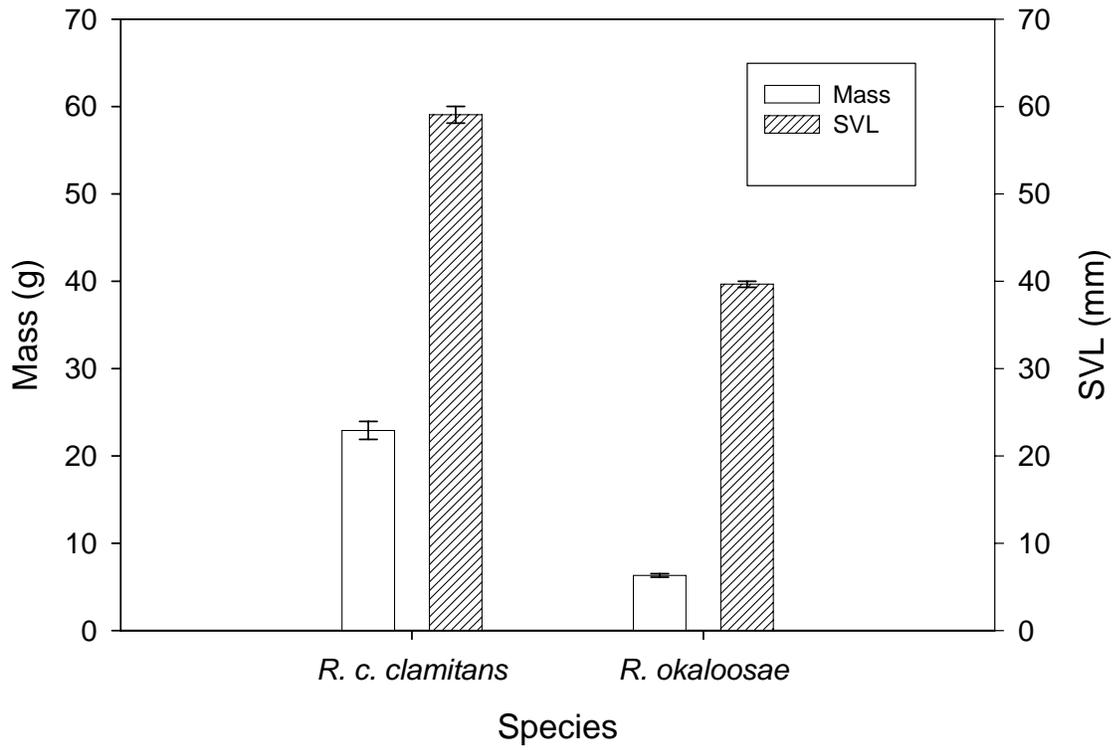


Figure 1.2. Mass and snout-vent length of male *Rana okaloosae* (n = 99) and male *R. c. clamitans* (n = 56) at 4 study sites on Eglin Air Force Base, 2006-2008.

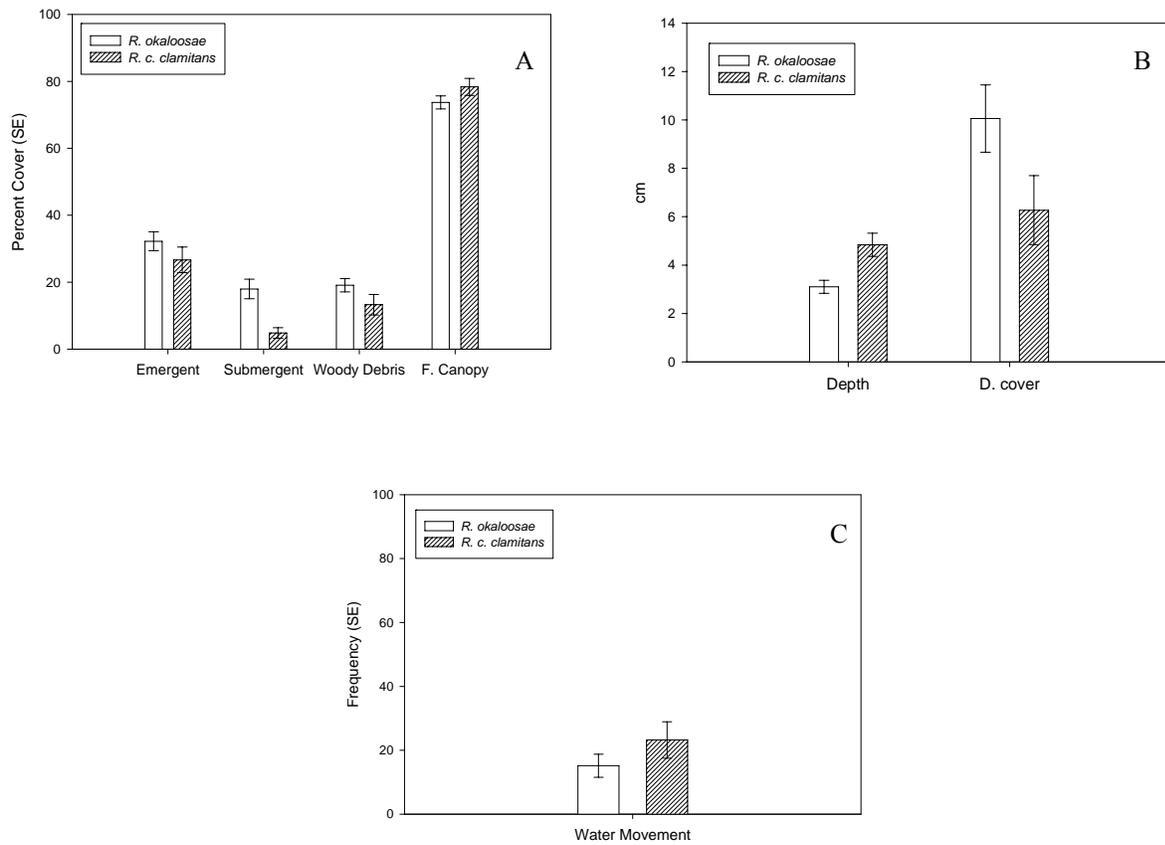


Figure 1.3. Habitat characteristics selected for by male *Rana okaloosae* (n = 99) and compared to *R. c. clamitans* (n = 56) at 4 study sites on Eglin Air Force Base, 2006-2008. (A) Mean percent cover with standard errors (SE) of emergent vegetation (Emergent), submergent vegetation (Submergent), woody debris, and frog-level canopy cover (F. canopy), (B) Mean measurement (cm) with standard errors (SE) of water depth (Depth) and Distance to cover (D. cover) (C) Mean frequency of occurrence with standard error (SE) of water movement (both species were found most often in still water).

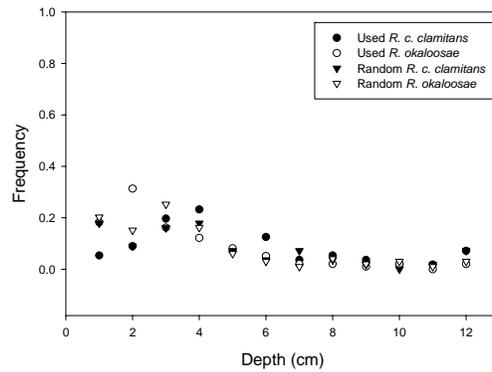
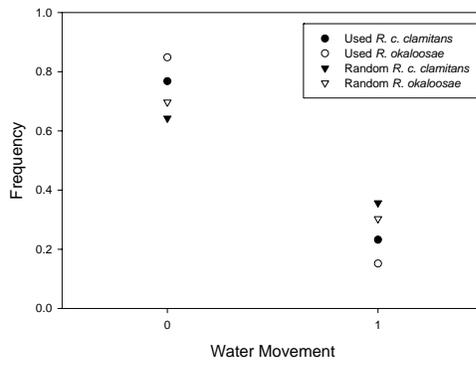
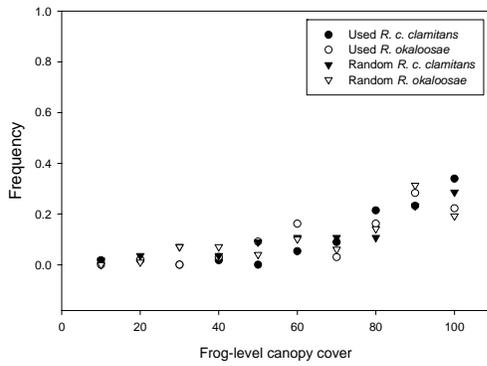
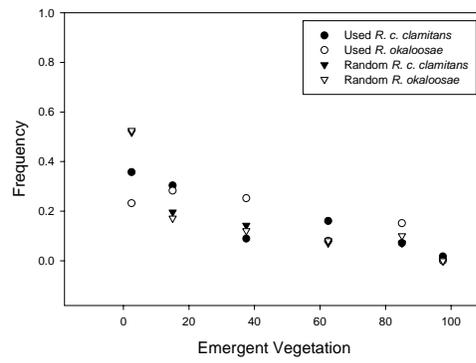
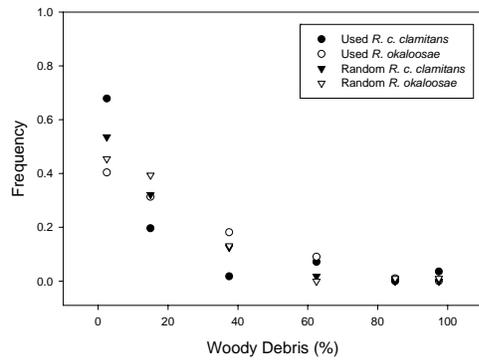
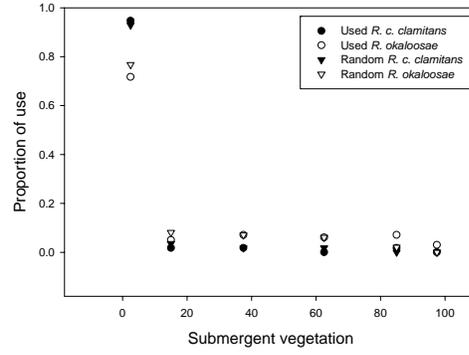
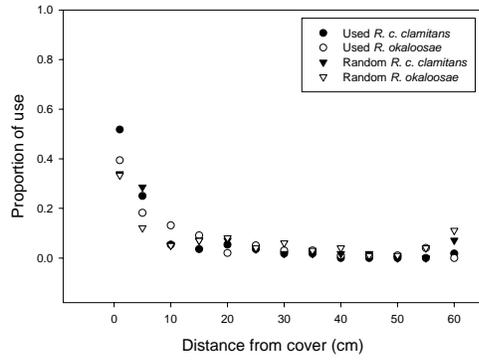


Figure 1.4. Distribution of habitat characteristics measured at used male calling sites and paired random locations (within 1-5 m from calling sites). These habitat characteristics were selected for by male *Rana okaloosae* and compared to *R. c. clamitans* on Eglin Air Force Base, Florida, 2006-2008.

CHAPTER 2

Habitat selection of a restricted endemic: *Rana okaloosae*

Abstract: Declines of amphibians are occurring on a global scale and habitat degradation is considered a major factor in many declines. Species with naturally occurring small populations may be at greater risk of decline from environmental perturbations, therefore understanding habitat use by species with this type of natural history may contribute to their conservation. I surveyed for Florida Bog Frogs (*Rana okaloosae*) 3 times/season from 2006-2008 along 14 streams on Eglin Air Force Base in northwestern Florida. I evaluated habitat characteristics at used sites and sites with no detections to develop among- and within-stream habitat models for *R. okaloosae*. *Rana okaloosae* used habitats with high amounts of emergent vegetation at both the among-stream scale and the within-stream scale. Emergent vegetation appears frequently in models of anuran habitat selection, particularly those that occur in fire-dominated landscapes. Further understanding the habitat requirements of *R. okaloosae* will aid in evaluation of sites for their importance in the conservation of this species. Further, it will allow land managers to use appropriate management activities (e.g., prescribed fire) that will increase emergent vegetation and potentially restore habitat to aid in increasing populations of *R. okaloosae*.

Key words: Florida, Florida Bog Frog, habitat, *Rana okaloosae*, wetlands

INTRODUCTION

Amphibians have declined across the world and efforts to understand these declines have increased over the past 25 years (Blaustein and Kiesecker 2002, Beebee and Griffiths 2005). Loss of habitat is considered a leading factor associated with many of these declines (Blaustein and Kiesecker 2002, Beebee and Griffiths 2005). In the southeastern United States, the longleaf pine ecosystem was a dominant vegetative feature on the landscape, but has dramatically declined since European settlement (Noss 1989). The increasing rarity of the longleaf pine ecosystem is significant to the conservation of amphibians because a diverse suite of amphibians are known to be associated with this system (Means et al. 2004). The longleaf pine ecosystem is well-known as a fire-maintained system; however fire regimes in wetlands merit more attention (Frost 1995, Kirkman 1995). Further, the response of amphibians to fire and/or fire suppression is not well understood, but it is believed that fire is essential to maintain habitat characteristics that are suitable for many species of amphibians (Russell et al. 1999, Pilliod et al. 2003, Schurbon and Fauth 2003, Bishop and Haas 2005).

Knowledge of the patterns of habitat use for a particular species may yield information on underlying ecological relationships (Arthur et al. 1996). Further, habitat use and selection are integral in conservation and management of animal populations, because species-specific habitat information can be used to promote biodiversity and conserve imperiled species (Garshelis 2000). Species habitat-relationships may occur at multiple spatial scales (Johnson 1980), and frequently scale can be important to understand how management decisions can affect a species and its habitat (Hobbs and Hanley 1990). Therefore examining habitat use of a species at multiple scales is warranted.

Florida Bog Frogs (*Rana okaloosae*) are endemic to northwestern Florida and the species was discovered and described in 1982 (Moler 1985). As a result there is a lack of information related to the habitat associations of this species and other aspects of the species' life history. *Rana okaloosae* are the smallest member of the genus and have been located near tributary streams associated with only 3 rivers in northwestern Florida (Moler 1993, Bishop 2004). Generally, the habitats of *R. okaloosae* have been described as non-stagnant, acidic seeps and overflow areas that are associated with clear water streams (Moler 1985, Moler 1992). Management and conservation priorities depend on understanding the habitat requirements of *R. okaloosae*, and fire suppression has been cited as a possible concern (Jackson 2004). However, no detailed assessment of habitat requirements has been conducted for this species and little information is available on the vegetation structure and physical characteristics of *R. okaloosae* habitat. My objective was to examine the macrohabitat selection of *R. okaloosae* during the breeding season. Therefore, I evaluated, at two different spatial scales, the difference in macrohabitat characteristics of sites used by *R. okaloosae* compared to sites where I did not detect *R. okaloosae*.

STUDY AREA

The study area was located on Eglin Air Force Base (Eglin) in the counties of Okaloosa, Santa Rosa, and Walton in northwestern Florida (Figure 2.1). Eglin occurs within the Coastal Plain physiographic province and the topography of the study area has little relief. Within the boundaries of Eglin there are numerous small perennial streams, some of which are “steepheads” (i.e., steep ravines associated with perennially wet first order streams that originate from springs [Means 1975, Schumm et al. 1995]). Riparian areas were dominated by black titi (*Cliftonia monophylla*), sweetbay magnolia (*Magnolia virginiana*), white cedar (*Chamaecyparis thyoides*),

swamp titi (*Cyrilla raceformia*), and blackgum (*Nyssa sylvatica*). The upland habitat of Eglin was largely dominated by a longleaf pine (*Pinus palustris*) and turkey oak (*Quercus laevis*) sandhill community. Prescribed fire was used as a management strategy in the surrounding uplands of riparian areas and prescribed fire was implemented primarily during the dormant season. Prescribed fire was implemented with an average return interval of approximately 5 years in the uplands on Eglin (Eglin Integrated Natural Resources Management Plan 2002).

METHODS

I established 14, 2.5 km transects along a stratified random sample of stream drainages. Strata were selected to incorporate a mixture of historically occupied streams with streams where *R. okaloosae* have never been detected and were spread across the geographic range of the species. Existing road survey locations provided a starting point and transects were oriented either up- or down-stream from these points and had a 0.10-0.25 km random start from these access points. Eleven survey locations were systematically oriented along each transect with 0.25 km spacing that resulted in a total of 154 survey locations. *Rana okaloosae* are prolonged breeders with an activity season that lasts from March–September (Moler 1992, Bishop 2004), but call most regularly from May–August. During this time males use vocalizations primarily at night to attract females. Therefore, frogs were surveyed at night along each transect 3 times/year from May–August 2006-2008 and transects were surveyed each month and year based on a random ordering.

Survey locations were directly adjacent to the stream course and at each survey location I listened for the presence of calling male *R. okaloosae* for 5 minutes. Surveys began 0.5 hours after sunset and ended before 02:00. Additionally, when traveling between survey locations

observers moved cautiously to try to assess the presence of *R. okaloosae* between the official survey locations.

I used in-stream belt-transects to assess habitat selection at sites where *R. okaloosae* were detected and at sites where they were not detected from May-August 2006 and August-October 2007. Belt-transects were oriented perpendicular to the stream and the length was determined by the wetted area of the channel and extended for 1 m onto the upland bank of each side of the stream. However, if a used location was in a large wetland (e.g., a fen) adjacent to the stream, transects were 10 m in length and did not always cross the stream. At each end point and at the center point I measured a suite of habitat characteristics. I measured vegetation and variables describing the site, such as percent canopy cover, frog-level canopy cover, basal area, percentage of emergent and submergent vegetation, woody debris, and width of stream.

I measured the percent canopy cover using a spherical densiometer both at the height of the frog, (e.g., measurements were taken on the ground or at the surface of the water) as well as at 1.5 m above the ground. Overhead canopy measurements were recorded at the center of transects and at the ends of transects (i.e., the land-water interface). I measured basal area using a Jim-Gem Cruz-All (English basal area factor of 5) at the ends and the center point of the belt-transects. I estimated the percentage of emergent (e.g., *Sphagnum* spp. and *Carex* spp.) and submergent vegetation (e.g., algal mats and submerged *Carex* spp.) and the amount of woody debris in a 0.5 m X 0.2 m rectangular plot at the center and at the ends of the belt-transect using the Daubenmire (1959) cover class scale. Further, I recorded the width of the wetted portion of the stream.

I developed a suite of *a priori* models (Table 2.1) based on the vegetation structure and physical characteristics of the sites and used these models to describe habitat selection of *R.*

okaloosae at each scale. I evaluated these models with an information theoretic approach using second-order Akaike's Information Criteria (AIC_c), which is adjusted for small sample sizes, to examine the relative strength of the models (Burnham and Anderson 2002). The model with the lowest AIC_c value was considered to be the model with the best balance between statistical parsimony and goodness of fit for the empirical data (Burnham and Anderson 2002). Models with ΔAIC_c values ≤ 2.0 were considered equally supported by the data (Burnham and Anderson 2002) and models with ΔAIC_c values >2.0 and <4.0 were considered to have limited support.

I conducted separate analyses for each scale, among-stream and within-stream. The among-stream analysis represented all sites surveyed along all 14 streams and the within-stream analysis included only survey locations that occurred on 9 streams where ≥ 1 survey site was occupied. Using this framework I compared sites that were occupied by *R. okaloosae* with those where frogs were not detected using Proc GLIMMIX (SAS 9.2) with a binary distribution and logit link function. This platform allowed me to conduct a logistic regression while incorporating stream as a random effect, which allowed for unmeasured variation to be incorporated into each model. I assessed the top model using the odds ratios (Keating and Cherry 2004) and effect sizes (Anderson et al. 2001).

RESULTS

To describe *R. okaloosae* habitat, I sampled 138 in-stream belt-transects on 14 streams (8-11 belt-transects/stream) where call survey transects were established from 2006-2008. Overall, 56 used belt-transects and 82 belt-transects without detections were sampled. At the among-stream scale the model with percent emergent vegetation received the greatest amount of support ($w_i = 0.67$, $\Delta AIC_c = 0.0$) for predicting habitat use (Table 2.2) out of the 14 *a priori* habitat models. Emergent vegetation was positively associated with use (Table 2.3) and

emergent vegetation was 1.53 times greater at used sites versus sites with no detections (Figure 2.2). The odds ratio of the coefficient for emergent vegetation predicted a 4.5% increase in the probability of use of a site for every 1% increase in emergent vegetation (Table 2.3). At this scale, the second model which received limited support ($w_i = 0.23$, $\Delta AIC_c = 2.17$) was a model that included emergent vegetation, canopy cover, and the interaction of the 2 variables. At the among-stream scale canopy was 1.10 times greater at sites with no detection as compared to sites with use by *R. okaloosae* (Table 2.4).

Similarly, at the within-stream scale a model with only emergent vegetation received the greatest support ($w_i = 0.65$, $\Delta AIC_c = 0.0$) when only considering streams with at least one occupied site within the 2.5 km reach sampled (Table 2.2). Percent emergent vegetation was positively associated with use (Table 2.3) and was 1.73 times greater at used sites versus sites with no detections (Figure 2.2). Similar to the among-stream scale the odds ratio of coefficient for emergent vegetation predicted a 4.9% increase in use of site for every 1% increase in emergent vegetation (Table 2.3). At this scale, the second model which received limited support ($w_i = 0.16$, $\Delta AIC_c = 2.83$) was a model that included emergent vegetation, canopy cover, and the interaction of the 2 variables. At the within-stream scale, canopy was 1.03 times greater at sites with no detection as compared to sites with use by *R. okaloosae*, whereas submergent vegetation was 1.49 times greater at used sites than at sites with no detections.

DISCUSSION

I sampled stream drainages across the entire geographic range of *R. okaloosae*. Based on my habitat modeling, *R. okaloosae* selected for streams at the among-stream scale that had higher amounts of emergent vegetation (Figure 2.2). At the within-stream scale (i.e., within occupied streams), a higher amount of emergent vegetation still was the best predictor of use by

breeding male *R. okaloosae* (Figure 2.2). Use of sites with high amounts of understory cover is consistent with characteristics that have been observed for other amphibian species that occur in the longleaf pine ecosystem (Means and Moler 1979, Gorman et al. 2009). For example, Pine Barren Tree Frogs (*Hyla andersonii*), another habitat specialist, used herb bogs (wetlands with high amounts of emergent vegetation) to deposit egg masses and therefore tadpoles developed in these sites (Means and Longden 1976, Means and Moler 1979). True to their nature as tree frogs, *H. andersonii* used shrub bogs as calling sites, but eggs and tadpoles were most often located in herb bogs. This was attributed to decreased evapotranspiration in herb bogs that allowed them to retain water longer compared to shrub bogs which have greater numbers of woody stems (Means and Longden 1976, Means and Moler 1979). This increase in water retention at sites dominated by emergent vegetation may be an important factor, particularly for *R. okaloosae*, because this species is thought to overwinter as a tadpole (Moler 1993). Further, other rare species (e.g., Columbia Spotted Frog [*R. luteiventris*]) have been found to occur more often at sites with high amounts of emergent vegetation (Welch and MacMahon 2005).

Additionally, other more generalist species of calling anurans use sites that contain high amounts of emergent vegetation including many common species found in the southeastern U.S. (Lichtenberg et al. 2006). It is likely that this cover type has other benefits beyond the possibility that it has lower rates of evapotranspiration. Emergent vegetation may also be an important habitat requirement for anurans, because it can provide attachment sites for oviposition, is a potential source of cover from predation for tadpoles and adults, may increase food resources, may decrease flow of water, and provides shade and shelter from inclement weather (Hazzell et al. 2001, Welch and MacMahon 2005).

