

**THE EFFECTS OF CLIMATE CHANGE AND LONG-TERM FIRE SUPPRESSION ON  
EPHEMERAL POND COMMUNITIES IN THE SOUTHEASTERN UNITED STATES**

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# THE EFFECTS OF CLIMATE CHANGE AND LONG-TERM FIRE SUPPRESSION ON EPHEMERAL POND COMMUNITIES IN THE SOUTHEASTERN UNITED STATES

Houston C. Chandler

## ABSTRACT

In the southeastern United States, ephemeral wetlands in pine flatwoods provide important habitat for amphibians and aquatic invertebrates, but extensive deforestation has destroyed or isolated many wetlands and fire suppression has altered vegetation in others. My goals were to identify how wetland hydroperiods have changed through time and to examine the effects of long-term fire suppression on aquatic communities, including Reticulated Flatwoods Salamanders (*Ambystoma bishopi*) and Ornate Chorus Frogs (*Pseudacris ornata*). Chapter 1 used a modeling approach to relate wetland hydroperiods to current climate conditions and to hindcast historic conditions. Over the past 118 years, hydroperiods were often unfavorable for *A. bishopi* reproduction, and in recent years hydroperiods were shortened by persistent drought. Chapters 2 and 3 focused on identifying the effects of shifting from an open, grass dominated wetland to a wetland with high canopy cover and little herbaceous vegetation. In Chapter 2, I quantified amphibian and invertebrate communities in several wetlands. *A. bishopi* and *P. ornata* tended to occupy wetlands with lower canopy cover and higher herbaceous vegetation cover. Aquatic invertebrate abundance was generally higher in wetlands with lower shrub density and lower canopy cover. In Chapter 3, I examined how a reduction in herbaceous vegetation affected tadpoles when a predatory crayfish was present using two experiments. Crayfish were effective predators of both species across all vegetation treatments and often caused nonlethal tail injury. My results suggest that managers should focus on ensuring that wetland basins regularly burn, and wetlands with longer hydroperiods should be a management priority.

## ATTRIBUTION

A brief description of the contributions made by colleagues that assisted with the preparation of my thesis chapters is outlined below. Contributions rose to the level of co-author for manuscript publication in all three chapters.

### **Chapter 1. Modeling historical breeding conditions for an endangered salamander in**

### **ephemeral wetland habitats of southeastern USA: Implications of climate change.**

Andrew L. Rypel (Department of Fish and Wildlife Conservation, Virginia Tech) currently at Wisconsin Department of Natural Resources, Madison, WI, provided assistance with study design, constructing statistical models, and edited drafts of the manuscript. Yan Jiao (Department of Fish and Wildlife Conservation, Virginia Tech) provided assistance with statistical analyses and edited drafts of the manuscript. Thomas A. Gorman and Carola A. Haas (Department of Fish and Wildlife Conservation, Virginia Tech) obtained funding for the project, supervised long-term data collection efforts, provided assistance with study design, and edited drafts of the manuscript.

### **Chapter 2. The effects of changing habitat structure on aquatic invertebrate and**

### **amphibian communities in pine flatwoods wetlands.**

Thomas A. Gorman and Carola A. Haas (Department of Fish and Wildlife Conservation, Virginia Tech) obtained funding for the project, supervised long-term data collection efforts, provided assistance with study design, and edited drafts of the manuscript.

### **Chapter 3. The effects of crayfish predation and vegetation cover on tadpole growth,**

**survival, and nonlethal injury.** Thomas A. Gorman and Carola A. Haas (Department of Fish

and Wildlife Conservation, Virginia Tech) obtained funding for the project, provided assistance with study design, and edited drafts of the manuscript.

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## TABLE OF CONTENTS

<b>Abstract.....</b>	<b>ii</b>
<b>Attribution.....</b>	<b>iii</b>
<b>Acknowledgements .....</b>	<b>v</b>
<b>Table of Contents .....</b>	<b>vii</b>
<b>List of Tables .....</b>	<b>ix</b>
<b>List of Figures.....</b>	<b>xii</b>
<b>Preface.....</b>	<b>1</b>
Literature Cited .....	4
<b>Chapter 1: Hindcasting historical breeding conditions for an endangered salamander in ephemeral wetland habitats of the southeastern USA: Implications of climate change since 1896.....</b>	<b>8</b>
Abstract .....	8
Introduction .....	9
Materials and Methods .....	12
Results .....	16
Discussion .....	18
Acknowledgements .....	25
Literature Cited .....	26
<b>Chapter 2: The effects of changing habitat structure in pine flatwoods wetlands on aquatic invertebrate and amphibian communities.....</b>	<b>43</b>
Abstract .....	43
Introduction .....	44
Materials and Methods .....	47
Results .....	53
Discussion .....	60
Literature Cited .....	65

<b>Chapter 3: The effects of crayfish predation and vegetation cover on tadpole growth, survival, and nonlethal injury .....</b>	<b>82</b>
Abstract .....	82
Introduction .....	83
Materials and Methods .....	86
Results .....	91
Discussion .....	93
Acknowledgments .....	97
Literature Cited .....	97
<b>Conclusions.....</b>	<b>108</b>
Literature Cited .....	111
<b>Management Implications.....</b>	<b>113</b>
<b>Future Research Questions .....</b>	<b>116</b>
<b>Appendix A. Study site information.....</b>	<b>117</b>
<b>Appendix B. Pooled amphibian dipnetting captures .....</b>	<b>122</b>
<b>Appendix C. Full AIC modeling results.....</b>	<b>123</b>
<b>Appendix D. Photos of tail injury caused by crayfish .....</b>	<b>126</b>