In addition to the macrohabitat scale, *R. okaloosae* have also been found to select for emergent vegetation at the microhabitat scale (Chapter 1). Emergent vegetation was among a suite of 5 habitat variables, including submergent vegetation, distance to cover, woody debris, and frog-level canopy, that were selected at the micro-scale. The selection of this habitat feature at multiple spatial scales highlights the importance of this characteristic. For calling males, emergent vegetation may serve as a feature that is important as escape cover from predators and may be a feature that is selected by females as well.

Fire in the upland longleaf pine ecosystem is known to be an important driver that maintains an open overstory, a reduced midstory, and a lush understory, and has been shown to have positive effects on the restoration of sites with histories of fire suppression (Litt et al. 2001). The frequency of fire in the streams and wetlands within the system are not as well understood, but it is likely that wetlands benefit from the fire that enters wetlands from the uplands (Means and Moler 1979, Means 2006). Fires during the growing season are more likely to burn into wetlands due to dryness during this time of year (Bishop and Haas 2005, Gorman et al. 2009). Therefore, fires in the growing season may be an important driver in the wetlands of the longleaf pine system, because fires may create the high amounts of emergent vegetation (Means and Moler 1979, Jackson 2004, Enge 2005) that was selected for by *R. okaloosae* in this study.

In addition to fire, it is likely that other factors, such as hydrology and beaver (*Castor canadensis*) activity, may contribute to maintaining higher amounts of emergent vegetation. Wetlands that have a longer hydroperiod are thought to deter growth of woody vegetation while emergent vegetation responds favorably (Stroh et al. 2008). Similarly, beaver activity, such as dam building and foraging has been shown to alter vegetative communities along streams by decreasing basal area and tree density (Johnston and Naiman 1990) and impoundments created

by beaver can become areas dominated by emergent vegetation (e.g., “beaver meadows”) (Baker and Hill 2003). Future research should focus on understanding how management activities, such as prescribed fire and mechanical treatments that can mimic these possible drivers can be used to create suitable habitat for *R. okaloosae*.

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Table 2.1. Suite of models used to assess the selection of among- and within-stream macrohabitat characteristics by *Rana okaloosae* on Eglin Air Force Base, Florida, 2006-2008.

Model	Variables
Microhabitat Cover	Submergent and emergent vegetation, woody debris, frog-level canopy, and a submergent/emergent interaction
Macrohabitat Cover	Total channel width, percent canopy cover, basal area, and an interaction of canopy cover and basal area
Macro and Microhabitat Cover	Submergent and emergent vegetation, woody debris, percent canopy cover, basal area, canopy cover/basal area interaction, submergent/emergent interaction
Overstory Canopy Cover	Percent canopy cover at breast height
Frog-level Canopy	Percent canopy cover at ground level
Canopy and Emergent	Percent canopy cover, emergent vegetation, and interaction
Channel Width	Width of stream
Basal Area	Basal area (m ²)
Woody Debris	Percent woody debris
Submergent	Percent submergent vegetation
Emergent	Percent emergent vegetation
Global	Submergent and emergent vegetation, woody debris, canopy cover, frog-level canopy, basal area, channel width, canopy cover/basal area interaction, submergent/emergent interaction, canopy/emergent interaction

Table 2.2. Model selection results for the top 3 models for among- (n = 138) and within-stream (n = 87) comparisons of macrohabitat characteristics for *Rana okaloosae* on Eglin Air Force Base, Florida, 2006-2008 (k = # of parameters, AIC_c = second-order Akaike's Information Criteria [i.e., for small sample sizes], ΔAIC_c = the change in AIC_c , and w_i = the relative amount of support for the model).

Model	K	AIC_c	ΔAIC	w_i
<u>Among-Stream</u>				
Emergent	2	117.83	0.00	0.67
Canopy and emergent	4	120.00	2.17	0.23
Overstory canopy	2	123.91	6.08	0.03
<u>Within-Stream</u>				
Emergent	2	96.58	0.00	0.65
Canopy and emergent	4	99.41	2.83	0.16
Submergent	2	100.93	4.35	0.07

Table 2.3. Parameter estimates and standard errors (SE), degrees of freedom (df), *t*-value, *P*, odds ratio, and confidence intervals from the most supported models describing *Rana okaloosae* breeding habitat use at the among- and within-stream scales on Eglin Air Force Base, Florida, 2006-2008.

Effect	Estimate	SE	df	t-value	<i>P</i>	Odds Ratio	95% CI	
							lower	upper
<i>Among-Stream</i>								
Emergent	0.044	0.017	123	2.57	0.011	1.045	1.010	1.080
<i>Within-Stream</i>								
Emergent	0.048	0.019	77	2.54	0.013	1.049	1.010	1.088

Table 2.4. Mean habitat characteristics and standard errors (SE) of sites where *Rana okaloosae* were detected and not detected on 14 streams (i.e., among-stream scale) on Eglin Air Force Base, Florida, 2006-2008.

Variables	No Detection (n=82)	SE	Detection (n=56)	SE
Channel Width (m)	9.3	0.8	12.1	2.3
Canopy (%)	75.7	1.8	68.7	1.5
Frog-level canopy (%)	83.5	1.3	81.0	1.5
Basal Area (m ² /ha)	13.0	0.5	12.0	0.6
Emergent vegetation (%)	19.0	2.0	29.0	3.2
Submergent vegetation (%)	4.0	0.6	4.1	0.5
Woody debris (%)	17.5	1.5	16.4	2.0

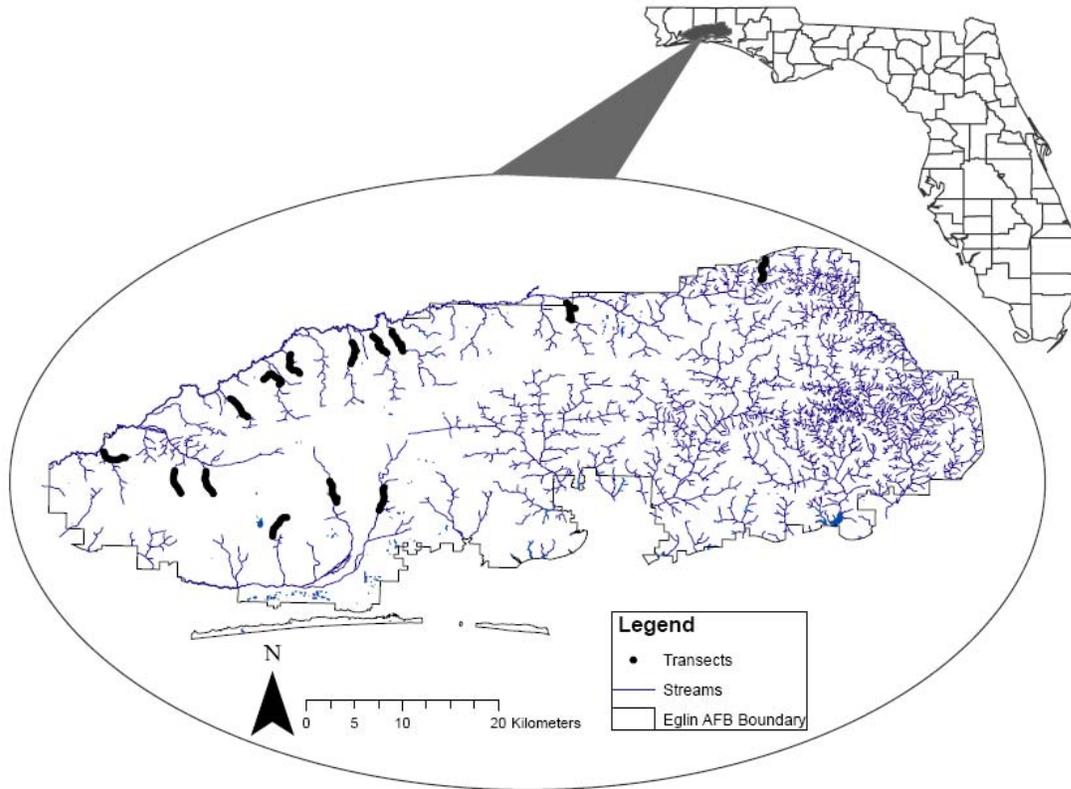


Figure 2.1. Study area depicting the 14 stream drainages that were surveyed for the presence of *Rana okaloosae* and sampled for habitat characteristics on Eglin Air Force Base, Florida, 2006-2008.

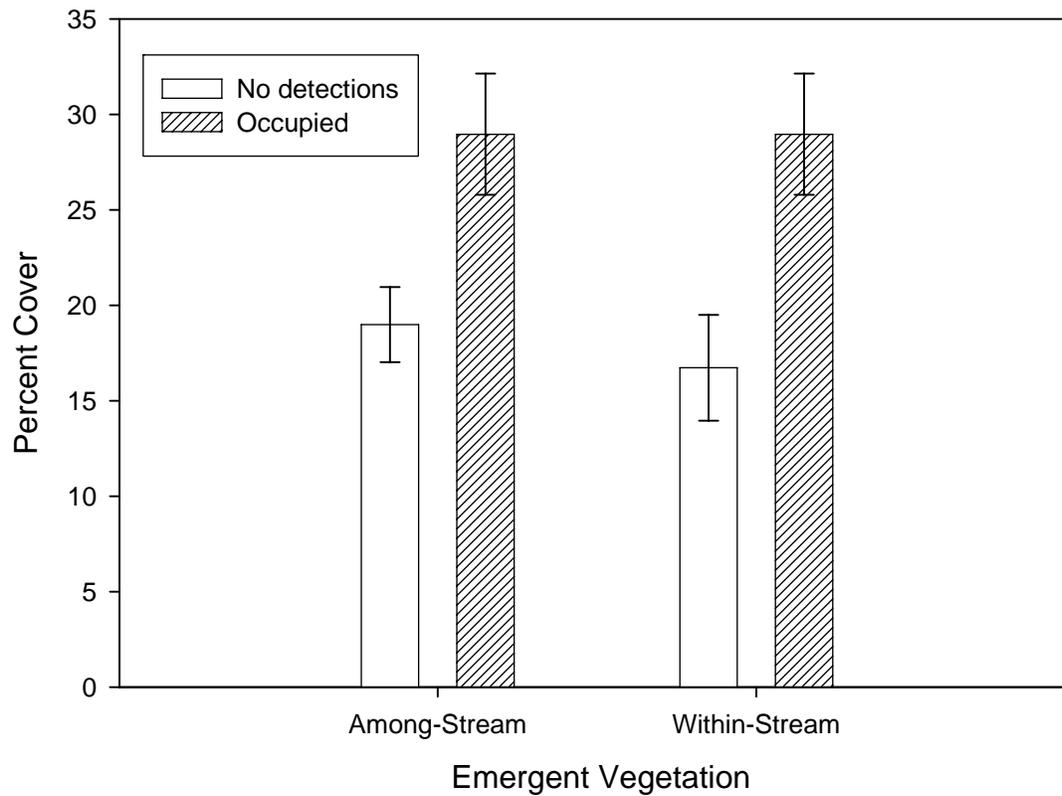


Figure 2.2. Comparison of the mean percentage of emergent vegetation at the among-stream scale (n=138) and the within-stream scale (i.e., only sites found on streams that had ≥ 1 site occupied [n=87]) by *Rana okaloosae* on Eglin Air Force Base, Florida, 2006-2008.

CHAPTER 3

Range-wide and Stream-level Scale Occupancy of *Rana okaloosae*

Abstract: The current geographic range of the Florida Bog Frog (*Rana okaloosae*) is known to be restricted and the majority of the geographic range occurs on Eglin Air Force Base (Eglin), Florida. While the general range is understood, the distribution of occupied sites within the range has a relatively large disjuncture that is not well understood. Therefore, my colleagues and I conducted aural surveys of *R. okaloosae* three times/year from May-August, 2006-2008 along road and walking transect routes. The resulting detection/nondetection data were used to estimate the probability of occupancy, colonization, and detection. Additionally, I related these population-level parameters to habitat characteristics at two different spatial scales: *range-wide* and *stream-level* scales to understand how occupancy and colonization of *R. okaloosae* may be influenced by scale. Also, because of the restricted nature of this endemic species, my goal was to improve survey and monitoring techniques for *R. okaloosae* on Eglin. My results suggest that at both spatial scales occupancy by *R. okaloosae* was best predicted by the presence of mixed forest wetlands at survey sites. At the range-wide scale, colonization and detection were constant across years. At the stream-level scale, colonization was estimated by the number of years since last fire and detection was best estimated by the additive combination of relative humidity and temperature. Occupancy of *R. okaloosae* was patchy at the range-wide ($\bar{x} = 16.1\%$) and at the stream-level ($\bar{x} = 32.1\%$) scales and colonization was low at both scales ($\bar{x} = 8.3\%$ and 5.3% , respectively) while derived estimates of local extinction were moderately high ($\bar{x} = 32.5\%$ and 21.0% , respectively). In the early 1980s when *R. okaloosae* were originally identified, populations were located on small tributary streams associated with 3 rivers. While *R.*

okaloosae are still associated with these 3 river systems, one of the three river systems appears to be very isolated and detections there are becoming very infrequent. Because of a paucity of information related to life history, dispersal strategies are not well understood, but conservation activities should focus on protection of high quality sites and proximity or connectivity among sites to maximize efficiency of colonization of new sites and sites previously occupied.

Key Words: colonization, detection, Florida Bog Frog, habitat, geographic range, occupancy, *Rana okaloosae*, spatial scale, surveys

INTRODUCTION

Amphibian declines are occurring across the world and efforts to understand these declines have increased over the past 25 years (Blaustein and Kiesecker 2002, Beebee and Griffiths 2005). Declines have been attributed to many different causes, including habitat loss and fragmentation, global warming, environmental contamination, increased levels of ultraviolet radiation, disease, and invasive species (Blaustein and Kiesecker 2002). Species that have restricted ranges and naturally occur as small populations can be particularly susceptible to events that can cause declines in population size (Caughley 1994, Gaston 1998). Therefore, identifying and monitoring species that occur in small populations is of particular interest for conservation and may aid in understanding the competing paradigms of small populations versus declining populations (Caughley 1994). Further, knowledge of a species' geographic range and local abundance can provide information on the risk of extinction for a species (Gaston 1998, Johnson 1998).

Florida bog frogs (*Rana okaloosae*) are 1 of 7 species in the species group *R. catesbiana* (Anura: Ranidae; Austin et al. 2003), and are endemic to northwestern Florida with the majority of this species' range occurring within the boundaries of Eglin Air Force Base. The species was discovered and described in 1982 (Moler 1985) and since then there has been limited work on the limitations to the species distribution and population dynamics (Jackson 2004). This species is listed as a state species of special concern (Florida Fish and Wildlife Conservation Commission 2007) and the Department of Defense (DOD) has designated it a "species at risk." "Species at risk" are defined as plant and animal species that are not yet federally listed under the Endangered Species Act, but are rare or declining and have populations that are known to occur on or near DOD installations (NatureServe 2004). DOD lands are thought to support more federally threatened and endangered species than any other federal agency, and have more species that are imperiled than either the U.S. Fish and Wildlife Service or the National Park Service (Groves et al. 2000). Although military activity often results in improved habitat conditions for some species (e.g., those that require fire-maintained habitats), managing for threatened and endangered species can constrain or conflict with military activity. Thus, DOD has attempted to identify "species at risk" on land they manage to try and preclude these imperiled species from declining to the point that they become federally listed.

Across North America there have been many different monitoring programs established to obtain data on populations and distributions of anurans (e.g., Jung et al. 2002, Nelson and Graves 2004, Stevens and Paszkowski 2004, Weir et al. 2005). Survey data allow researchers to evaluate whether annual variation in population size and changes in spatial distributions are normal fluctuations or consistent trends (Nelson and Graves 2004). One approach that has been frequently used to monitor anuran populations is aural surveys of breeding sites (Scott and

Woodward 1994). Monitoring of *R. okaloosae* on Eglin with aural surveys was implemented sporadically from 1985-2001, and from 2002-2005 a more formal approach was initiated, but was primarily focused on documenting the species' distribution (Bishop 2004). *Rana okaloosae* are particularly suited to aural surveys, because the species is a prolonged breeder with an activity season that lasts from March–September (Moler 1992, Bishop 2004), but males call most regularly from May–August. The call of *R. okaloosae* is distinct from other species with which it co-occurs (Bishop 2005).

Recent improvements in inference and analytical techniques of binary data (i.e., detection/nondetection) allows for estimation of site occupancy while accounting for the detection probability of the species of interest (MacKenzie et al. 2002, 2003, 2006). The major advantage of using site occupancy in this context is that it allows for a detailed treatment of detection probabilities (Weir et al. 2005), which is a commonly overlooked component of binary survey data (Mazerolle et al. 2005). Occupancy can be defined as the proportion of landscape units where a species is determined to be present (MacKenzie et al. 2006). Further, site occupancy can be considered a state variable (i.e., a measure of the current status of the population), because it relates to the distribution of a species (MacKenzie et al. 2006).

Knowledge of a species' distribution, particularly in the context of a rare species with a restricted range, can allow for a better understanding of how conservation strategies can be applied. Species that occur in metapopulations may require different strategies than species that are more uniformly distributed, because when small populations within the metapopulation become isolated they are less likely to be recolonized and therefore are at higher risk of localized extinction (Lawes et al. 2000). Theoretically, these smaller isolated patches may have a chance of longer term persistence if they are connected by dispersal to other occupied patches (Moilanen

and Hanski 1998). Also, if the number of occupied patches falls below a threshold or the distance between patches exceeds a threshold then extinction of the entire metapopulation is likely. Further, the relationship of occupancy and colonization to landscape attributes, such as habitat type and configuration, can aid in decision-making related to the types of strategies that will be most effective for conserving species that occur as metapopulations. Connectivity among sites and the scale at which management actions are applied can have implications for the persistence of populations and habitat quality of both occupied sites and habitat between sites. Therefore, my objectives were to model the probability of site occupancy, probability of colonization, and probability of detection and to derive local extinction rates of *R. okaloosae* using aural call surveys from 2006-2008. I used two different survey methodologies to understand how spatial scale influences occupancy, colonization, local extinction, and detection rates, and I incorporated components of habitat into models to understand how habitat can influence occupancy and colonization.

STUDY AREA

The study area was located on Eglin Air Force Base (Eglin) in the counties of Okaloosa, Santa Rosa, and Walton in northwestern Florida (Figure 3.1). Eglin is a large military installation (187,375 ha) that plays an integral role in the nation's defense, because it is a base that develops and tests weapons (Hardesty and Kindell 1997). However, Eglin is also one of the most biologically diverse Air Force Bases in the nation (Hardesty and Kindell 1997). Eglin is situated in the Coastal Plain physiographic province and overall the topography of the study area has little relief, but there are numerous steep ravines associated with headwater streams (i.e., steepheads) where slopes can be $\geq 30\%$ (Eglin Air Force Base 2002, Means 1975). Riparian areas were dominated by black titi (*Cliftonia monophylla*), sweetbay magnolia (*Magnolia*

virginiana), white cedar (*Chamaecyparis thyoides*), swamp titi (*Cyrilla raceformia*), and blackgum (*Nyssa sylvatica*) (Chapter 2). Beyond the riparian areas the study area was dominated by a longleaf pine (*Pinus palustris*) and turkey oak (*Quercus laevis*) sandhill community that encompasses the majority of Eglin and is primarily managed for the Red-cockaded Woodpecker (*Picoides borealis*). Therefore, the predominant management strategy on Eglin was to use prescribed fire in the uplands, primarily during the dormant season. Return intervals of fire in the uplands were planned to be approximately every 5 years (Eglin Integrated Natural Resources Management Plan 2002).

METHODS

Range-Wide Occupancy

It has been suggested that the best approach to use when surveying rare species is to sample more sites less intensively as opposed to surveying at fewer sites more intensively as one might for a more common species (Mackenzie et al. 2005). However, some within-season replication is necessary to assess detection probabilities. I used a large secondary road network (Albertson et. al. 1995) to survey locations at bridge crossings and areas where the road was adjacent to a wetland (Figure 3.1A). Many sites were established amphibian monitoring stations that have been used to monitor amphibians on Eglin in the past (Bishop 2004). I grouped all of these previously surveyed stations into regional blocks that could be surveyed within one or two nights and then randomly selected blocks to survey. Surveys were conducted at 172 sites in 2006 and an additional 65 sites were added in 2007, such that in both 2007 and 2008, 237 sites were surveyed.

Stream-Level Occupancy

I established 14, 2.5km transects along a stratified random sample of stream drainages (Figure 3.1B). Strata were selected to incorporate a mixture of historically-occupied streams with streams that have never had frogs detected and were spread across the geographic range of the species. Seven transects were located on drainages with known populations of bog frogs while 5 transects were located on drainages previously thought to be unoccupied, and 2 were located on drainages with historic but no recent occupancy. Existing road survey locations provided a starting point and transects were oriented either up or down stream from these points and had a 100-250 m random start from these access points. From the resulting random start location, a total of 11 survey locations was systematically oriented along transects with 0.25km spacing, which resulted in a total of 154 survey locations.

Surveys

My colleagues and I surveyed for *R. okaloosae* from May–August in 2006–2008, because this is the primary breeding season for the species (Bishop 2005). During this time males use vocalizations primarily at night to attract females. *Rana okaloosae* were surveyed at night at georeferenced listening sites along transects and at designated road survey locations 3 times/year (although conditions occasionally prevented repeated sampling at all sites and some sites were not phased into the surveys until 2007). Sites were surveyed each month based on a random ordering. Surveys were started 0.5 hour after sunset and ended before 2 am. On road surveys, observers turned off the vehicle and stood outside at survey stations. At each road and transect survey location observers listened for the presence of calling male *R. okaloosae* for 5 minutes. Observers recorded variables that may have an influence on detection probabilities or the probability of a frog to be calling, such as air temperature, relative humidity, and sky condition

(i.e., cloudiness), and start time of survey. I did not conduct surveys during rain events, because *R. okaloosae* reduce calling activity during this time (Bishop 2005) and rain can reduce observer efficacy. Also, I did not conduct surveys on nights with high wind, because wind can also reduce the ability of an observer to hear calls.

Landscape Covariates

I used a Geographic Information System (GIS) to extract variables that I used to assess the influence of vegetation structure and landscape metrics on the probability of occupancy by *R. okaloosae*. I used Florida Natural Areas Inventory (FNAI) Land Use coverage to extract land cover types that I hypothesized would be of importance to occupancy by *R. okaloosae*. Using ArcGIS 9.2 (Environmental Systems Research Institute, Inc., Redlands, California), I established a 125 m buffer that was centered on either a road survey location or a transect survey location. I chose 125 m because this was approximately the maximum known distance moved by an individual *R. okaloosae* (Gorman et al. 2009) and was beyond the distance an observer could confidently hear *R. okaloosae* from a survey location. Next, I intersected the buffers with the FNAI Land Use coverage. I extracted 4 habitat variables that included a single *Upland* category (e.g., sandhill, hardwood forest, and pine production) and 3 wetlands categories that included *Mixed forest wetland* (including both mixed forest wetlands and floodplain swamp that are composed of a mixture of deciduous and evergreen trees), *Flatwoods* (including mesic, wet, and scrubby flatwoods), and *Other wetlands* (including many infrequently occurring wetland types, such as depression marsh, dome swamp, wet prairie, and seepage slope). Using the intersect tool I calculated the years since last burn for the burn unit containing each location using fire data provided by the Eglin Natural Resources Branch. Further, because transect survey locations occurred within a riparian corridor, I also calculated sinuosity of the stream within the buffer, by

dividing the length of the stream within the buffer by the diameter of the buffer (i.e., the straight line distance).

Occupancy Analyses

I used a multiple-season occupancy model (MacKenzie et al. 2003) to estimate occupancy, colonization, and local extinction while accounting for probability of detection. Increasingly it has been reported that estimation of state variables without accounting for detection may lead to biased estimates (Mackenzie et al. 2003, Bailey et al. 2004, Mazerolle et al. 2007). Therefore, within the multiple-season framework I selected a model that estimated seasonal occupancy (ψ), colonization (γ), and detection probability (p). I estimated occupancy and colonization as functions of landscape metrics at both the range-wide scale and the stream-level scale separately. Using this model I was able to attain derived estimates of local extinction from the measure of seasonal occupancy and seasonal colonization (Mackenzie et al. 2006) at both spatial scales.

At the range-wide scale I modeled the probability of occupancy as a function of the proportion of the buffer area classified as mixed forest wetland, flatwoods, other wetlands, and uplands. I also modeled the effect of recent fire by using the number of years since last fire of the upland block in which each point occurred and I modeled occupancy as a function of the year of the survey (2006-2008). I hypothesized that these covariates would yield more accurate estimates of occupancy. Additionally, at the range-wide scale I modeled colonization as a function of years since last fire and constant between years. I hypothesized that a lower number of years since last fire may facilitate movement of frogs among sites by creating openings that would promote moister ground conditions and more emergent vegetation.

At the stream-level scale I modeled the probability of occupancy as a function of the proportion of the buffer area classified as mixed forest wetland, flatwoods, other wetlands, and uplands. I also modeled the effect of recent fire by using the number of years since last fire of the upland block in which each point occurred. Additionally, I modeled the probability of occupancy as a function of stream sinuosity within the buffer and I modeled occupancy as a function of the year of the survey (2006-2008). Similar to the range-wide scale I hypothesized that incorporating habitat covariates would yield more accurate estimates of occupancy. Also, at the stream-level scale I modeled colonization as a function of years since last fire. I hypothesized that a lower number of years since last fire would facilitate movement by connecting habitat or making dispersal through habitat easier. Finally, I modeled colonization as a constant among years.

Further, at both spatial scales I modeled detection probabilities as a function of year, a constant among years, sky condition, or the additive combination of temperature and relative humidity. I hypothesized that these variables may have an influence on the calling behavior of *R. okaloosae*. I chose to combine temperature and relative humidity to reduce the number of variables included in these models. All occupancy analyses were performed within Presence 2.2 (Hines 2006). I used an information theoretic approach and developed a suite of models based on combinations of covariates for occupancy, colonization, and detection, which resulted in 24 models for the range-wide scale and 28 models for the stream-level scale. At both spatial scales, I evaluated models using Akaike's Information Criteria (AIC) to examine the relative strength of the models (Burnham and Anderson 2002). The model with the lowest AIC value was considered to be the model with the best balance between statistical parsimony and goodness of fit for the empirical data (Burnham and Anderson 2002). I considered models with Δ AIC values

≤ 2.0 were considered equally supported by the data (Burnham and Anderson 2002) and models >2.0 and <4.0 were considered to have some support.

RESULTS

Range-Wide Occupancy

From 2006-2008, my colleagues and I conducted 1751 surveys of 237 sites. In 2006 a total of 443 surveys was conducted at 172 of the 237 sites, in 2007 a total of 597 surveys were conducted at the 237 sites, and in 2008 a total 711 surveys was conducted at the 237 sites. All sites were surveyed a minimum of 4 times over the 3 year period ($\bar{x} = 7.4$ surveys/site, SE = 0.10). *Rana okaloosae* were detected at least once at 26 sites in 2006 (naïve $\psi = 15.1\%$), 28 sites in 2007 (naïve $\psi = 11.8\%$), and 32 sites (naïve $\psi = 13.5\%$) in 2008. In total *R. okaloosae* were detected at least once at 53 unique sites over the 3 year period.

Occupied sites had greater percentages of wetlands within the 125 m buffer around road survey locations (Figure 3.2). Mixed forest wetlands made up the greatest percentage of wetlands at both occupied sites ($\bar{x} = 27.1\%$, SE = 3.2%) and sites with no detections ($\bar{x} = 17.5\%$, SE = 1.7%). Flatwoods habitat was similar between occupied sites ($\bar{x} = 15.0\%$, SE = 3.6%) and sites with no detections ($\bar{x} = 11.0\%$, SE = 1.8%). Also, the category of other wetlands was similar between occupied sites ($\bar{x} = 2.0\%$, SE = 0.8%) and sites with no detections ($\bar{x} = 2.3\%$, SE = 0.5%). Uplands made up the greatest percentage of habitat for both occupied sites ($\bar{x} = 56.0\%$, SE = 3.9%) and sites with no detections ($\bar{x} = 69.3\%$, SE = 2.4%). Sites that were occupied were burned last a mean of 3.7 years (SE = 0.5) previously and sites with no detections were burned last a mean of 5.4 years previously (SE = 0.5).

The most highly supported model of occupancy, colonization, and detection for *R. okaloosae* was a model that included mixed forest wetland for occupancy and was constant

across years for both colonization and detection ($AIC = 741.87$, $\Delta AIC = 0.0$, $w_i = 0.52$) (Table 3.1). The next most highly supported model was a function of upland habitat for occupancy and was constant across years for both colonization and detection ($AIC = 744.10$, $\Delta AIC = 2.23$, $w_i = 0.17$) (Table 3.1). The third most highly supported model was a model where mixed forest wetland was the predictor of occupancy, colonization was best predicted by year since last fire, and sky condition (i.e., a positive relationship with increased cloudiness) was the best predictor of detection ($AIC = 745.22$, $\Delta AIC = 3.35$, $w_i = 0.10$) (Table 3.1). Finally, the fourth most highly supported model was a model where mixed forest wetland was the predictor of occupancy, colonization was best predicted by year since last fire, and year was the best predictor of detection ($AIC = 745.43$, $\Delta AIC = 3.56$, $w_i = 0.09$) (Table 3.1).

The most highly supported model predicted a positive relationship between occupancy and mixed forest wetland (Table 3.2; Figure 3.3), a negative slope for the intercept of colonization (Table 3.2), and a positive slope for the intercept of the probability of detection (Table 3.2). Further, the most highly supported model predicted that across the area sampled mean occupancy was 16.1% (SE = 2.9%). Further, colonization at this scale was estimated to be 5.3% (SE = 1.1%) and detection was estimated to be 60.3% (SE = 3.5%). The derived estimate for the probability of yearly local extinction was 32.5% (SE = 7.4).

Stream-Level Occupancy

From 2006-2008, my colleagues and I conducted 1386 surveys of 154 sites located on 14 streams. In each year I was able to survey every site 3 times therefore there were no missing values for this spatial scale. *Rana okaloosae* were detected at least once at 47 sites in 2006, 47 sites in 2007, and 42 sites in 2008. Overall, *R. okaloosae* were detected at least once at 62 unique sites over the 3 year period on 9 streams. Of the streams where I did not detect any *R.*

okaloosae over the 3 year period, 3 streams were historically unoccupied and 2 were streams that have had a detection historically within the drainage (i.e., prior to 2003 and 1986). Of the remaining 9 streams, 7 were known to be occupied historically, but the surveys resulted in a broader occurrence along the length of the 2.5 km transect than previously known, and 2 streams were historically thought to be unoccupied, but I had at least 1 detection during the 3 year period.

Similar to the road survey locations, overall transect locations that were occupied had a greater percentage of wetlands within the 125m buffer (Figure 3.4). Mixed forest wetlands made up the greatest percentage of wetlands at both occupied ($\bar{x} = 25.9\%$, SE = 2.1%) and sites with no detections ($\bar{x} = 15.7\%$, SE = 1.8%). Flatwoods habitat was similar between occupied sites ($\bar{x} = 7.5\%$, SE = 2.1%) and sites with no detections ($\bar{x} = 9.3\%$, SE = 2.0%). Also, the category of other wetlands was similar between occupied sites ($\bar{x} = 1.1\%$, SE = 0.5%) and sites with no detections ($\bar{x} = 0.8\%$, SE = 0.3%). Uplands made up the greatest percentage of habitat for both occupied sites ($\bar{x} = 65.5\%$, SE = 2.9%) and sites with no detections ($\bar{x} = 74.1\%$, SE = 2.4%). Further, sites that were occupied were burned last a mean of 3.0 years (SE = 0.3) previously and sites with no detections were burned last a mean 7.0 years (SE = 1.0) before. Of the sites that were occupied at least once, 90% were within burn units that had been burned in ≤ 4 years. Stream reaches at both occupied ($\bar{x} = 0.99$, SE = 0.04) and sites with no detections ($\bar{x} = 0.93$, SE = 0.03) on average were not very sinuous (i.e., the closer the value is to 0 the greater the sinuosity).

The most highly supported model of occupancy, colonization, and detection for *R. okaloosae* at the stream-level scale was a model where mixed forest wetland was the predictor of occupancy, colonization was best predicted by years since last fire, and the additive combination of relative humidity and temperature was the best predictor of detection (AIC = 929.06, Δ AIC =

0.00, $w_i = 0.78$) (Table 3.3). The next most highly supported model was a model where upland habitat was the predictor of occupancy, colonization was best predicted by years since last fire, and the additive combination of relative humidity and temperature were the best predictors of detection (AIC = 932.87, Δ AIC = 3.81, $w_i = 0.12$) (Table 3.3).

The most highly supported model predicted a positive relationship between occupancy and mixed forest wetland (Table 3.4; Figure 3.5), a negative relationship between colonization and years since last fire (Table 3.4; Figure 3.6), and the probability of detection was positively related to temperature and negatively related to relative humidity (Table 3.4; Figure 3.7). Further, the most highly supported model predicted that at the stream-level scale the mean occupancy was 32.1% (SE = 4.5%). Further, mean probability of colonization at this scale was estimated to be 8.3% (SE = 2.5%) and the mean probability of detection was 62.5% (SE = 4.1%). The derived estimate for the probability of yearly local extinction was 21.0% (SE = 7.1%).

DISCUSSION

The known distribution of *Rana okaloosae* is extremely restricted resulting in the species not being discovered until the 1980s (Moler 1985). Understanding the limitations to the range of this species is important for future management and conservation decisions. The range-wide survey for *R. okaloosae* yielded important results that describe the population structure of this rare species. As Schmidt (2008) points out, parameter estimates for uncommon species are limited, but these data are important because these species tend to be at the forefront of conservation work. While using data from similar species in some cases may provide a more viable option than no data there is little substitute for intensive data on a species of interest.

Rabinowitz (1981) and Rabinowitz et al. (1986) described a model of rarity that is composed of 7 forms of rarity and is based on 3 characteristics of populations of species. These

3 characteristics include local population size, distribution (area of a species geographic range), and the variety of habitat that the species uses. This study addressed two aspects of this model, distribution (occupancy is considered a state variable of distribution), and habitat specificity. In addition, I have limited data that suggests *R. okaloosae* occurs in small population sizes at 4 study sites (see Chapter 1) where I conducted mark-recapture (Gorman, unpublished data). My results confirmed the patchy nature over which *R. okaloosae* are distributed. Across all of the sites that I surveyed at the range-wide scale this species had a low probability of occupancy ($\bar{x} = 16.1\%$) and the estimated probability of colonization was low ($\bar{x} = 5.3\%$), while the derived estimate of yearly extinction probability was moderately high ($\bar{x} = 32.5\%$). Further, at both scales only a portion of the total sites were occupied in a given year and the occupied sites changed from year to year, but the overall occupancy rate was relatively stable among years. This combination of parameter estimates suggest that the species is sparsely distributed across Eglin. My more intensive surveying along 2.5 km stretches of stream within the species' known range yielded higher estimates of occupancy ($\bar{x} = 32.1\%$) and colonization ($\bar{x} = 8.3\%$), and lower derived probability of local extinction ($\bar{x} = 21.0\%$). Estimates of colonization can be interpreted as an indirect measure of dispersal, however both colonization and local extinction can be confounded by intermittent breeding (Hossack and Corn 2007). Higher colonization and occupancy rates at the stream-level scale may be a result of easier movement among sites due to proximity and shorter travel distances, or lower colonization and occupancy may have been a result of a higher number of unsuitable sites being surveyed at the range-wide scale. The estimates of colonization and local extinction should be interpreted cautiously, because the duration of my data collection was 3 years, resulting in only 2 estimable periods for both

extinction and colonization and as previously mentioned these measures are subject to fluctuations related to other biological processes.

Based on my modeling at 2 spatial scales, occupancy was best explained by mixed forest wetlands (Figure 3.3 and 3.5). This habitat type is informative at these scales, but provides little in the way of management recommendations. At lower spatial scales (e.g., site-level conditions) I documented that *R. okaloosae* select for areas along streams that have abundant amounts of emergent vegetation (Chapter 2). Similarly, at the microhabitat scale I documented that male *R. okaloosae* select for areas that have greater amounts of cover, including emergent vegetation, submergent vegetation, and woody debris (Chapter 1). At these lower spatial scales the conditions within the wetlands suggest that fire may be an important driver to maintain high quality conditions for successful breeding to occur.

Data related to the dispersal capabilities of *Rana okaloosae* are not available; however one long distance movement has been documented that involved a marked individual moving 130 m from one wetland to an adjacent wetland (Gorman et al. 2009). Despite this one long distance observation, *R. okaloosae* tend to have very limited movements (i.e., mean minimum daily distance moved = 1.8 m and maximum daily distance moved = 8.9 m) (Gorman et al. 2009). Therefore, adult dispersal may be a major limitation on the species ability to colonize new sites and this was reflected in the very low estimates of colonization. It is likely that juvenile individuals disperse most frequently as with other species of *Rana* (e.g., Martof 1953, Schroeder 1976), but because *R. okaloosae* occur predominantly along streams tadpoles may also disperse (see Kraaijeveld-Smit et al. 2005). Conversely local extinction estimates were moderately high at both spatial scales. At the range-level scale, 3 major river systems are still occupied, which reflects a similar pattern to when the species was first discovered in 1982

(Moler 1985). However one of these populations (Titi Creek, a tributary of the Shoal River) has become very isolated and despite intensive surveying at both spatial scales we only documented occupancy at 1 site 1 time during the 3 year period. This portion of the species' geographic range has become so isolated that the closest occupied patch occurs on Malone Creek (a tributary of the Yellow River), which is ~37 straight-line km away. For comparison the next most isolated site is only ~4 straight-line km away from another occupied site.

Recently, the importance of incorporating estimates of detection into estimates of population parameters has been highlighted by many authors (e.g., Mackenzie et al. 2002, 2003, 2006, Bailey et al. 2004, Mazerolle et al. 2005, Mazerolle et al. 2007). My estimates of detection for both spatial scales were moderately high ($\bar{x} > 60\%$) compared to those for other species of more commonly occurring anurans (Weir et al. 2005), suggesting that my survey effort was reasonable. As detection rates decrease more effort is needed to ensure that sites are properly categorized as occupied or not, but even at 60%, inclusion of detection as a correction for estimates of occupancy and colonization are important because by accounting for detection probabilities, estimates of population parameters are less biased (Mazerolle et al. 2007).

CONCLUSIONS

At two spatial scales I determined that *R. okaloosae* are patchily distributed on the landscape. *Rana okaloosae* appeared to have poor colonization ability and I estimated that there was a high likelihood that many sites were at risk of localized extinction. At the geographic range scale, *R. okaloosae* occur in what appear to be 3 populations. Two of these populations are within a conceivable distance for dispersal to occur, however the third appears to be isolated and at risk of localized extinction. Across all years I found that at the stream-level scale *R. okaloosae* were less patchily distributed along the length of my transects than at the range-wide

scale; however, they were still not uniformly distributed on an annual basis with some sites only having detections in 1 of 3 years despite high detection rates. My results suggest that protection of habitats where *R. okaloosae* occur is warranted because of the high estimates of local extinction and low estimates of colonization. Further, many of the habitats where this species occurs are not widely distributed and habitats that make up small mixed forest wetlands (e.g., seeps and headwater streams) are not formally protected in the same manner as larger riverine systems (Corn et al. 2003). Additionally, we have a limited understanding of how *R. okaloosae* interact with upland habitat, but many anuran species use terrestrial habitat as dispersal routes and connectivity is vital to sustain metapopulations of amphibians (Semlitsch 2002). Future research should focus on the importance of terrestrial habitats for *R. okaloosae*. Further, because dispersal is critical for maintaining local populations within a metapopulation framework, future research should be directed towards understanding dispersal of *R. okaloosae*, including studies of tadpole movements and the feasibility of using translocations to improve colonization of isolated sites. Translocations should be used cautiously as this approach has potential drawbacks, such as disease transmission and low probability of success. Therefore, translocations only should be used as an experimental approach (Seigel and Dodd 2002). Further, known limiting conditions that caused declines or extirpations should be remedied prior to implementing any translocation effort.

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Table 3.1. Occupancy modeling results including AIC, change in AIC (ΔAIC), model weight (w_i), model likelihood, and the number of parameters (k) for *Rana okaloosae* based on range-wide call surveys at 237 sites on Eglin Air Force Base, 2006-2008 (ψ = probability of occupancy, γ = probability of colonization, p = probability of detection). Covariates in parentheses are mfw = mixed forest wetlands, upland = upland habitat, othwet = other wetlands, flat = flatwoods habitat, fire = year since last fire, rh = relative humidity, temp = temperature, sky = sky condition, and “.” = constant.

Model	AIC	ΔAIC	w_i	Likelihood	k
ψ (mfw), γ (.), p (.)	741.87	0.00	0.51	1.00	4
ψ (upland), γ (.), p (.)	744.10	2.23	0.17	0.33	4
ψ (mfw), γ (fire), p (sky)	745.22	3.35	0.10	0.19	6
ψ (mfw), γ (fire), p (year)	745.43	3.56	0.09	0.17	7
ψ (mfw), γ (fire), p (rh+temp)	746.74	4.87	0.05	0.09	7
ψ (upland), γ (fire), p (sky)	747.46	5.59	0.03	0.06	6
ψ (upland), γ (fire), p (year)	747.67	5.80	0.03	0.06	7
ψ (upland), γ (fire), p (rh+temp)	749.29	7.42	0.01	0.02	7
ψ (flat), γ (.), p (.)	751.65	9.78	0.00	0.01	4
ψ (othwet), γ (.), p (.)	751.72	9.85	0.00	0.01	4
ψ (fire), γ (fire), p (year)	752.89	11.02	0.00	0.00	7
ψ (year), γ (.), p (.)	753.63	11.76	0.00	0.00	5
ψ (fire), γ (fire), p (sky)	754.20	12.33	0.00	0.00	6
ψ (fire), γ (.), p (.)	754.26	12.39	0.00	0.00	4
ψ (flat), γ (fire), p (sky)	755.01	13.14	0.00	0.00	6

ψ (othwet), γ (fire), p (sky)	755.08	13.21	0.00	0.00	6
ψ (flat), γ (fire), p (year)	755.10	13.23	0.00	0.00	7
ψ (othwet), γ (fire), p (year)	755.14	13.27	0.00	0.00	7
ψ (falt), γ (fire), p (rh+temp)	756.74	14.87	0.00	0.00	7
ψ (year), γ (fire), p (year)	756.76	14.89	0.00	0.00	8
ψ (othwet), γ (fire), p (rh+temp)	756.78	14.91	0.00	0.00	7
ψ (year), γ (fire), p (sky)	756.97	15.10	0.00	0.00	7
ψ (year), γ (fire), p (rh+temp)	758.58	16.71	0.00	0.00	8
ψ (fire), γ (fire), p (rh+temp)	764.55	22.68	0.00	0.00	7

Table 3.2. Parameter estimates and standard errors (SE) for variables that best described occupancy (ψ), colonization (γ), and detection (p) at the range-wide scale of *Rana okaloosae* on Eglin Air Force Base, 2006-2008.

Parameter	Estimate	SE
ψ (Intercept)	-1.7247	0.1634
ψ (Mixed forest wetland)	0.4558	0.1450
γ (Intercept)	-2.8806	0.2114
p (Intercept)	0.4161	0.1477

Table 3.3. Occupancy modeling results including AIC, change in AIC (ΔAIC), model weight (w_i), model likelihood, and the number of parameters (k) for *Rana okaloosae* based on stream-level call surveys at 154 sites on Eglin Air Force Base, 2006-2008 (ψ = probability of occupancy, γ = probability of colonization, p = probability of detection). Covariates in parentheses are mfw = mixed forest wetlands, upland = upland habitat, othwet = other wetlands, flat = flatwoods habitat, sin = sinuosity, fire = year since last fire, rh = relative humidity, temp = temperature, sky = sky condition, and “.” = constant.

Model	AIC	ΔAIC	w_i	Likelihood	k
ψ (mfw), γ (fire), p (rh+temp)	929.06	0.00	0.78	1.00	7
ψ (upland), γ (fire), p (rh+temp)	932.87	3.81	0.12	0.15	7
ψ (fire), γ (fire), p (rh+temp)	934.86	5.80	0.04	0.06	7
ψ (mfw), γ (fire), p (sky)	935.74	6.68	0.03	0.04	6
ψ (mfw), γ (fire), p (year)	937.34	8.28	0.01	0.02	7
ψ (sin), γ (fire), p (rh+temp)	939.41	10.35	0.00	0.01	7
ψ (upland), γ (fire), p (sky)	939.87	10.81	0.00	0.00	6
ψ (fire), γ (fire), p (sky)	941.01	11.95	0.00	0.00	6
ψ (upland), γ (fire), p (year)	941.53	12.47	0.00	0.00	7
ψ (othwet), γ (fire), p (rh+temp)	941.83	12.77	0.00	0.00	7
ψ (flat), γ (fire), p (rh+temp)	942.57	13.51	0.00	0.00	7
ψ (fire), γ (fire), p (year)	942.80	13.74	0.00	0.00	7
ψ (mfw), γ (.), p (.)	943.14	14.08	0.00	0.00	4
ψ (sin), γ (fire), p (sky)	945.47	16.41	0.00	0.00	6
ψ (upland), γ (.), p (.)	946.40	17.34	0.00	0.00	4

ψ (sin), γ (fire), p (year)	947.38	18.32	0.00	0.00	7
ψ (othwet), γ (fire), p (sky)	947.90	18.84	0.00	0.00	6
ψ (year), γ (fire), p (rh+temp)	948.52	19.46	0.00	0.00	8
ψ (flat), γ (fire), p (sky)	948.70	19.64	0.00	0.00	6
ψ (fire), γ (.), p (.)	948.83	19.77	0.00	0.00	4
ψ (othwet), γ (fire), p (year)	949.69	20.63	0.00	0.00	7
ψ (flat), γ (fire), p (year)	950.48	21.42	0.00	0.00	7
ψ (year), γ (fire), p (sky)	950.63	21.57	0.00	0.00	7
ψ (sin), γ (.), p (.)	951.14	22.08	0.00	0.00	4
ψ (othwet), γ (.), p (.)	954.01	24.95	0.00	0.00	4
ψ (year), γ (.), p (.)	954.29	25.23	0.00	0.00	5
ψ (flat), γ (.), p (.)	954.75	25.69	0.00	0.00	4
ψ (year), γ (fire), p (year)	959.34	30.28	0.00	0.00	8

Table 3.4. Parameter estimates and standard errors (SE) for variables that best described occupancy (ψ), colonization (γ), and detection (p) at the stream-level scale for *Rana okaloosae* on Eglin Air Force Base, 2006-2008.

Parameter	Estimate	SE
ψ (Intercept)	-0.7976	0.1602
ψ (Mixed forest wetlands)	0.5680	0.1645
γ (Intercept)	-3.7662	0.9021
γ (Years since last fire)	-4.0039	2.0717
p (Intercept)	0.5154	0.1209
p (Temperature)	0.1387	0.1027
p (Relative humidity)	-0.3724	0.1309

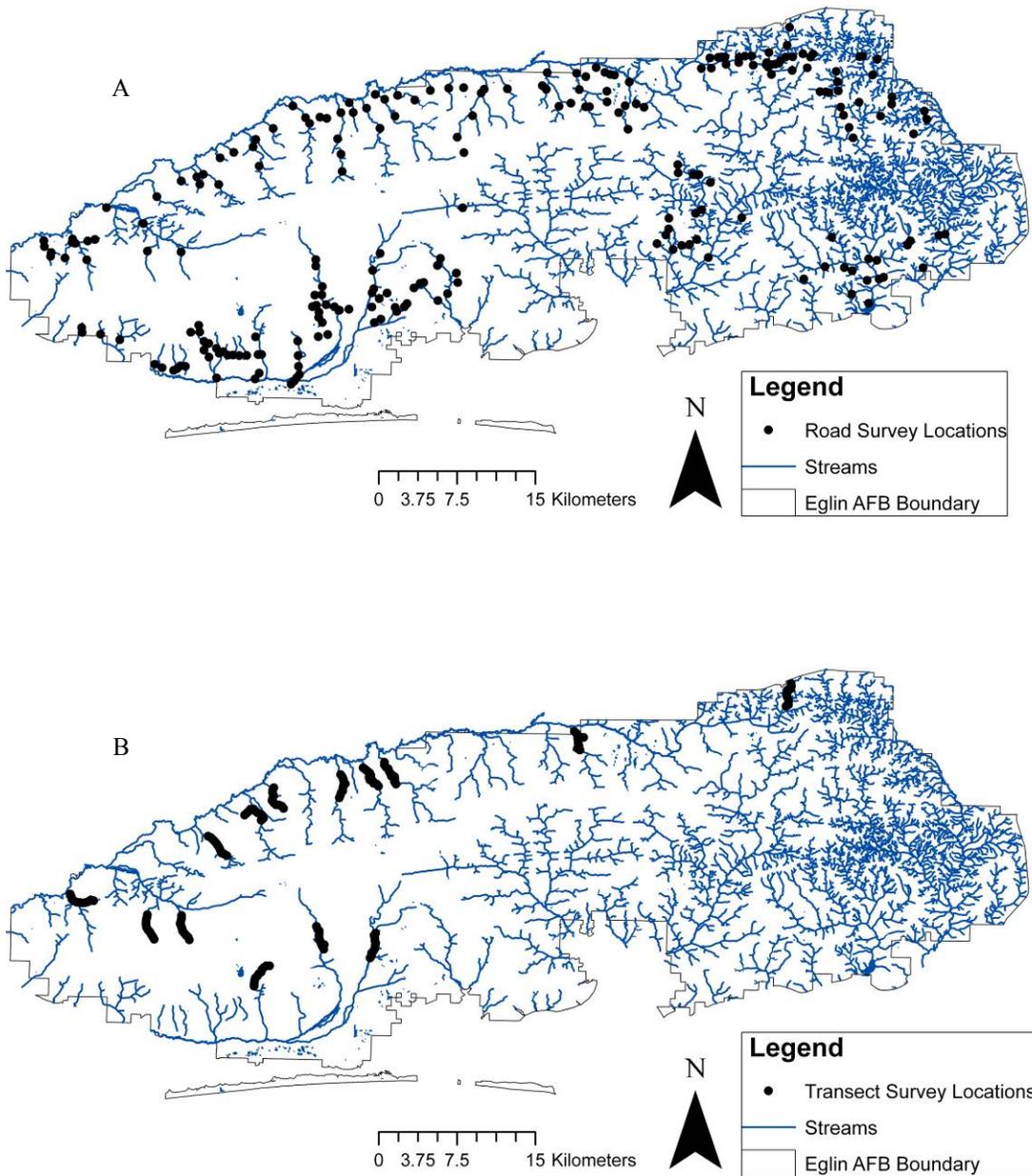


Figure 3.1. Study area in northwestern Florida, Eglin Air Force Base, where surveys of *R. okaloosae* were conducted from 2006-2008, (A) road survey locations and (B) walking transect survey locations.

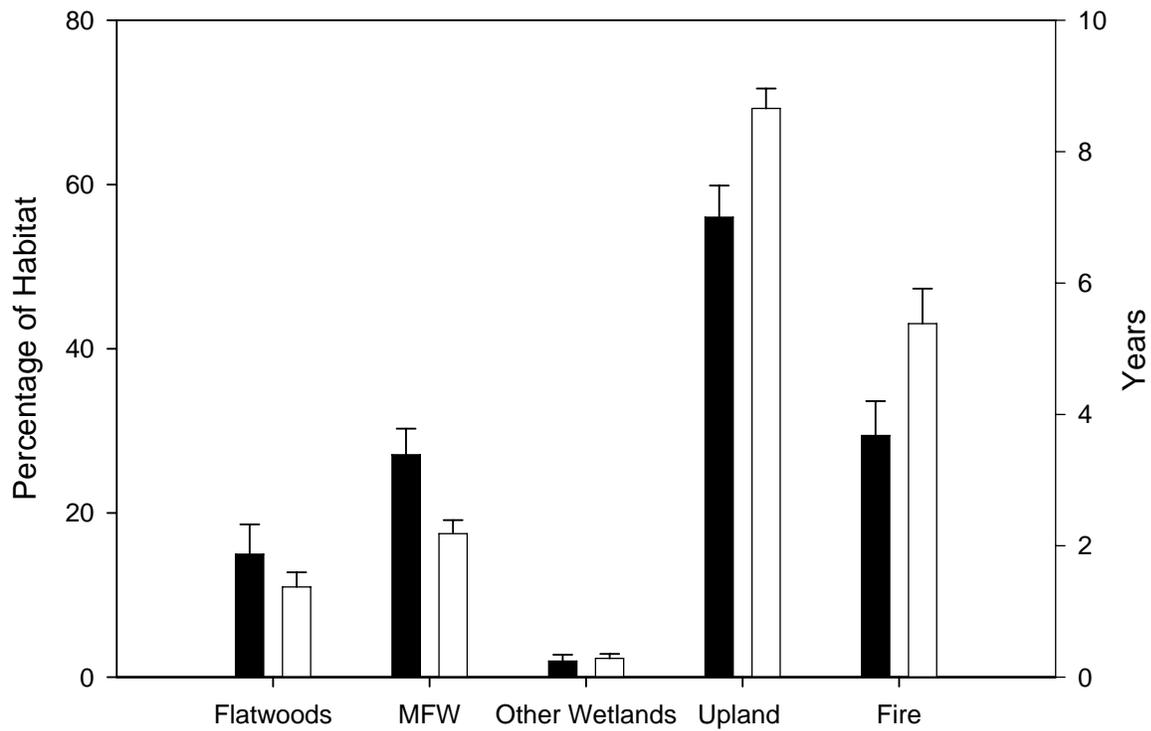


Figure 3.2. Mean percentage of cover types and mean years since last fire within 125 m buffers surrounding range-wide survey locations on Eglin Air Force Base, Florida, where road surveys of *Rana okaloosae* were conducted from 2006-2008 (black bars denote ≥ 1 detection; white bars denote no detections).

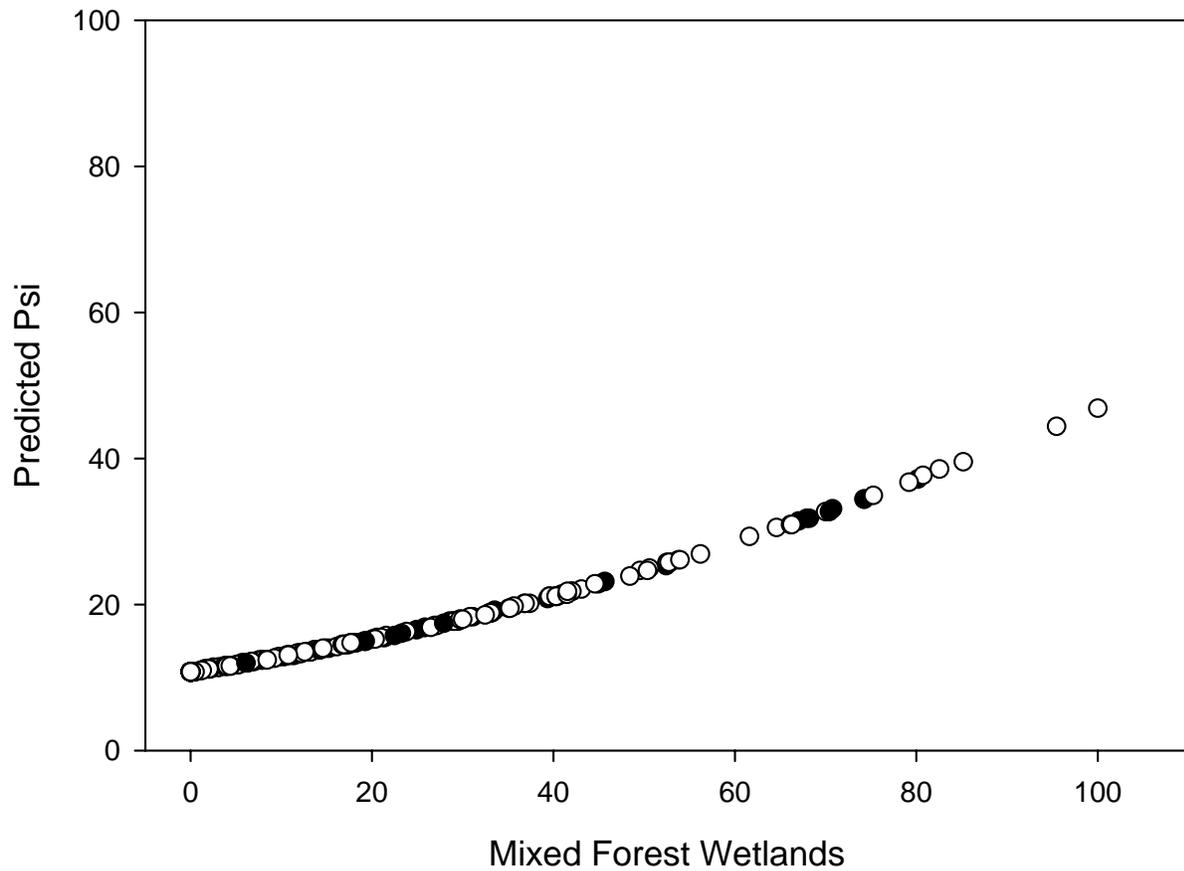


Figure 3.3. Relationship between the predicted values of ψ (occupancy) and percentage of mixed forest wetlands from the range-wide scale surveys for *Rana okaloosae* on Eglin Air Force Base, 2006-2008 (open circles denote sites with no detection and closed circles denote sites with at least 1 detection).

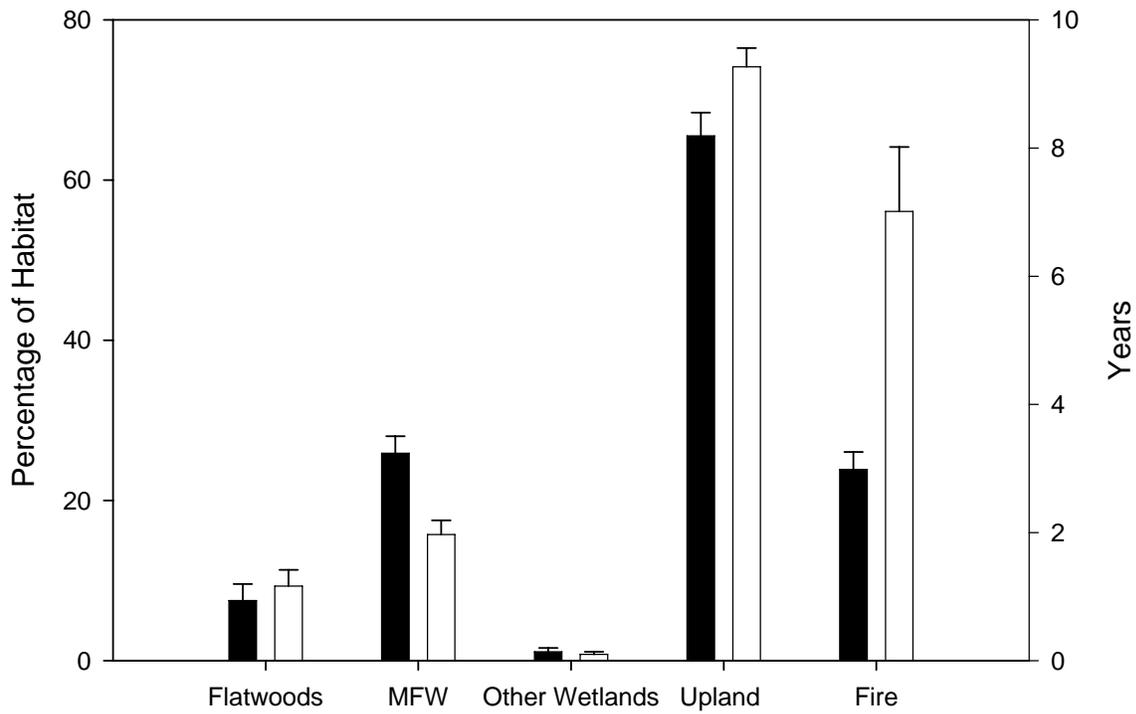


Figure 3.4. Mean percentage of cover types and mean years since last fire within 125 m buffers surrounding stream-level survey locations on Eglin Air Force Base, Florida, where transect surveys of *Rana okaloosae* were conducted from 2006-2008 (black bars denote detection ≥ 1 ; white bars denote no detections).

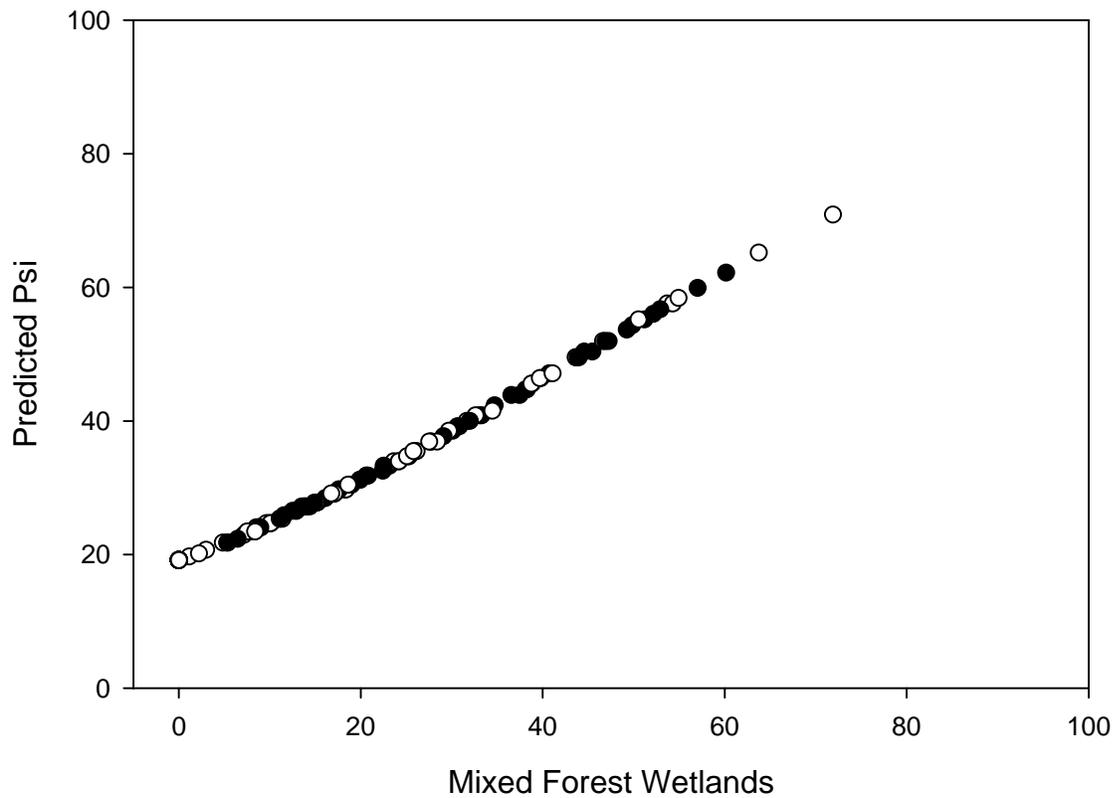


Figure 3.5. Relationship between the predicted values of ψ (occupancy) and percentage of mixed forest wetlands from the top model from the stream-level scale surveys for *Rana okaloosae* on Eglin Air Force Base, 2006-2008 (open circles denote sites with no detection and closed circles denote sites with at least 1 detection).

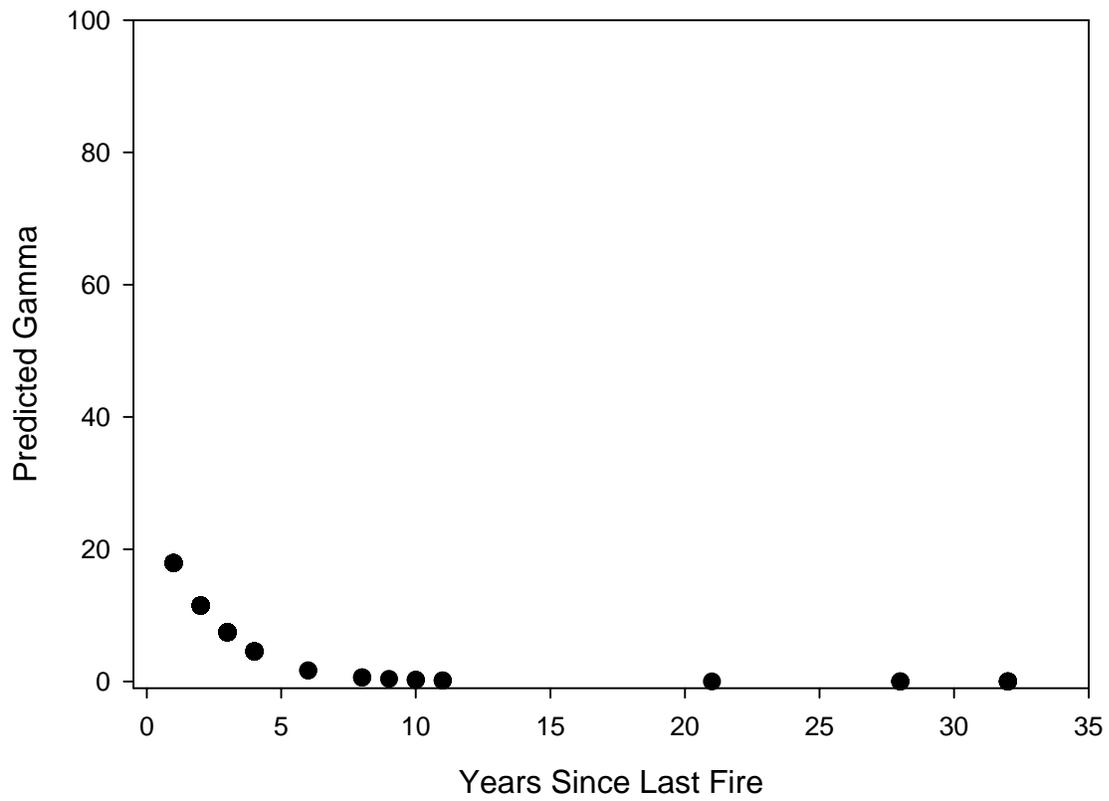


Figure 3.6. Relationship between the predicted values of γ (colonization) and years since last fire from the top model from stream-level scale surveys for *Rana okaloosae* on Eglin Air Force Base, 2006-2008.

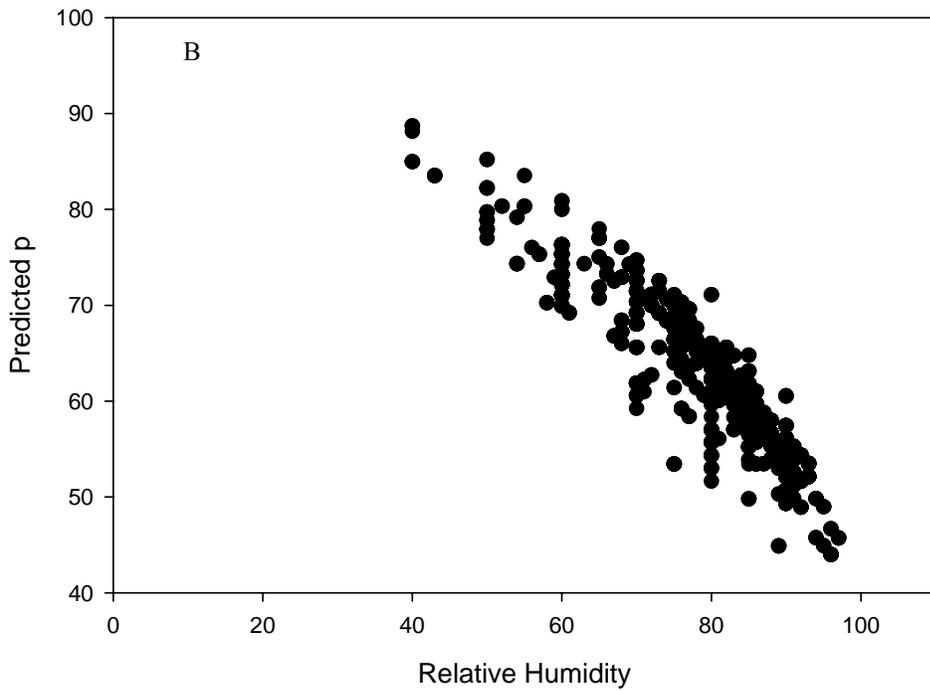
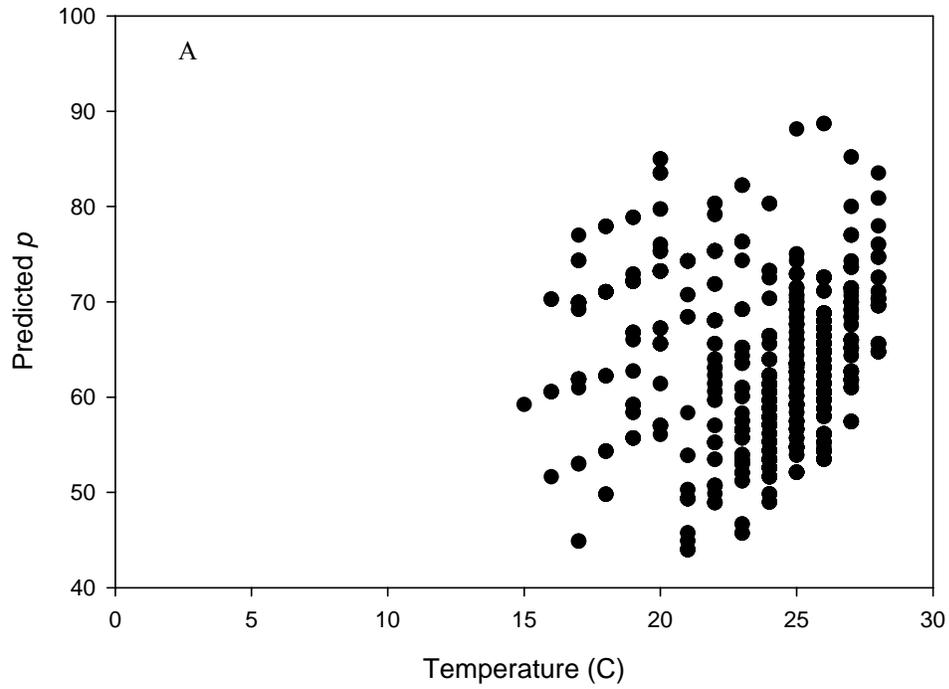


Figure 3.7. Relationship between the predicted values of (A) p (detection) and temperature and (B) p and relative humidity from the top model from stream-level scale surveys for *Rana okaloosae* on Eglin Air Force Base, 2006-2008.

CHAPTER 4

Tadpole competition: the influence of a common tadpole on the growth of a rare congener

Abstract: Competition is an ecological process that may play a role in the structuring of communities. Understanding the role of competition on rare species may be particularly important for amphibian conservation, because amphibians are experiencing global declines. I experimentally evaluated the effects of *Rana clamitans clamitans* (Bronze Frog) tadpoles, a common relatively widespread species, on *Rana okaloosae* (Florida Bog Frog) tadpoles, a rare species with a geographic range that only encompasses small tributary streams of 3 rivers in northwestern Florida. The two species are sympatric, have overlapping breeding seasons, and are known to hybridize. In 2007, I established an experimental design of 4 treatments with 3 replicates and in 2008, I established an experimental design of 5 treatments with 4 replicates and in both years I reared tadpoles in a laboratory setting. My results suggest that there was limited influence of *Rana c. clamitans* on *Rana okaloosae*; because in only one of six comparisons among mixed treatments (i.e., 4 *R. c. clamitans* with 8 *R. okaloosae*) was *R. okaloosae* growth in the treatment lower than single species *R. okaloosae* treatment. Conversely, it appeared that *Rana c. clamitans* was more susceptible to intraspecific competition than interspecific competition, because growth in total length in 2007 and body length in 2008 were higher for mixed species treatments than pure treatments. The lack of a strong competitive effect of *Rana c. clamitans* on *Rana okaloosae* suggests that competitive interactions may have a limited effect at the densities I examined. Therefore, given other threats facing *R. okaloosae*, such as drought, habitat succession, and hybridization, competition between tadpoles may not be a leading

population-level driver of rarity. However, negative interactions with more common anurans may increase as resources become more limited.

Key Words: Bronze Frogs, competition, Florida Bog Frogs, *Rana c. clamitans*, *R. okaloosae*

INTRODUCTION

Competition is an ecological concept that has received significant attention in the past several decades (Alford 1999, Morin 1999). Interspecific competition can be manifested through several mechanisms and results in negative consequences for both species involved in the interaction (Morin 1999). Mechanisms of interspecific competition were split into 6 categories by Schoener (1983) to account for most forms of competition. For the purposes of this study I will elucidate the role of consumptive competition (Schoener 1983), between a rare species and a more common congener, where *consumptive competition* is defined as the greater use of a shared resource by one species, thus inhibiting the other species' fitness (Schoener 1983). Resources are often used asymmetrically, which can result in the coexistence of competitors (Morin and Johnson 1988). The concept of asymmetric competition has been supported experimentally and occurs often between species (Connell 1983, Schoener 1983), meaning that one species has greater per capita influence on intraspecific or interspecific competition (Underwood 1986, Morin and Johnson 1988).

Much attention has focused on the significance of competition from invasive species and the detrimental role they cause to ecosystems and rare species, which has been cited as a major conservation concern (see Gurevitch and Padilla 2004). Understanding the consequences of competition with a common species on a rare congener may be important for appropriate

conservation and management of rare species (Abramsky et al. 2005). Competition is rarely purported to be a direct cause of rarity (Rabinowitz 1981), however competition in concert with other factors may limit distributions and be cause for concern in already rare species. For example, Griffis and Jaeger (1998) reported on a long term study of a population of *Plethodon shenandoah*, a rare, geographically isolated salamander and its primary interspecific competitor, *P. cinereus*, a widespread congener. Their data suggested that *P. cinereus* outcompeted *P. shenandoah* through territoriality and that competitive exclusion was operating on their study site. Further, Jaeger (1980), working on the same two species, suggested that a population of *P. shenandoah* went extinct as the result of a combination of biological (i.e., competition with *P. cinereus*) and physical factors (i.e., drought).

Rana okaloosae (Florida Bog Frog) is a rare endemic that has a restricted geographic range limited to portions of the panhandle of Florida (Moler 1985, Bishop 2005, Chapter 3). The entire known range of *R. okaloosae* encompasses small tributary streams of three rivers in Walton, Okaloosa, and Santa Rosa counties, Florida (Moler 1993, Bishop 2004). Further, almost all known localities occur within the boundaries of Eglin Air Force Base. *Rana clamitans clamitans* (Bronze Frog) is a widely distributed species that occurs throughout most of the southeastern U.S., and is generally detected at sites occupied by *R. okaloosae*. *Rana c. clamitans* are close relatives of *R. okaloosae* (Austin et al. 2003) and are potentially their principal interspecific competitor. *Rana okaloosae* and *R. c. clamitans* are both considered prolonged breeders with breeding taking place from April through August and both species may overwinter as tadpoles (Moler 1985, Martof 1956). *Rana okaloosae* clutches range from approximately 150-350 eggs (Bishop 2005) and *R. c. clamitans* have clutches that range from 1000-7000 eggs (Wells 1976). Both species have similar breeding strategies and hybrids between the species

have been reported (Moler 1993, Bishop 2005). Observational research on adult male *R. okaloosae* and *R. c. clamitans* during the breeding season did not reveal evidence of strong negative interactions between the species (Gorman et al. 2009). However, amphibians have complex biphasic life histories that may allow for negative interactions to occur at either larval or adult stage.

The goal of this experiment was to evaluate the influence of interspecific and intraspecific competition on the growth of *R. okaloosae* and *R. c. clamitans*. Growth of larval amphibians is considered an important metric that relates directly to individual survival and reproduction and thus fitness (Wilbur and Collins 1973, Berven 1990). My goal was to understand how these species interact in the tadpole stage of their life cycle. Therefore, I assessed changes in body length, total length, and survival in response to 5 different experimental treatments. To examine intraspecific and interspecific competition I compared the growth of both species in mixed species treatments to treatments with each species alone using a replacement series (while keeping the overall treatment density constant).

METHODS

Study Design

Experiment 1.--I established an experimental design of 4 treatments with 3 replicates to examine the influence of *R. c. clamitans* on the growth of *R. okaloosae*. The design was a replacement series and the 4 treatments were (A) 2 *R. okaloosae* tadpoles with 6 *R. c. clamitans* tadpoles, (B) 6 *R. okaloosae* tadpoles with 2 *R. c. clamitans* tadpoles, (C) 8 *R. okaloosae* tadpoles and, (D) 8 *R. c. clamitans* tadpoles (Table 4.1). A total of 48 *R. okaloosae* and 48 *R. c. clamitans* tadpoles were included in this experiment.

I collected one *R. okaloosae* egg mass and one *R. c. clamitans* egg mass on 28 May 2007 and I reared each egg mass separately until both egg masses were fully hatched on 30 May 2007. I then moved 100 tadpoles of each species into 2 separate aquaria until the start of the experiment. On 1 July 2007 I randomly assigned tadpoles to a treatment and aquaria using 12, 38 L aquaria filled with 7.5 L of water. Aquaria were housed in an outdoor open-air screened building and treatments were randomly assigned to each of the aquaria in the laboratory that were oriented in a 6x2 design. I used water from a stream where both species of frogs occur and I changed the water on a weekly basis. Additionally, each aquarium was equipped with an aerator. I measured total length (i.e., snout to end of tail) of each tadpole using a dial caliper (Swiss Precision Instruments, Inc.) upon initial entry into the aquaria/treatment (week 0) and each week of the experiment to week 4. Each aquarium was provided with 2 g of frozen spinach (thawed) each week.

Experiment 2.--I established an additional experimental design of 5 treatments with 4 replicates to further examine the influence of *R. c. clamitans* on the growth of *R. okaloosae*. The design was a replacement series with an additional treatment for *R. okaloosae*. The 5 treatments were (1) 4 *R. okaloosae* tadpoles, (2) 4 *R. okaloosae* tadpoles with 8 *R. c. clamitans* tadpoles, (3) 8 *R. okaloosae* tadpoles with 4 *R. c. clamitans* tadpoles, (4) 12 *R. okaloosae* tadpoles, and (5) 12 *R. c. clamitans* tadpoles (Table 4.2). A total of 112 *R. okaloosae* and 96 *R. c. clamitans* tadpoles were included in this experiment.

I collected one *R. okaloosae* egg mass on 23 June 2008 and one *R. clamitans* egg mass on 24 June 2008 and I reared each egg mass separately and both egg masses were fully hatched by 25 June 2008. On 26 June 2008, I separated tadpoles into 5 aquaria of 30 tadpoles/aquaria. Finally, on 3 July 2008 I randomly assigned tadpoles to a treatment and aquaria using 20, 38 L

aquaria filled with 7.5 L of water. Aquaria were housed in an outdoor open-air screened building and treatments were randomly assigned to each of the aquaria in the laboratory that were oriented in a 10x2 design. I used water from a stream where both species of frogs occur and I changed the water on a weekly basis. Additionally, each aquarium was equipped with an aerator.

I measured the body length (i.e., snout to vent) and total length of each tadpole in hand using a dial caliper (Swiss Precision Instruments, Inc.) upon initial entry into the aquaria/treatment (week 0) and each week of the experiment to week 4. Each aquarium received 0.4 g of Sun Seed[®] rabbit food (Wilbur 1977), except treatment RO-1, which was provided with 0.13g of rabbit feed (i.e., the proportional amount of feed given the different density of treatment RO-1) for week 0 and 1. In weeks 2 and 3 the amount of feed was increased in each treatment to 0.6g of rabbit chow, except treatment RO-1 which was raised to 0.2g. The increase in feed was to reflect the increasing size of the tadpoles. Finally, I documented the species-specific mortality in each treatment.

Statistical Analyses

Experiment 1.--To examine initial differences in total lengths upon entry into the experiment between species I used a Mann-Whitney U test. I used a repeated-measures Analysis of Variance (ANOVA) on ranked data to test for differences between time periods (weeks) treatments, and the interaction of time period and treatment, because these data did not have equal variances and attempts to transform these data were unsuccessful (Conover 1999). For this analysis I used the difference between tank means from one week to the next, resulting in 4 weekly differences and used difference in total length as the dependent variable. I used Fisher's least significant difference to evaluate pair-wise differences and I only considered within species comparisons to be valid, so I did not compare growth between *R. okaloosae* and *R. c. clamitans*,

but rather evaluated the effect of the density of those species on the growth rate of the other species.

Experiment 2.--To examine initial differences in the body and total lengths upon entry into the experiment between species I used a t-test. I used a repeated-measures ANOVA to test for differences between time periods (weeks), treatments, and the interaction of time period and treatment. For this analysis I used the difference between tank means from one week to the next, resulting in 4 weekly differences and again used body length and total length as separate dependent variables. I used Fisher's least significant difference to evaluate pair-wise differences and I only considered within species comparisons to be valid, so I did not compare growth between *R. okaloosae* and *R. c. clamitans*, but rather evaluated the effect of the density of those species on the growth rate of the other species. I used a Modified Levene's test to evaluate the homogeneity of variances and normal probability plots to evaluate normality. For all analyses I used SAS 9.2 and I set alpha equal to 0.10.

RESULTS

Experiment 1

Initial total length of *R. okaloosae* ($\bar{x} = 19.57$ mm, SE = 0.17) was greater than *R. c. clamitans* ($\bar{x} = 15.99$ mm, SE = 0.22, $Z = -7.78$, $df = 94$, $P < 0.001$). Overall, the mortality rate was relatively low in all treatments over the entire length of the experiment. There were no deaths in the treatment RC-B, but otherwise individuals of both species that were in mixed species treatments experienced the highest mortality rate (Table 4.1).

Experiment 1: R. okaloosae

For *R. okaloosae* I detected a difference with the omnibus repeated-measures ANOVA on the ranks of total length ($F_{11, 24} = 3.99$; $P = 0.002$) (Table 4.3). I did not detect a significant

interaction between time period and treatment ($F_{6, 11} = 0.60$; $P = 0.724$). Further, I did not detect a difference between any of the treatments on total length ($F_{2, 6} = 1.60$; $P = 0.223$) (Figure 4.1). I did detect a difference between time periods ($F_{3, 6} = 12.36$; $P < 0.001$). Growth increased over time across all treatments and time period 1 was > 2 ($P < 0.001$), and 4 ($P = 0.005$), but not time period 3 ($P = 0.236$). Time period 2 was < 3 ($P < 0.001$) and 4 ($P = 0.016$) and time periods 3 and 4 were different ($P = 0.069$).

Experiment 1: R. c. clamitans

For *R. c. clamitans* I detected a difference using the omnibus repeated-measures ANOVA on the ranks of total length ($F_{11, 24} = 5.08$; $P < 0.001$) (Table 4.3). I did not detect a significant interaction between time period and treatment ($F_{6, 11} = 1.81$; $P = 0.139$), but both treatment ($F_{2, 6} = 2.96$; $P = 0.071$) and period ($F_{3, 6} = 13.01$; $P < 0.001$) were significant. *Rana c. clamitans* growth, measured by total length, was higher in the mixed species treatment RC-B, 2 *R. clamitans* and 6 *R. okaloosae*, than in the pure *R. c. clamitans* treatment (treatment RC-D) ($P = 0.025$), but there were no other differences among treatments (Figure 4.1). Growth increased over time across all treatments and time period 1 was $>$ time period 2 ($P < 0.001$), but not 3 ($P = 0.262$), or 4 ($P = 0.236$). Time period 2 $<$ 3 ($P < 0.001$) and 4 ($P < 0.001$), but period 3 was not different than period 4 ($P = 0.947$).

Experiment 2

I examined the assumption of normality with normal probability plots for both body length and total length of *R. okaloosae* and both datasets met this assumption. My test of homoscedasticity using a Modified Levene's Test was not significant for body length or total length ($P = 0.465, 0.458$, respectively), so both repeated-measures datasets for *R. okaloosae* met this assumption. Further, I examined the assumption of normality with normal probability plots for

both body length and total length of *R. c. clamitans* and both datasets met this assumption. My test of homoscedasticity using a Modified Levene's Test was not significant for body length ($P = 0.149$), but was significant for total length ($P = 0.039$), so I log transformed the total length data for *R. c. clamitans* to meet the assumption of homoscedasticity ($P > 0.05$).

Initial body length of *R. c. clamitans* ($\bar{x} = 5.22$ mm, SE = 0.06) was greater than *R. okaloosae* ($\bar{x} = 4.83$ mm, SE = 0.06, $t = 4.71$, df = 206, $P < 0.0001$). Likewise, initial total length of *R. c. clamitans* ($\bar{x} = 13.77$ mm, SE = 0.07) was greater than *R. okaloosae* ($\bar{x} = 13.40$ mm, SE = 0.10, $t = 2.86$, df = 206, $P = 0.008$). Overall, survival was high in all treatments over the entire length of the experiment. There were no deaths in the treatments RO-1, RO-2, or RC-3. There was only 1 death in treatment RC-2 and in other treatments there were 2 deaths of each species (Table 4.2). The highest rate of mortality was 2 out of 32 *R. okaloosae* tadpoles in replicates of treatment 3 (a mixed-species treatment of 8 *R. okaloosae* and 4 *R. c. clamitans*) (Table 4.2).

Experiment 2: R. okaloosae

For *R. okaloosae* I detected a difference with the omnibus repeated-measures ANOVA on body length ($F_{15, 46} = 6.29$; $P < 0.001$) (Table 4.4). I did not detect a significant interaction between time period and treatment ($F_{9, 15} = 0.87$; $P = 0.554$). Further, I did not detect a difference between any of the treatments on body length ($F_{3, 9} = 1.48$; $P = 0.232$) (Figure 4.2). I did detect a difference between time periods ($F_{3, 9} = 40.39$; $P < 0.001$). Further, overall growth increased over time (Figure 4.3). Across all treatments time period 1 was $> 2, 3,$ and 4 ($P < 0.001$), time period 2 was < 3 and 4 ($P < 0.001$), but time periods 3 and 4 were not different ($P = 0.943$).

I detected a difference using the omnibus repeated-measures ANOVA on total length ($F_{15, 46} = 9.33$; $P < 0.001$) (Table 4.4) for *R. okaloosae*. I did not detect a significant interaction between time period and treatment ($F_{9, 15} = 0.88$; $P = 0.550$), but both treatment ($F_{3, 9} = 4.53$; $P = 0.007$) and period ($F_{3, 9} = 40.48$; $P < 0.001$) were significant for total length. The growth rate of *R. okaloosae* measured by total length was greater for tadpoles in the low density, *R. okaloosae* control (treatment RO-1) than treatment RO-2, 4 *R. okaloosae* and 8 *R. clamitans* ($P = 0.008$), and treatment RO-3, 8 *R. okaloosae* and 4 *R. c. clamitans* ($P = 0.001$) (Figure 4.2). Further, growth was lower in the mixed species treatment RO-3, 8 *R. okaloosae* and 4 *R. c. clamitans*, than in the single species treatment with the same density (treatment RO-4) ($P = 0.070$), but I did not detect a difference between any of the other *R. okaloosae* treatments ($P > 0.10$) (Figure 4.4). Growth increased as expected over time and across all treatments time period 1 was $> 2, 3$, and 4 ($P < 0.001$), time period 2 was not different than 3 or 4 ($P = 0.626$ and 0.482 , respectively), and time period 3 was not different than 4 ($P = 0.836$).

Experiment 2: R. c. clamitans

For *R. c. clamitans* I detected a difference with the omnibus repeated-measures ANOVA on body length ($F_{11, 34} = 5.29$; $P < 0.001$) (Table 4.4). I did not detect a significant interaction between time period and treatment ($F_{6, 11} = 0.65$; $P = 0.687$), but both treatment ($F_{2, 6} = 4.55$; $P = 0.018$) and period ($F_{3, 6} = 14.37$; $P < 0.001$) were significant. *Rana c. clamitans* growth, measured by body length, was higher in the mixed treatments RC-2, 8 *R. clamitans* and 4 *R. okaloosae*, and RC-3, 4 *R. clamitans* and 8 *R. okaloosae*, than in the pure treatment (treatment RC-5) ($P = 0.080$ and $P = 0.005$, respectively), but there was no difference between treatments RC-2 and RC-3 ($P = 0.218$) (Figure 4.2). Further, growth increased over time and across all treatments time period 1 was different than time periods 2 ($P < 0.001$), 3 ($P = 0.002$), and 4 ($P <$

0.001), and period 2 was different than 3 ($P = 0.006$) and 4 ($P = 0.032$), but period 3 was not different than period 4 ($P = 0.440$) (Figure 4.5).

I detected a difference using the omnibus repeated-measures ANOVA on total length ($F_{11, 34} = 4.97$; $P < 0.001$) (Table 4.4) for *R. c. clamitans*. I did not detect a significant interaction between time period and treatment ($F_{6, 11} = 0.61$; $P = 0.717$). Further, I did not detect a difference between any of the treatments on total length ($F_{2, 6} = 2.06$; $P = 0.144$) (Figure 4.4), but I did detect a difference among periods ($F_{3, 6} = 15.36$; $P < 0.001$) for total length. Across all treatments time period 1 was different than 2, 3, and 4 ($P < 0.001$), time period 2 was not different than 3 or 4 ($P = 0.806$ and 0.251 , respectively), and time period 3 was not different than 4 ($P = 0.165$).

DISCUSSION

Rana okaloosae displayed a detectable difference in growth when comparing between those reared under interspecific and intraspecific competition in my lab experiment, but this difference occurred in only one treatment group and for one growth measurement in 2008, and there was no difference detected in 2007 (Table 4.5). The effect observed suggests that growth of *Rana okaloosae* is more negatively impacted by interspecific than intraspecific competition. *Rana c. clamitans* growth was more negatively impacted by intraspecific competition than interspecific competition (Table 4.5). For example, body lengths of *R. c. clamitans* tadpoles were 1.5 times greater when exposed to the highest level of interspecific competition than when exposed to intraspecific competition (Figure 4.1). Thus my results suggest that *R. c. clamitans* were more capable competitors than *R. okaloosae* and hence there is a trend towards asymmetric competition between these two sympatric anurans, a result that is common among anuran tadpoles (e.g., Smith et al. 2004, Downie et al. 2008). For example, Downie et al. (2008)

reported that *Leptodactylus fuscus* attained larger body sizes when collocated with equal numbers of *Engystomops pustulosus* than when they were reared under the influence of intraspecific competition. Further, Smith et al. (2004) found that *Hyla versicolor* and *R. clamitans* displayed asymmetric competition, but in their experiment *R. clamitans* were the lesser competitors.

Rana c. clamitans are known to grow to larger sizes as both tadpoles (Altig et al. 1998, Priestley et al. *In review*) and adults (Chapter 1) and this may help to explain their disproportional use of resources. However, in my study growth was not appreciably different between *R. okaloosae* and *R. c. clamitans*. Individual *R. c. clamitans* reached the greatest overall sizes, but tank means were similar when considering those treatments with densities of 1.6 tadpoles/liter. Smith et al. (2004) suggested that greater initial body size of *H. versicolor* tadpoles explained the asymmetric competition in their experiment, even though *H. versicolor* adults are smaller than *R. c. clamitans* adults. Similarly, in my study despite trying to control for biases in initial body size (i.e., egg masses were the same age, I used the weekly difference, and I randomly assigned tadpoles to treatments), the mean body and total length of *R. c. clamitans* was greater than the initial mean sizes of *R. okaloosae* in 2008. However, in the less replicated 2007 experiment *R. okaloosae* were larger initially and a similar trend in overall growth among treatments was observed (Figure 4.1 and 4.4). The larger initial size of *R. c. clamitans* in 2008 is likely attributable to the larger adult sizes attained by females, therefore allowing them to oviposit larger eggs (Castellano et al. 2004) and the larger size of *R. okaloosae* in 2007 was likely a result of holding *R. c. clamitans* tadpoles in too high a density prior to the initiation of the experiment. Contrary to the initial size hypothesis, Werner (1992) argued that activity plays a greater role in determining the outcome of interspecific competition in tadpoles, something I did

not directly address in this study, but Bishop (2005) reported that *R. c. clamitans* tadpoles were more active than *R. okaloosae* tadpoles in a study related to predation.

I may have been able to detect a greater effect of *R. c. clamitans* on *R. okaloosae* if there had been greater replication in my experimental design. I conducted a post-hoc power analysis (Faul et al. 2007) that resulted in a power ($1-\beta$) of 0.60, suggesting that my experimental design would have benefited from greater replication. An additional 2 experimental replicates/treatment would have increased my power to 0.81, a more suitable level to detect differences. However, Steidl et al. (1997) criticized the use of post-hoc power analyses suggesting that they result in a biased estimate of power, so caution should be used in interpretation of these estimates. Further, I assumed that the beginning period of larval development was likely to be the period of development where competition had the greatest influence, because this is a period of rapid growth (Alford and Harris 1988). However, over the entire larval period the effect I observed may either increase or decrease. Both species are capable of over wintering as tadpoles (Wells 1976, Moler 1985), and as several studies have shown, as tadpoles increase in size their competitive effects also increase (Morin and Johnson 1988, Alford 1999, Richter-Boix et al. 2004).

Given that this experiment was based in the laboratory it raises the question of whether competition will occur or is actually occurring in the field. Because *R. okaloosae* is a rare species with a limited geographic range, the influence of *R. c. clamitans* may be minimal in years with an abundance of resources, but in years with lower resources (e.g., prolonged droughts) competition with *R. c. clamitans* may have greater fitness consequences on *R. okaloosae* (Wiens 1977). Bardsely and Beebee (2001) documented increased effects of competition following successional changes to habitat that allowed other native species to exploit habitats once

unavailable to them. It is likely that competition between *R. okaloosae* and *R. c. clamitans* tadpoles may not have strong negative population-level consequences for *R. okaloosae*. However, combined with a density-independent threat such as drought or habitat succession or hybridization (likely an inverse density-dependent effect) the negative influence of larval competition may increase (Jaeger 1980, Griffis and Jaeger 1998).

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Table 4.1. Experimental treatments from 2007 tadpole competition study. The 4 treatments are indicated by the letter A-D and the focal species are indicated by the designators RC and RO (RC = *Rana c. clamitans* and RO = *R. okaloosae*). The table shows the number of focal animals measured and the number of tadpoles co-located with focal tadpoles in each treatment. Additionally, the percentage of overall mortality and the number of deaths that occurred among focal animals across all replicates/treatment (n = 3 replicates/treatment) over the duration of the experiment.

Species- Treatment	Focal Animals	Co-located Animals	Total	Density (tadpole/L)	% Overall Mortality (# of deaths)
RO-A	2 RO	6 RC	8	1.07	33.3 (2)
RC-A	6 RC	2 RO	8	1.07	27.8 (5)
RO-B	6 RO	2 RC	8	1.07	22.2 (4)
RC-B	2 RC	6 RO	8	1.07	0.0 (0)
RO-C	8 RO	0 RC	8	1.07	8.3 (2)
RC-D	8 RC	0 RC	8	1.07	12.5 (3)

Table 4.2. Experimental treatments from 2008 tadpole competition study. The 5 treatments are indicated by the letter A-D and the focal species are indicated by the designators RC and RO (RC = *Rana c. clamitans* and RO = *R. okaloosae*). The table shows the number of focal animals measured and the number of tadpoles co-located with focal tadpoles in each treatment. Additionally, the percentage of overall mortality and the number of deaths that occurred among focal animals across all replicates/treatment (n = 4 replicates/treatment) over the duration of the experiment.

Species- Treatment	Focal Animals	Co-located Animals	Total	Density (tadpole/L)	% Overall Mortality (# of deaths)
RO-1	4 RO	0 RC	4	0.53	0.0 (0)
RO-2	4 RO	8 RC	12	1.60	0.0 (0)
RC-2	8 RC	4 RO	12	1.60	3.1 (1)
RO-3	8 RO	4 RC	12	1.60	6.3 (2)
RC-3	4 RC	8 RO	12	1.60	0.0 (0)
RO-4	12 RO	0 RC	12	1.60	4.2 (2)
RC-5	12 RC	0 RC	12	1.60	4.2 (2)

Table 4.3. Repeated-measures analysis of variance results for an aquaria competition experiment between *Rana okaloosae* and *R. c. clamitans* tadpoles conducted in 2007 (experiment 1), where total length was used as a measure of growth.

	F-statistic	Degrees of Freedom	P-value
<u>R. okaloosae - total length</u>			
Omnibus repeated-measures	3.99	11, 24	0.002
Treatment x Time period	0.60	6, 11	0.724
Treatment	1.60	2, 6	0.223
Time period	12.36	3, 6	<0.001
<u>R. c. clamitans - total length</u>			
Omnibus repeated-measures	5.08	11, 24	<0.001
Treatment x Time period	1.81	6, 11	0.139
Treatment	2.96	2, 6	0.071
Time period	13.01	3, 6	<0.001

Table 4.4. Repeated-measures analysis of variance results for an aquaria competition experiment between *Rana okaloosae* and *R. c. clamitans* tadpoles conducted in 2008 (experiment 2), where body length and total length were used as measures of growth.

	F-statistic	Degrees of Freedom	P-value
<u>R. okaloosae - body length</u>			
Omnibus repeated-measures	6.29	15, 46	<0.001
Treatment x Time period	0.87	9, 15	0.554
Treatment	1.48	3, 9	0.232
Time period	40.39	3, 9	<0.001
<u>R. okaloosae - total length</u>			
Omnibus repeated-measures	9.33	15, 46	<0.001
Treatment x Time period	0.88	9, 15	0.55
Treatment	4.53	3, 9	0.007
Time period	40.48	3, 9	<0.001
<u>R. c. clamitans - body length</u>			
Omnibus repeated-measures	5.29	11, 34	<0.001
Treatment x Time period	0.65	6, 11	0.687
Treatment	4.55	2, 6	0.018
Time period	14.37	3, 6	<0.001
<u>R. c. clamitans - total length</u>			
Omnibus repeated-measures	4.97	11, 34	<0.001
Treatment x Time period	0.61	6, 11	0.717
Treatment	2.06	2, 6	0.144
Time period	15.36	3, 6	<0.001

Table 4.5. Summary of results from 2 separate experiments conducted in 2007 and 2008. The experiments evaluated the effect of competition on the growth rates of *Rana okaloosae* and *R. c. clamitans*.

	2007 Body Length	2007 Total Length	2008 Body Length	2008 Total Length
<i>R. okaloosae</i>				
mixed 1 vs. control	No Data	Not Significant	Not Significant	Not Significant
mixed 2 vs. control	No Data	Not Significant	Not Significant	RO-3 < RO-4
<i>R. c. clamitans</i>				
mixed 1 vs. control	No Data	Not Significant	RC-2 > RC-5	Not Significant
mixed 2 vs. control	No Data	RC-B > RC-D	RC-3 > RC-5	Not Significant

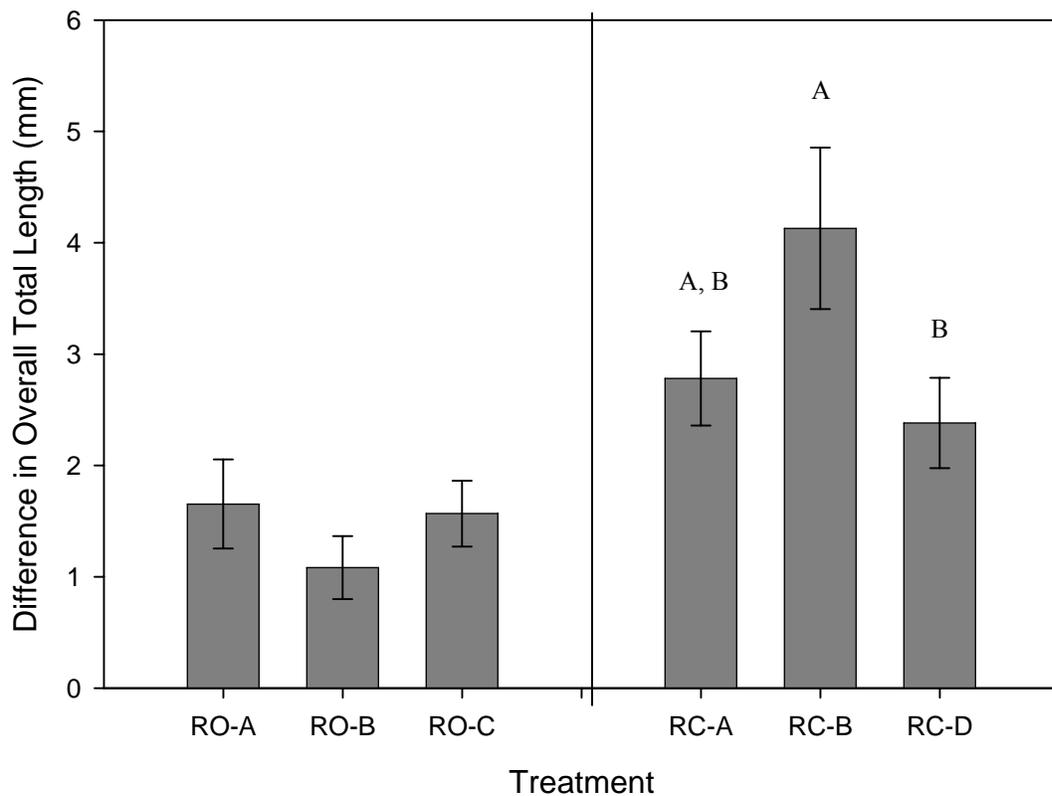


Figure 4.1. Overall growth measured by total length with 1 standard error of *Rana okaloosae* (RO) and *R. c. clamitans* (RC) tadpoles by treatment over a four week period in 2007 (RO-A = 2RO that were housed with 6RC, RO-B = 6RO that were housed with 2RC, RO-C = 8RO, RC-A = 6RC that were housed with 2RO, RC-B = 2RC that were housed with 6RO, and RC-D = 8RC). Different letters above bars indicate statistical differences among treatments ($P < 0.10$).

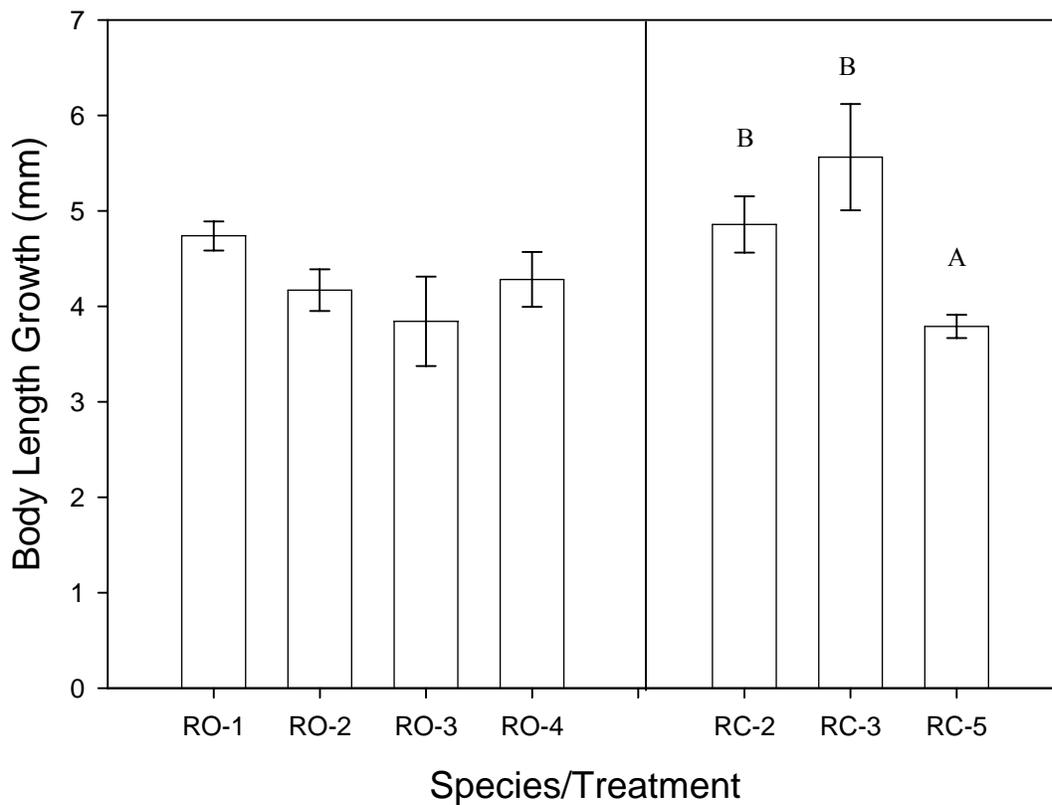


Figure 4.2. Overall growth measured by body length with 1 standard error of *Rana okaloosae* (RO) and *R. c. clamitans* (RC) tadpoles by treatment over a four week period in 2008 (RO-1 = 4RO, RO-2 = 4RO that were housed with 8RC, RO-3 = 8RO that were housed with 4RC, RO-4 = 12RO, RC-2 = 8RC that were housed with 4RO, RC-3 = 4RC that were housed with 8RO, and RC-5 = 12RC). Different letters above bars indicate statistical differences among treatments ($P < 0.10$).

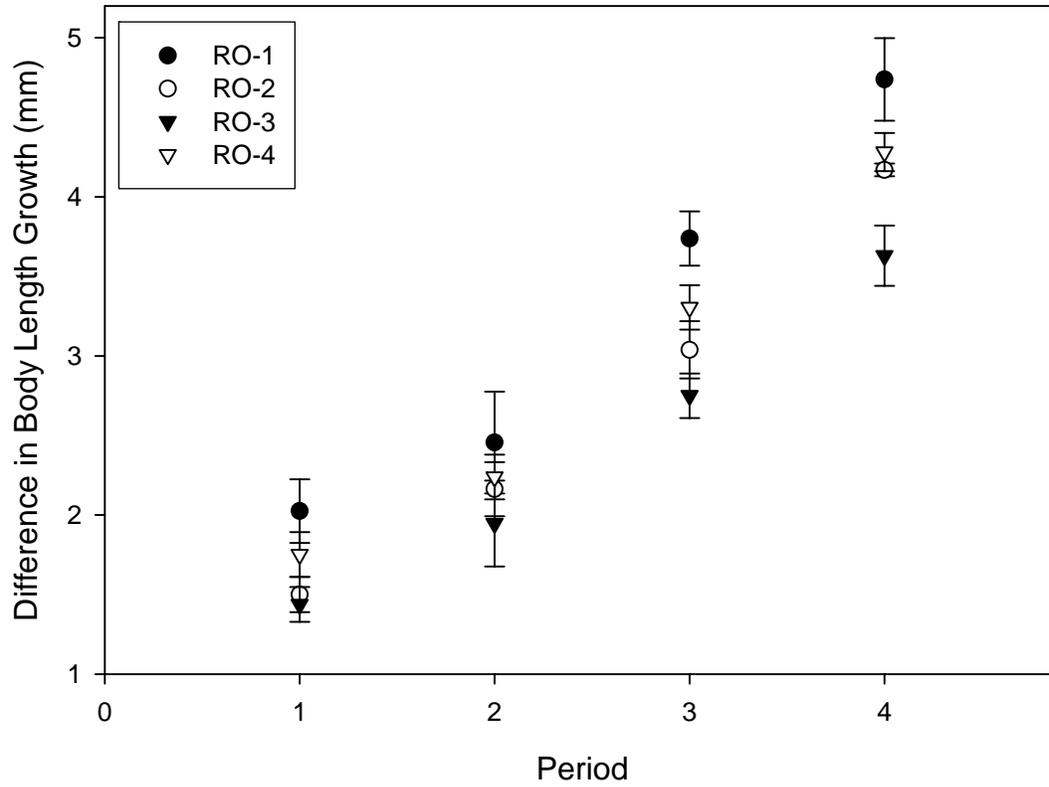


Figure 4.3. Weekly change in body length with standard error bars of *Rana okaloosae* (RO) tadpoles in four different treatments across a four-week period in 2008 (RO-1 = 4RO, RO-2 = 4RO that were housed with 8RC, RO-3 = 8RO that were housed with 4RC, RO-4 = 12RO).

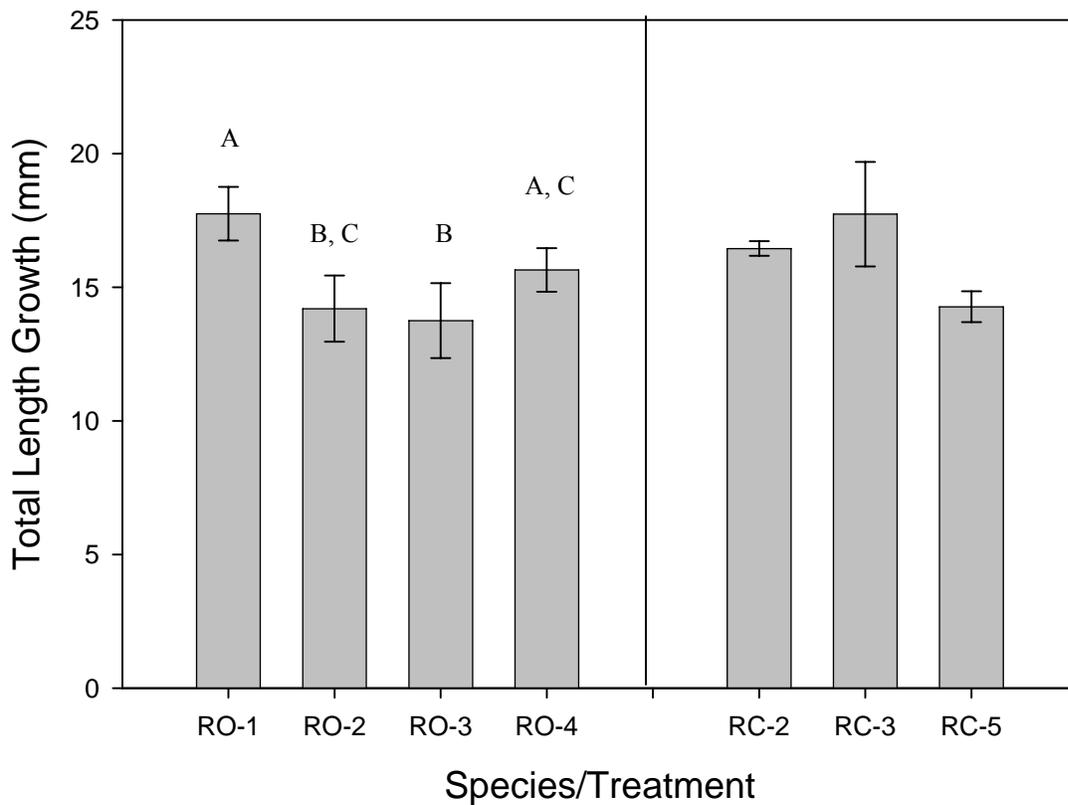


Figure 4.4. Overall growth measured by total length with 1 standard error of *Rana okaloosae* (RO) and *R. c. clamitans* (RC) tadpoles by treatment over a four week period in 2008 (RO-1 = 4RO, RO-2 = 4RO that were housed with 8RC, RO-3 = 8RO that were housed with 4RC, RO-4 = 12RO, RC-2 = 8RC that were housed with 4RO, RC-3 = 4RC that were housed with 8RO, and RC-5 = 12RC). Different letters above bars indicate statistical differences among treatments ($P < 0.10$).

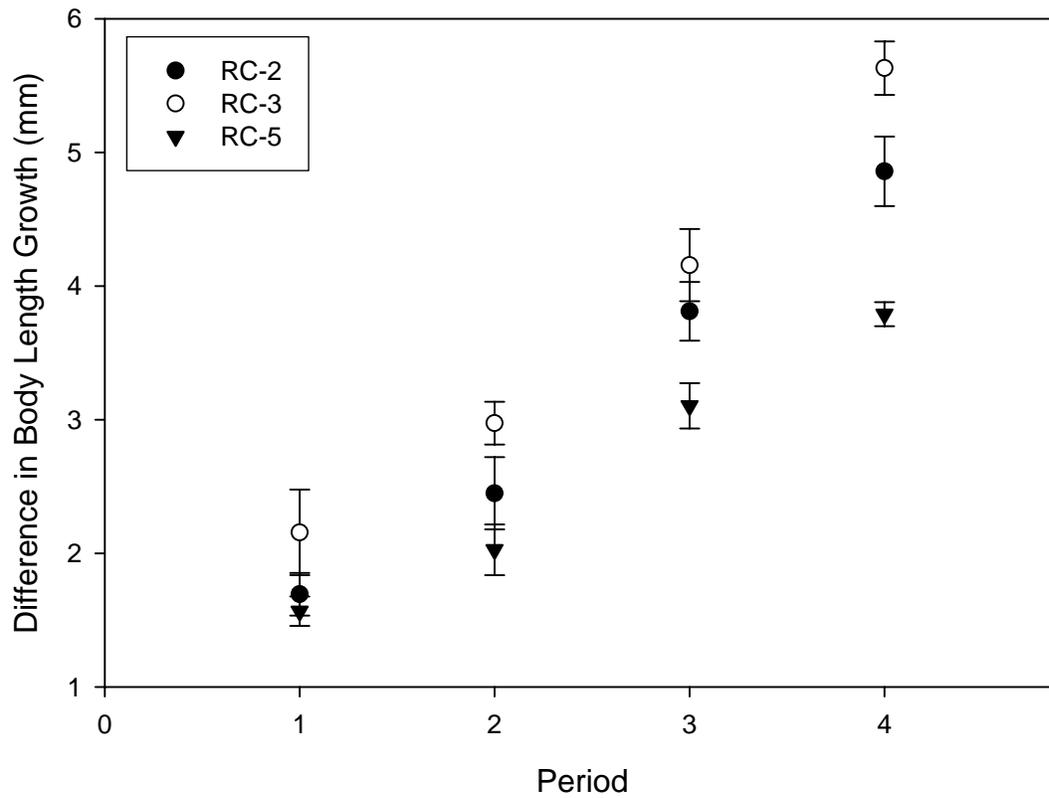


Figure 4.5. Weekly change in body length with standard error bars of *Rana c. clamitans* (RC) tadpoles exposed to three different treatments across a four-week period in 2008 (RC-2 = 8RC that were housed with 4RO, RC-3 = 4RC that were housed with 8RO, and RC-5 = 12RC).

CHAPTER 5

Factors related to occupancy of breeding wetlands by Flatwoods salamander larvae

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Abstract: The flatwoods salamander (*Ambystoma cingulatum*) was listed as federally threatened in 1999. Alteration of habitat was considered the main threat to the species, especially the loss of habitat for larval flatwoods salamanders that develop in isolated, seasonally flooded wetlands. Our objectives were to evaluate a suite of within-pool factors (i.e., vegetation structure, water level, and an index to presence of fish) that could influence occupancy of breeding wetlands by larval flatwoods salamanders on Eglin Air Force Base in Florida, USA. We dip-netted for larval salamanders from January through March 2003–2006 and we measured a suite of vegetation characteristics in 2006–2007. Further, in 2006 we measured the level of water and relative presence of fish over the salamander breeding season. Site occupancy over the 4 year period was best described by a model that incorporated high herbaceous vegetation cover and open canopy cover. Detection probability was assessed, but it varied among years and was not included in the model. Our study suggests that managing the breeding habitat of flatwoods salamander for open canopies and dense herbaceous vegetation may contribute to this species' recovery.

Key Words: *Ambystoma cingulatum*, *Ambystoma bishopi*, conservation, Florida, prescribed fire

INTRODUCTION

The flatwoods salamander (*Ambystoma cingulatum* Cope) was listed as federally threatened in April 1999 (United States Department of the Interior, Fish and Wildlife Service [USFWS] 1999). The recovery of *A. cingulatum* across their geographic range has been complicated further by evidence that the distribution really represents 2 species, *A. cingulatum* and *A. bishopi* Goin (Pauly et al. 2007). The populations examined in this study represent the newly recognized species of flatwoods salamander (*A. bishopi*) that is restricted to the northern coastal plain of the Gulf of Mexico. The recognition that there are 2 species rather than 1 has resulted in what were 59 populations being split into 37 populations of *A. cingulatum* and 22 populations of *A. bishopi* (Pauly et al. 2007, USFWS 2005). Therefore, this taxonomic change has elevated the conservation priority of these salamanders and highlights the need for more active management to avoid extinction. In addition, critical habitat for both species has been proposed and *A. bishopi* has been proposed to be listed as an endangered species under the U.S. Endangered Species Act (USFWS 2008).

Loss and alteration of habitats was considered the main threat and the cause of population declines to flatwoods salamanders (Means et al. 1996, Palis 1996, USFWS 1999). Similar to other ambystomatid salamanders, the habitat used by flatwoods salamanders differs greatly between larvae and adults. Adults are fossorial and occur in mesic, longleaf pine flatwoods and savannas (Palis 1996). Adult flatwoods salamanders migrate to breeding sites, which consist of isolated ephemeral wetlands, on rainy nights from October–December (Means 1972, Anderson and Williamson 1976, Palis 1996). The quality of surrounding nonbreeding habitat used by adult flatwoods salamanders is likely a factor related to occupancy of pools; however, here we focus on within-pool factors that may attract adults and may lead to recruitment of larvae.

Although several hypotheses have been proposed about environmental conditions that constitute high quality larval habitat versus degraded habitat, such as hydroperiod and attributes of vegetative cover (USFWS 1999), there has been little quantitative research relating these conditions to the presence or abundance of flatwoods salamander larvae (Sekerak et al. 1996). In this paper we report the results of quantitative analyses of 3 factors: vegetation, water level, and relative presence of fish. Because flatwoods salamander larvae can be difficult to detect (Bishop et al. 2006), we incorporated estimates of detection into the models as well.

A potential factor for the decline of flatwoods salamanders is that components of breeding and larval habitat have degraded over time (USFWS 2008). In particular, the suppression of fire during the growing season may explain the premature drying of breeding wetlands (Bishop and Haas 2005) because a lack of fire may facilitate encroachment of woody vegetation (Kirkman 1995), leading to an increase in evapotranspiration (Huxman et al. 2005). In many locations prescribed burning is conducted during the dormant season (i.e., winter months) when conditions are better for controlling fire (Bishop and Haas 2005). However, this season also overlaps with the recharge of water into ephemeral wetlands, which will inhibit fire from entering the wetlands. Further, dormant season fires may cause re-sprouting of woody vegetation and increase stem densities of shrubs in longleaf pine (*Pinus palustris* Mill.) associated wetlands (Clewell 1989, Smith et al. 1997, Drewa et al. 2002). In contrast, growing season fires are more likely to cause mortality of woody stems (Clewell 1989, Smith et al. 1997, Drewa et al. 2002). Growing season burns also may increase seeding of wiregrass (*Aristida stricta* Michx.) (Outcalt 1994) and other dominant herbaceous species (Brewer and Platt 1994), which may provide larvae with opportunities for foraging and cover (Sekerak et al. 1996). Dormant season burns may also overlap with the winter breeding season of some amphibians and

may affect breeding migrations (Semlitsch 2000, Schurbon and Fauth 2003). Thus, support for growing season burns to maintain high quality breeding habitat for amphibians of the fire-maintained longleaf pine ecosystem of the southeastern coastal plain has been increasing (Schurbon and Fauth 2003, Means et al. 2004, Bishop and Haas 2005), although data to support its value are sparse.

Another potential threat to flatwoods salamanders in breeding wetlands is fish, which may be either predators or competitors of flatwoods salamander larvae. Historically, fish were thought to be absent from most ephemeral wetlands (Semlitsch 1988, Hopey and Petranka 1994), but fish have been documented in flatwoods salamander breeding sites (Palis 1996). Fish may migrate into wetlands during seasonal floods (Palis 1996). Invasion of fish may also result from a change in disturbance regimes (Maret et al. 2006). Activities such as road building may alter the hydrology of nearby streams and wetlands, and potentially facilitate movement of fish. Anuran tadpoles and eggs were depredated by mosquitofish (*Gambusia affinis* Baird and Girard) in the western United States (Lawler et al. 1999) and Australia (Komak and Crossland 2000). Larval Sonoran tiger salamanders (*Ambystoma tigrinum stebbinsi* Lowe) have been negatively affected by *G. affinis* and other predatory fish in Arizona (Maret et al. 2006). Mole salamander (*A. talpoideum* Holbrook) eggs and larvae were susceptible to predation by fish in South Carolina (Semlitsch 1988). As competitors, *Gambusia* can cause declines in some groups of invertebrate prey (Hurlbert and Mulla 1981), including prey of flatwoods salamanders (Whiles et al. 2004). Under laboratory conditions, *G. holbrooki* Girard can eat 3.5 times more mosquito larvae than *A. talpoideum* larvae (DuRant and Hopkins, 2008). Therefore, fish predators and competitors in amphibian breeding wetlands may decrease recruitment of adult salamanders via direct mortality of eggs and larvae or via competition for invertebrate prey.

Our goal was to examine which within-pool factors (i.e., vegetation characteristics, water-levels, and/or presence of fish) may limit successful breeding of flatwoods salamanders. Although these factors have been discussed previously by several authors in the literature, quantitative tests have been lacking. Therefore, we related vegetation characteristics, water level, and the presence of fish to occupancy and detection of larval flatwoods salamanders over time.

STUDY AREA

We evaluated the occupancy and detection of flatwoods salamander larvae in 18 previously occupied breeding wetlands on Eglin Air Force Base (Eglin), Florida from 2003 through 2006. These sites were wetlands that were occupied by flatwoods salamanders at least once since 1993. Fourteen wetlands were located in the East Bay Flatwoods East in Okaloosa County, 1 wetland was located in the East Bay Flatwoods West in Santa Rosa County, and 3 wetlands were located in Oglesby's Flatwoods in Okaloosae County. Each of these 3 designated flatwoods regions was considered a population for recovery (USFWS 2005). The flatwoods ecosystem was historically a fire-maintained longleaf pine, slash pine (*Pinus elliottii* Engelm.), and wiregrass savanna with low, flat topography. The breeding sites of flatwoods salamanders are ephemeral wetlands that fill with water in the fall and winter months. These wetlands had overstories of longleaf pine, slash pine, pond cypress (*Taxodium ascendens* Brongn.), and blackgum (*Nyssa sylvatica* Marsh.) and have open to dense midstories dominated by myrtle-leaved holly (*Ilex myrtifolia* Walter) and Chapman's St. John's-wort (*Hypericum chapmanii* Chapman).

The soil series of the breeding wetlands were most frequently Rutlege, but also include Chipley and Hurricane, Dorovan, Leon, and Pactolus (Natural Resources Conservation Service

2007). The uplands surrounding the wetlands had a canopy dominated by longleaf pine and slash pine and an open to moderately dense understory. During the study period, rainfall in northwest Florida ranged from 106 cm in 2006 to 197 cm in 2005 (unpublished data, Florida Automated Weather Network).

The historic fire return interval of flatwoods was thought to have been 1–3 years in both the uplands (Stout and Marion 1993, Frost 1995) and depression wetlands (Frost 1995). Prescribed fire was used as a management strategy in the surrounding uplands of the breeding wetlands, but it is unlikely fire entered the wetlands because prescribed fire was implemented during the dormant season, when the wetlands contain water. Average return interval for prescribed fire on Eglin was approximately 5 years in the uplands (Eglin Integrated Natural Resources Management Plan 2002).

METHODS

We conducted surveys of larval flatwoods salamanders once per month in January, February, and March, 2003–2006, in each of 18 known breeding wetlands, for a total of 3 surveys/year/wetland. Due to a severe drought in 2007, the breeding wetlands never attained sufficient water levels to support reproduction. Breeding occurs primarily during the fall as wetlands begin filling with water and the larval period lasts from 11–18 weeks (Palis 1995b). Therefore, the months of January–March, when larvae have reached sufficient size for detection, are considered the most effective months for sampling flatwoods salamander larvae (Bishop et al. 2006). We used model SH-2 and SH-2D (Mid-Lakes Corporation, Knoxville, TN) dip-nets with a 3 mm mesh size (USFWS 2005) and conducted timed, systematic searches to sample salamander larvae. When multiple observers were used, effort was considered the sum of each surveyor's effort (i.e., total effort).

In 2006, we recorded the presence of fish in the study wetlands during dip-net surveys for salamanders. We included the presence of either *G. holbrooki* or *Esox americanus* Lesueur, both capable predators and/or competitors of larvae as a single factor, relative presence of fish. Although other techniques may have been more effective at sampling fish, those that were caught during our larval dip-net sampling were using the same areas as the flatwoods salamander larvae and were thus most likely to interact with the larvae.

In 2006–2007, we described the structural vegetation characteristics of the 18 study wetlands using vegetation plots that were systematically placed every 20 m along a single transect. Wetlands had between 3–11 vegetation plots that were subsequently averaged to acquire wetland-scale habitat characteristics. Transects were started in the ecotone surrounding the wetland and oriented along the long axis of the wetland. We examined percentage of canopy cover, basal area, percentage of herbaceous cover, and percentage of woody debris as potential descriptors of larval flatwoods salamander habitat. We measured percent canopy cover using a spherical densiometer and basal area using a Jim-Gem Cruz-All (Forestry Suppliers Inc., Jackson, MS). We used the Daubenmire (1959) cover class scale to estimate the percentage of herbaceous vegetation and woody debris by visually estimating the percentage of each variable in a 0.5 x 0.2 m rectangular plot. We recognize that the measurement of habitat structure at the end of the study is less than ideal, but the variables that we selected were not likely to appreciably change under normal conditions (i.e., there were no fires or mechanical treatments within any of the wetlands during the 5 year period). Further, remeasurement of these sites in 2008 showed similar patterns as the data from 2006–2007.

We monitored water levels at the 18 sites twice a month from January–March in 2006. Each site had 2 water level markers, 1 marker near the center of the wetland and a second at the

wetlands edge. We used the monthly average from both readings and both markers for subsequent analyses.

Statistical Analyses

We used a multiple-season occupancy model (MacKenzie et al. 2003) to estimate the occupancy of flatwoods salamanders. Occupancy in this analysis was more appropriately interpreted as a measure of "survival" or persistence of the flatwoods salamander populations (Mackenzie et al. 2006) because the sites of interest are known to have been occupied historically. First, we modeled occupancy and detection from 2003–2006 as a function of vegetation characteristics and year, and we assumed the extinction rate to be constant because we were primarily interested in occupancy and detection. We developed models that incorporated different vegetation characteristics as descriptors of occupancy (ψ), and year, total effort, and a constant parameter were used to describe detection (p). The result was a set of 23 models that were most biologically relevant to flatwoods salamander larvae. Next, we used a single season occupancy model (MacKenzie et al. 2002) to assess the influence of vegetation characteristics, water levels, and presence of fish on the occupancy of wetlands and detection of flatwoods salamander larvae in 2006. For this analysis, we developed models that incorporated vegetation characteristics, water levels, and/or presence of fish as descriptors of occupancy (ψ), with month, total effort, water levels, and a constant parameter being used to describe detection (p). This resulted in a set of 22 models that we hypothesized to be the most biologically relevant to flatwoods salamander larvae. We performed both analyses in Program Presence 2.1 (Hines 2006) and we used an information theoretic approach using Akaike's Information Criteria (AIC) to examine the relative strength of each model (Burnham and Anderson 2002).

RESULTS

From 2003 through 2006, we located flatwoods salamander larvae in 3, 6, 3, and 4 of the 18 wetlands each year, respectively. Over the course of this study, we conducted 161 individual surveys and flatwoods salamander larvae occupied 7 different wetlands. Canopy cover ranged from 1.7–78.5% (\bar{x} = 43.9%, SE = 5.2), cover of herbaceous vegetation ranged from 9.0–94.4% (\bar{x} = 50.8%, SE = 6.1), woody debris ranged from 6.7–54.2% (\bar{x} = 19.7%, SE = 2.5), basal area ranged from 0.0–19.3 m²/ha (\bar{x} = 8.5 m²/ha, SE = 1.4), and water levels ranged from 0.0–60.0 cm (\bar{x} = 5.5 cm, SE = 2.1) at all 18 sites combined (Table 1). We captured *Gambusia holbrooki* at 8 of 18 wetlands and *Esox americanus* at 4 of 18 wetlands. All sites where *E. americanus* were detected also contained *G. holbrooki*.

Based on our hypothesized models from the 2003–2006 data, herbaceous vegetation and canopy cover were the best habitat descriptors of occupancy by flatwoods salamanders (Figure 5.1), and year was the best descriptor of detection (Table 5.2A). This model received a significant amount of the support, with a model weight of 38%. Individual site estimates of occupancy ranged from 3.1–86.9% (\bar{x} = 33.5%, SE = 6.0), and detection of larvae was variable among years and ranged from 7.4–92.1% (\bar{x} = 45.9%, SE = 3.7). Differential sampling effort was not an important component of detection and was not present in any of the top models. When larvae were detected, mean total effort was 39 minutes (SE = 8) and when larvae were not detected, mean total effort was 41 minutes (SE = 3).

Lastly, based on our hypothesized models related to occupancy in 2006, herbaceous vegetation was the most important covariate predicting occupancy of larvae, and month and water level were the best predictors of detection (Table 5.2B). For both the multiple-season and single-season analyses, herbaceous vegetation was consistently included in the best supported

models, and percent cover of herbaceous vegetation was 1.7 times greater in the 7 occupied sites compared to the 11 sites that were not occupied during this study (Figure 5.2).

DISCUSSION

The overall occupancy from 2003–2006 of the 18 known flatwoods salamander breeding wetlands was relatively low (33.5%) given that all but one site had a prior history of being occupied. Detection rates were moderate (45.9%) and suggest that when the larvae were present detecting them is likely with multiple visits (as suggested by Bishop et al. 2006). Over the 4 year period we detected flatwoods salamander larvae in 7 of 18 wetlands. In 10 of the 11 wetlands where larvae were not detected occupancy had not been confirmed since the mid-1990's (i.e., 2 had detections in 1993, 8 in 1994; Palis 1995a). In the remaining wetland, 2 adults were captured as part of a drift fence survey in 2002 (Bishop 2004), but no larvae have been detected since 1993. The interval since larvae were observed in the 1990s would have been sufficiently long to allow some sites to develop more dense canopies and increase basal areas of overstory and midstory vegetation, which might have inhibited growth of herbaceous vegetation. However, 2 sites where larvae were not detected had similar percentages of canopy cover and herbaceous cover to sites that were occupied (Figure 5.1).

Herbaceous cover was the primary vegetation characteristic that predicted occupancy of flatwoods salamander larvae in both analyses (Table 5.2). Biologically, herbaceous vegetation likely provides both foraging habitat (Whiles et al. 2004) and escape refugia (Palis 1995b) for larval flatwoods salamanders as well as attachment sites for egg masses for adult females (Palis 1996). Our data suggest that managing ephemeral wetlands in pine flatwoods for herbaceous cover and an open canopy may improve breeding habitat for flatwoods salamanders. Similarly, Sekerak et al. (1996) found that flatwoods salamanders (*A. cingulatum*) were captured in areas

with higher amounts of sedges and other herbaceous vegetation and were infrequently captured in open water or areas with only detritus. The dusky gopher frog (*Rana sevosa* Goin and Netting), another endemic amphibian of the Gulf Coastal Plain, also requires isolated, ephemeral wetlands with open canopies (Thurgate and Pechmann 2007).

Growing season prescribed fires may be a useful management option in the sites because fires in this season effectively decrease canopy cover (Drewa et al. 2002), stimulate herbaceous growth (Clewell 1989, Brewer and Platt 1994, Outcalt 1994), and are more likely to burn into the wetland. However, fire regime may not necessarily need to shift completely to growing season fires because some evidence suggests that some herbaceous plants benefit from fires in both dormant and growing seasons (Hiers et al. 2000). Thus if conditions allow fire to enter the wetland, either season might be a viable option. In our study upland fire was last documented around 9 wetlands (6 lacking and 3 with larvae detection during this study) in 2004, 3 in 2001 (all with larvae detection), and 6 in 1995 (5 lacking and 1 with larvae detection). The 2004 fires occurring during this study did not enter the wetlands. Whether fires in 1995 and 2001 entered the wetlands is unknown.

Time (i.e., month and year) was an important covariate to estimate detection, which highlights the importance of conducting multiple surveys to avoid incorrect classification of occupied sites. Bishop et al. (2006) evaluated the importance of multiple survey events for detection of flatwoods salamander larvae and recommended a minimum of 2 survey events within a breeding season. Our results support this conclusion because our mean detection probability was < 50% and we observed variation in detection among months and years. The water level of breeding wetlands was important to detection in the 2006 model, and provides additional support for sampling wetlands at least twice within a season.

The influence of fish on the presence of flatwoods salamander larvae was not a component of the well-supported models. The predator/competitor community may not be a major influence on salamander larvae at sites with high levels of herbaceous vegetation because invertebrate food is plentiful and flatwoods salamander larvae have access to protective cover. We recognize that additional factors that were not measured (e.g., adult terrestrial habitat quality) could have also contributed to occupancy of wetlands.

CONCLUSIONS

Successful management of habitat used by larval flatwoods salamanders may require techniques to control encroachment of woody vegetation and to stimulate herbaceous vegetation. Based on the literature (e.g., Sharitz et al. 1992, Frost 1995), growing season prescribed fires would mimic natural disturbance regimes and likely create desired habitat conditions. However, an increase in urbanization within the coastal plain ecosystem where flatwoods salamanders reside has slowed the use of growing season burns. Instead, dormant season fires are used fairly frequently (Bishop and Haas 2005). However, burn success into wetlands themselves is not well documented, and fires during this season might actually increase growth of woody vegetation (Smith et al. 1997, Drewa et al. 2002). Managers may need to consider a targeted, multiple-step approach similar to that suggested for protection of cavity trees used by Red-cockaded Woodpeckers (*Picoides borealis* Vieillot) that includes mechanical treatment prior to a prescribed burn (Williams et al. 2006). For example, to increase herbaceous vegetation and open the canopy of breeding sites of flatwoods salamanders, it may be necessary to burn surrounding uplands during the dormant season to create a “safety” strip and secondly to conduct a prescribed fire within the perimeter of the wetland during the growing season. Mechanical treatments (e.g., brush saws and chainsaws) might enhance herbaceous vegetation and moderate levels of canopy

cover (Thurgate and Pechmann 2007), and provide a more long-term opening in the canopy than dormant season fires. Resprouting of woody vegetation could be minimized by basal application of herbicides. However, mechanical methods may compact soil (even if only from foot traffic) or harm migrating adults and metamorphs. Given that growing season fire is the natural driver in the system, restoring it to the system may have ecosystem benefits other than enhancing salamander habitat.

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Table 5.1. Means and standard errors (SE) of vegetation characteristics for occupied wetlands versus wetlands where flatwoods salamander (*Ambystoma bishopi*) larvae were not detected on Eglin Air Force Base, Florida, 2003-2007.

Vegetation Characteristic	\bar{x} (Occupied)	SE	\bar{x} (Not Detected)	SE
Canopy Cover (%)	42.6	5.2	44.7	8.1
Basal Area (m ²)	5.9	0.7	10.1	2.1
Herbaceous Cover (%)	67.6	8.2	40.1	7.0
Woody Debris (%)	20.6	5.9	19.2	2.0

Table 5.2. Top 4 model results including AIC, change in AIC (ΔAIC), model weight (w_i), and the number of parameters (K) for occupancy (ψ) and detection (p) of flatwoods salamander (*Ambystoma bishopi*) larvae at 18 known breeding wetlands on Eglin Air Force Base, Florida for A) 2003–2006 and B) 2006 only.

Model	AIC	ΔAIC	w_i	K
A. 2003–2006				
ψ (herbaceous veg., canopy), p (year)	90.16	0.00	0.38	8
ψ (herbaceous veg.), p (year)	91.52	1.36	0.19	7
ψ (herbaceous veg., woody debris), p (year)	91.67	1.51	0.18	8
ψ (herbaceous veg., basal area), p (year)	93.52	3.36	0.07	8
B. 2006 only				
ψ (herbaceous veg.), p (month, water level)	32.53	0.00	0.27	6
ψ (herbaceous veg.), p (month)	33.34	0.81	0.18	5
ψ (herbaceous veg., canopy), p (month, water level)	33.92	1.39	0.13	7
ψ (herbaceous veg., canopy), p (month)	34.73	2.20	0.09	6

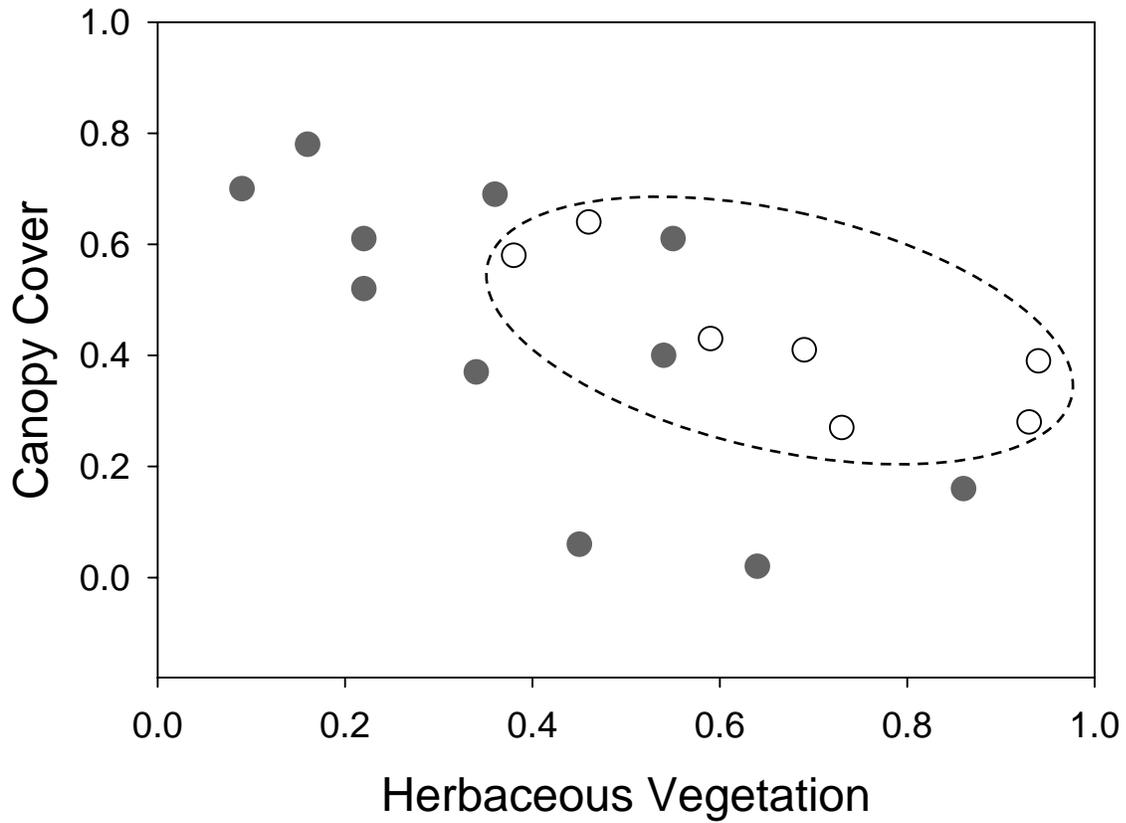


Figure 5.1. Herbaceous vegetation and canopy cover in 18 wetlands used historically by flatwoods salamander (*Ambystoma bishopi*) larvae. Open circles denote occupied sites and the closed circles denote sites where no salamander larvae were detected from 2003–2006.

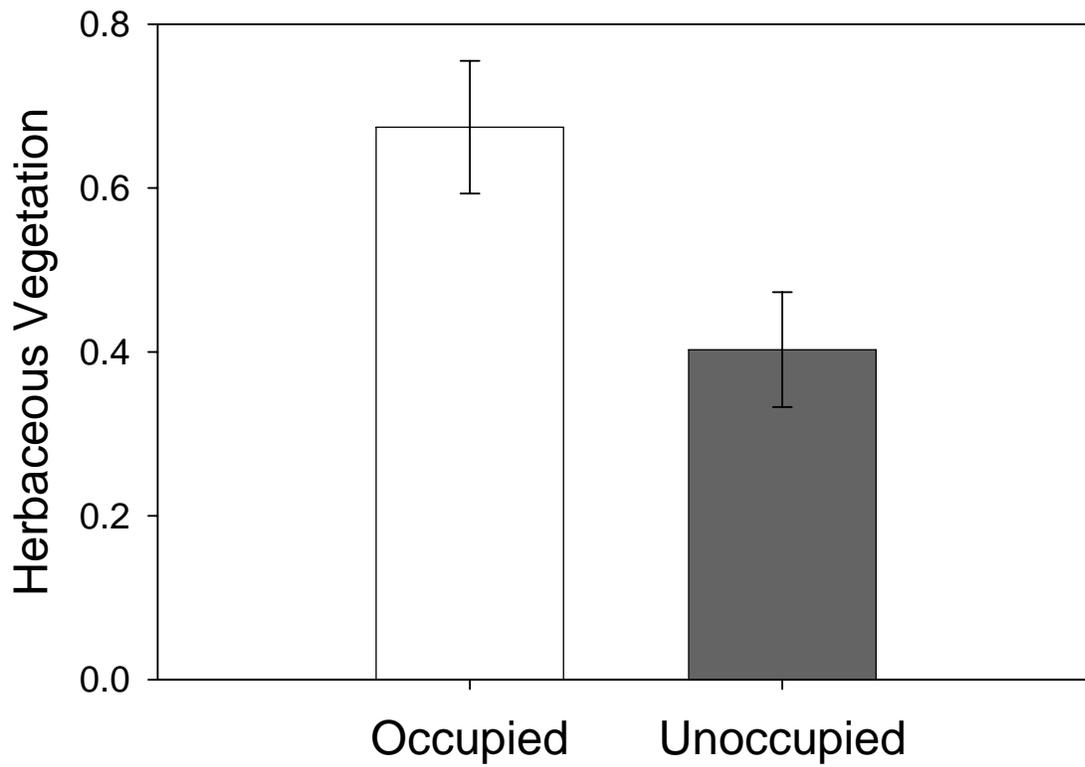


Figure 5.2. Herbaceous vegetation in 18 wetlands used historically by flatwoods salamander (*Ambystoma bishopi*) larvae on Eglin Air Force Base, 2003–2006.

CONCLUSIONS

Rana okaloosae and *A. bishopi* are both rare species of conservation concern that occur in a region where suitable habitat for both species is rare and habitat quality is declining. For both of these species a primary focus should be on understanding how management actions can conserve and restore habitat. In this dissertation I have documented characteristics of breeding habitat at multiple spatial scales for *R. okaloosae* (Chapter 1-3) and examined the within-pool characteristics of *A. bishopi* breeding wetlands. The information in this dissertation will be helpful in developing management strategies for breeding habitats of both species.

For *R. okaloosae*, my results suggest that breeding habitat should have an abundance of cover (e.g., woody debris and emergent and submergent vegetation) and should have shallow but constant water levels that will likely provide the best attributes for successful reproduction and survival of tadpoles (Chapter 1). Additionally, at the macrohabitat scale emergent vegetation appeared to be an important descriptor of habitat, and sites with higher levels of emergent vegetation tended to be occupied (Chapter 2). Further, suitable habitat may need to be in close proximity, because populations may be operating as metapopulations (Chapter 3) and *R. okaloosae* may have limited dispersal capabilities (Gorman et al. 2009). Research is underway to understand the genetic population structure of *R. okaloosae* (J. Austin, pers. com.) and this research likely will elucidate whether or not *R. okaloosae* are genetically distinct in separate drainages. However, future work on movements and dispersal through genetic and demographic studies are still needed to elucidate the population structure of *R. okaloosae* at finer scales.

In addition to high quality breeding habitat, many anurans also require high quality terrestrial habitat that surround breeding sites (Semlitsch 2002). Terrestrial habitat can be important as refugia, feeding, or to facilitate movement among breeding sites (Semlitsch 2002).

However, data on non-breeding habitat use and frequency of terrestrial habitat use of *R. okaloosae* are limited. Finally, the importance of disturbances (e.g., fire) in this system can not be overstated. An important future direction should look towards experimentation to manipulate *R. okaloosae* habitat to understand how fire, manmade disturbance (e.g., power line clearing), hydrology, and other potential drivers (e.g., beaver [*Castor canadensis*] activity) interact to create suitable habitat characteristics for *R. okaloosae*. These experiments will likely lead to a better understanding of potential methods that can be used to restore habitat that may no longer be suitable for *R. okaloosae* as a result of fire suppression or other causes.

Similarly, for *A. bishopi* our results suggested that high amounts of herbaceous vegetation and moderate levels of canopy cover were the best predictors of larval occupancy (Chapter 5). Therefore, management of habitat used by larval flatwoods salamanders may require techniques to control encroachment of woody vegetation and to stimulate herbaceous vegetation. Several authors have suggested that growing-season prescribed fires would mimic natural disturbance regimes and in turn create desired habitat conditions (e.g., Sharitz et al. 1992, Frost 1995). However, a shift away from growing season burns to dormant season fires has occurred (Bishop and Haas 2005) and therefore burn success for the wetlands has declined. Reintroduction of fire into wetlands used by *A. bishopi* will likely have strong positive benefits. In cases where fire can not be used, other options should be considered (see Chapter 5). However, because growing season fire is the natural driver in the system, restoring it to the system will likely have ecosystem benefits other than enhancing salamander habitat. Future research should be directed towards experimentation of approaches to control woody vegetation and to increase herbaceous cover (e.g., fire, mechanical removal, and herbicides) and on necessary fire frequencies to maintain these conditions. A replicated experimental manipulation of wetlands would likely

yield a better understanding of necessary fire return intervals and whether methods other than fire can create suitable habitat conditions for *A. bishopi*.

While management of wetland habitat is clearly an important factor for *A. bishopi*, upland habitat is likely an important component of metamorph and adult survival. Further, proximity of individual wetlands is also an important component of population management for *A. bishopi*, because having high quality upland habitat that surrounds multiple high quality wetlands will likely increase access for adults to breed successfully (Skelly et al. 1999, Semlitsch 2002). For example, Eglin and adjacent Hurlburt Field are the only population within the range of *A. bishopi* with >3 wetlands in a complex (USFWS 2009). The other 2 populations on Eglin have only been documented in a complex of 3 wetlands or 1 wetland and presence of larvae in the 1 wetland alone has not been confirmed since 1994 (Palis 1995), despite a concerted sampling effort from 2003-2008. In any given year, it is rare for >25% of the sites to be occupied and from 2003-2008 only 1 site was occupied in >3 years (Appendix D). Also, of the 18 known breeding wetlands only 7 have been occupied from 2003-2008 (see Chapter 5 for more details). Therefore, as with *R. okaloosae*, conservation of *A. bishopi* will only be ensured if these wetland complexes are protected and managed properly.

It appears that there may be limited direct influence of male *R. c. clamitans* on male *R. okaloosae* during the breeding season (Chapter 1). While aggressive interactions between these two species have not been observed (Bishop 2005), future research should specifically focus on how the males of these species interact behaviorally. In addition, to understand mechanisms related to microhabitat selection it may be important to investigate how females of both species are selecting males of both species and oviposition sites. For example, female *R. okaloosae* frequently oviposit directly at male *R. okaloosae* calling sites (Bishop 2005, Gorman,

unpublished data), so determining whether females are selecting for site quality or male quality may elucidate mechanisms that are facilitating hybridization between these two species.

Similarly, I did not find strong evidence that tadpoles of *R. c. clamitans* were negatively impacting the growth of *R. okaloosae* (Chapter 4). Our power to detect differences was moderate, so greater replication may have yielded results that were more straightforward. Given that this experiment was based in the laboratory it raises the question of whether competition will occur or is actually occurring in the field. Future research should focus on habitat selection of tadpoles of each species, because this could aid in assessing the extent of the interactions between these two species. Finally, it is likely that competition between *R. okaloosae* and *R. c. clamitans* tadpoles may not have strong negative population-level consequences for *R. okaloosae*, however, combined with a density-independent threat such as drought or habitat succession the negative influence of larval competition may increase (Jaeger 1980, Griffis and Jaeger 1998).

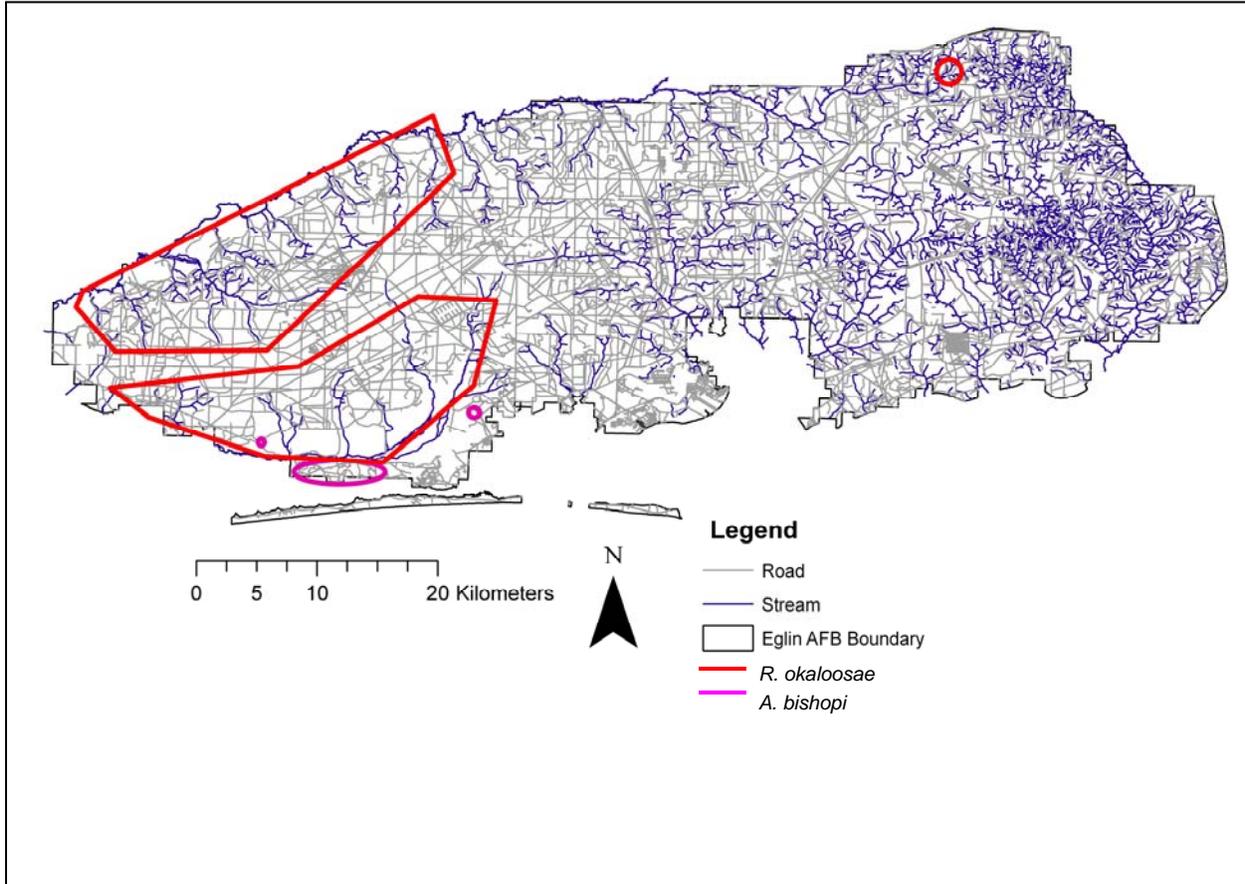
In summary, the results of my dissertation suggest that habitat may be an important factor that limits the distribution of *R. okaloosae*. Further, it appears to play an important role as a factor for larval *A. bishopi*. Also, I found that calling males of *R. okaloosae* and *R. c. clamitans* do select microhabitats for calling differently, but there is some overlap. Regardless of this separation in habitat there is an open question as to how and why hybridization is occurring and the focus for this question may require a more detailed assessment of female choice. Finally, I did not find strong evidence for competition between the tadpoles of *R. okaloosae* and *R. c. clamitans*, but more data on tadpoles in the field is warranted.

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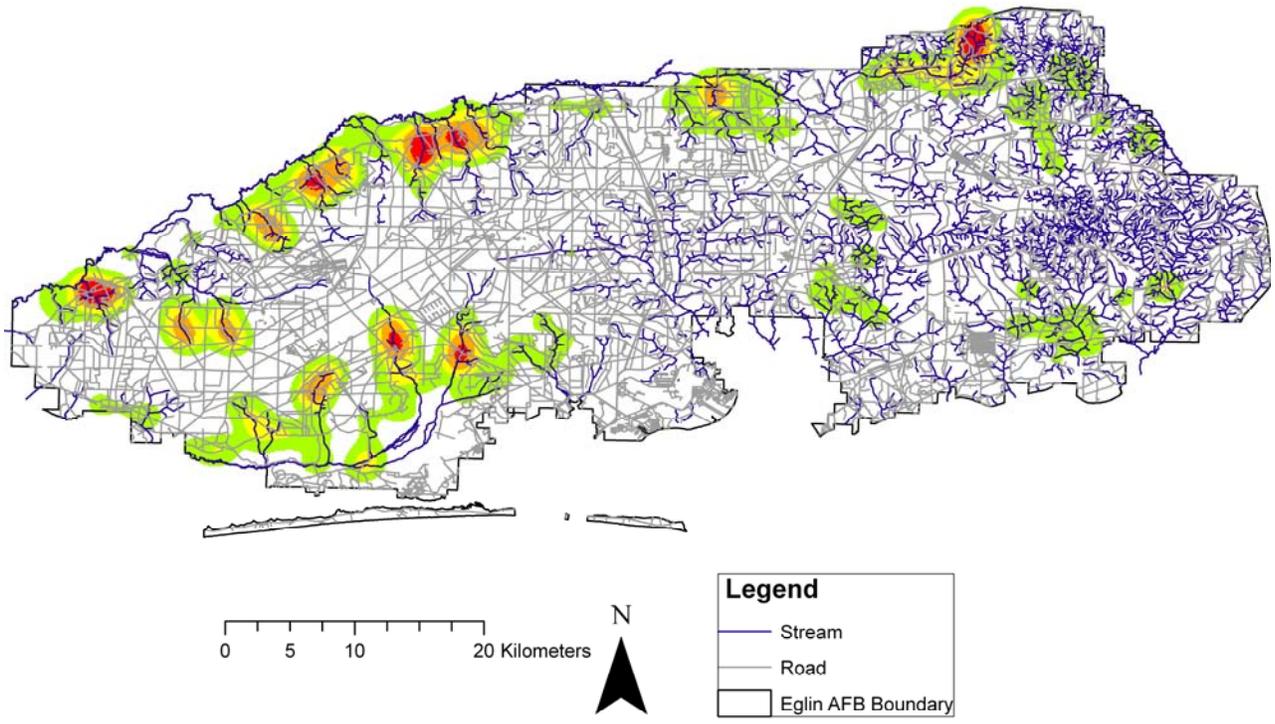
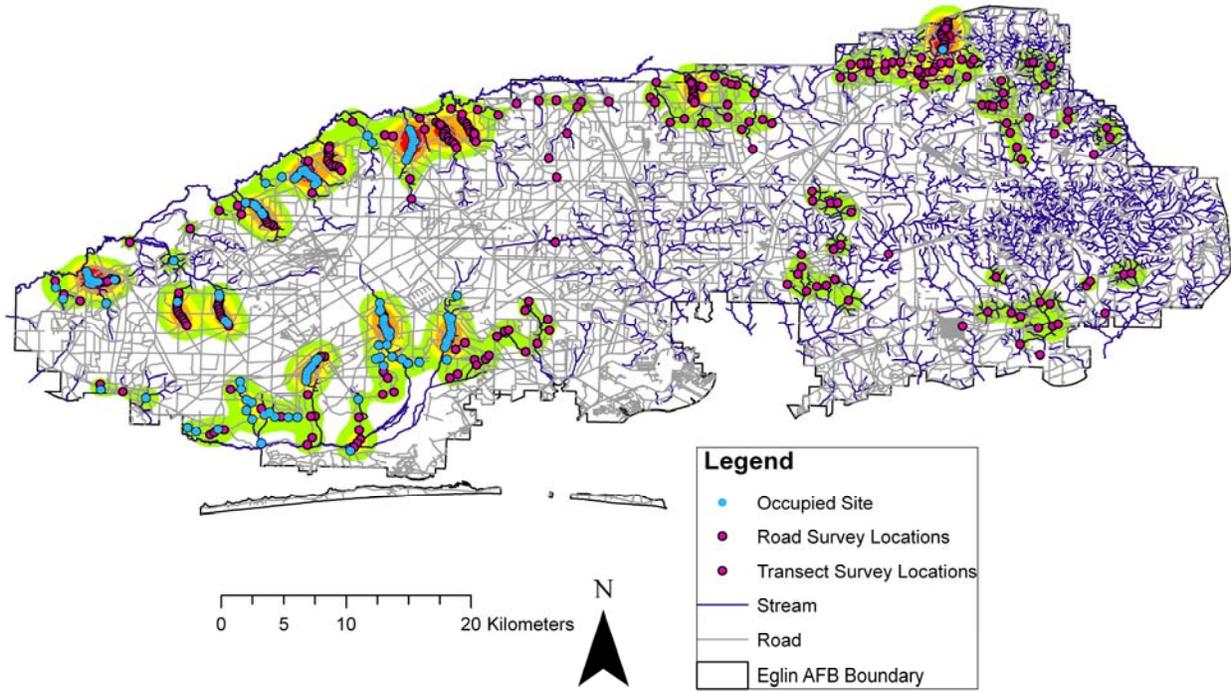
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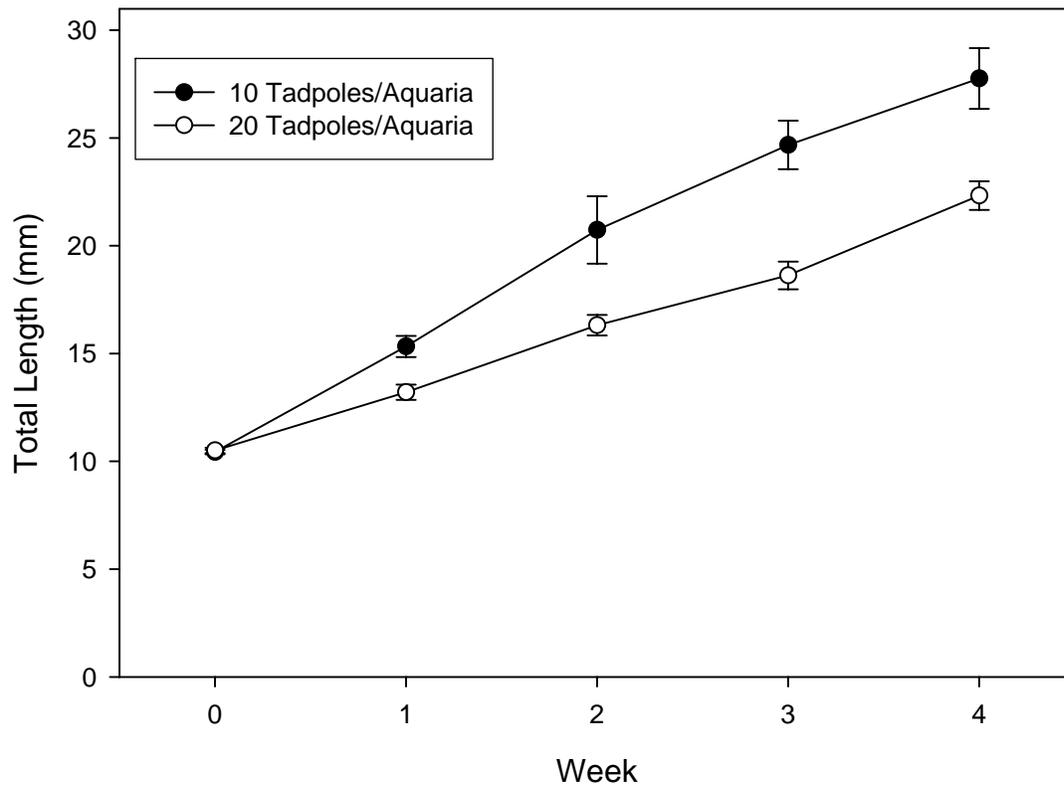
APPENDIX



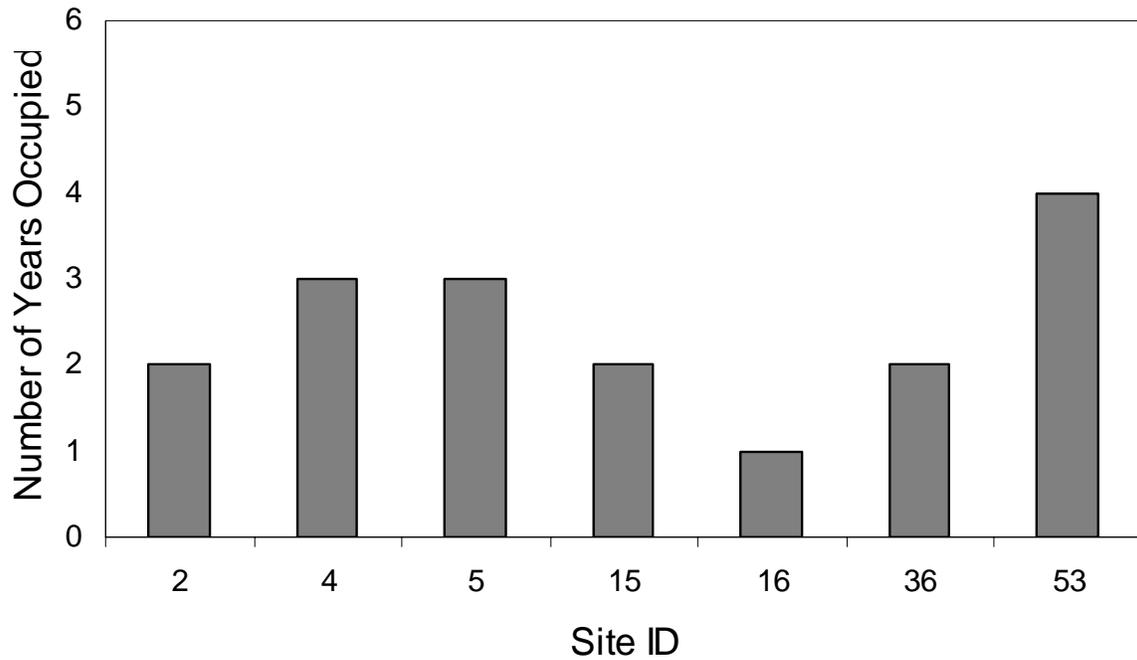
Appendix A. The approximate locations of 3 populations of *Rana okaloosae* based on survey locations from 2006-2008 and 3 populations of *Ambystoma bishopi* based on survey data from 2003-2008.



Appendix B. The estimated occupancy of *Rana okaloosae* presented graphically by using a fixed kernel estimator that is weighted with the occupancy estimates generated from occupancy modeling at the range-wide (see Table 3.1) and stream-level scales (see Table 3.3), where (A) has the estimated occupancy for both scales shown and is overlain with the sites locations where *R. okaloosae* were detected and not detected from 2006-2008 and (B) has only the estimated occupancy for both scales (where the hotter (red) the color has higher probability of occupancy and the cooler (green) the color has a lower probability of occupancy). I used individual survey points (weighted by occupancy estimates) to create a “home range” using a 95% fixed kernel estimator, so areas that were not surveyed are not represented on this map. From our modeling it appears that areas surveyed in north-central and northeastern Eglin may be areas that are suitable for *R. okaloosae*.



Appendix C. Weekly change in total length of *Rana okaloosae* tadpoles across a four-week period in 2006 (error bars = 1 standard error). Tadpoles were reared in aquaria with 5L of water and fed a diet of 2g of frozen spinach (thawed) and were in either a low or high density treatment (densities of 0.5 tadpoles/L and 0.25 tadpoles/L, respectively) with 4 replicates for each density.



Appendix D. Number of years *Ambystoma bishopi* larvae were detected from 2003-2008 on Eglin Air Force Base. Larvae were sampled by dipnetting 18 ponds with a known history of being occupied by larvae. Of the 18 known ponds surveyed during this 6 year period only 7 were found to be occupied. The majority of these sites were sampled 3 times/year in each year of sampling (2007 was a severe drought year and wetlands never filled with water).