## LIST OF TABLES

### **Chapter 1: Hindcasting historical breeding conditions for an endangered salamander in ephemeral wetland habitats of the southeastern USA: Implications of climate change since 1896**

Table 1.1. Mean and median filling and drying dates for 15 *Ambystoma bishopi* breeding wetlands in northwest Florida. Water levels were measured at an approximate wetland centroid from 2006-2013. The range of fill dates was September 1 – April 8, and the range of dry dates was January 21 – October 21 of the following fall. .... 35

Table 1.2. Factor loadings for three principal components from the PCA of six climate variables used in modeling wetland hydroperiod. Eigenvalues were 3.0, 1.1, and 0.9 for principal components 1, 2, and 3, respectively. Other principal components were not included because their eigenvalues were less than 0.9..... 36

Table 1.3. Model results for 17 logistic regression models that predicted whether or not historically active *Ambystoma bishopi* breeding wetlands were wet or dry on a monthly basis based on historic climate data. PC1, PC2, and PC3 were the three principal components that were used to construct models. The percent correct column indicates the number of months the models classified correctly from the training data. .... 37

Table 1.4. Results of change-point analyses of modeled hydroperiods for 17 *Ambystoma bishopi* breeding wetlands. Change points indicate periods where the average hydroperiod has significantly shifted..... 38

### **Chapter 2: The effects of changing habitat structure in pine flatwoods wetlands on aquatic invertebrate and amphibian communities**

Table 2.1. Mean habitat characteristics for 21 ephemeral wetlands located on Eglin Air Force Base, Florida. Hydroperiod measurements represent an average of the longest hydroperiod occurring at least partially between October and April from 2010 – 2014. Vegetation measurements were recorded once in each wetland during fall 2013 or spring 2014. Depth measurements represent the average depth (measured bimonthly) at an approximate center point from January – April 2014 and are likely higher than average because 2014 was an abnormally wet year. .... 73

Table 2.2. Amphibian species recorded in 21 ephemeral wetlands on Eglin Air Force Base, Florida from 2013 – 2014. Wetlands with herbaceous vegetation cover > 30% and canopy cover < 50% were high quality, wetlands with herbaceous vegetation cover < 20% and canopy cover > 50% were low quality, and wetlands that did not meet both criteria for either group were considered medium quality. Wetlands were also divided by the length of their average hydroperiod from 2010-2014 (long: > 5 months, medium: 3 – 5 months, and short: < 3 months). The number of wetlands fitting into each category is indicated in parentheses. .... 74

Table 2.3. Total invertebrate captures from dipnetting surveys conducted once a month from January – April 2014 in 21 ephemeral wetlands on Eglin Air Force Base, Florida. Surveys were conducted using a 1mm mesh dipnet, and invertebrates were identified to the lowest possible taxonomic level in the field..... 75

Table 2.4. Size measurements for adult male and adult female Panhandle Crayfish (*Procambarus evermanni*) captured in ephemeral wetlands from 2013 – 2014 on Eglin Air Force Base, Florida. .... 76

Table 2.5. Multiple linear regression modeling results relating invertebrate abundance to vegetation characteristics and wetland hydrology in ephemeral wetlands on Eglin Air Force Base, Florida (K = the number of parameters, AICc = second-order Akaike’s Information Criteria,  $\Delta$ AICc = change in AICc, and  $w_i$  = relative amount of support for each model). Potential variables included in the models are listed in Table 1..... 77

Table 2.6. Parameter estimates and 95% confidence intervals for the best-approximating model relating habitat variables to the abundance of three aquatic invertebrate groups. Aquatic invertebrates were capturing once a month from January – April 2014 in 21 ephemeral wetlands on Eglin Air Force Base, Florida. .... 78

**Chapter 3: The effects of crayfish predation and vegetation cover on tadpole growth, survival, and nonlethal injury**

Table 3.1. Fixed effects Wald tests for linear mixed effects models examining the effects of vegetation and presence of a predator on tadpole growth and survival. Experiment 1 was conducted using Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpoles and contained three vegetation treatments (68%, 34%, and 0% coverage). Experiment 2 was conducted with Ornate Chorus Frog (*Pseudacris ornata*) tadpoles and contained two vegetation treatments (80% and 40% coverage). A single Panhandle Crayfish (*Procambarus evermanni*) was used as a predator treatment in both experiments. .... 103

## Appendix A. Study site information

Table A1. Habitat characterizations, sampling data, and the presence of target species in 21 ephemeral wetlands on Eglin Air Force Base, Florida. Minnow trapping was conducted during 2014, and the presence of Reticulated Flatwoods Salamander (*Ambystoma bishopi*), Ornate Chorus Frogs (*Pseudacris ornata*), and Panhandle Crayfish (*Procambarus evermanni*) was identified during 2013 and 2014. A detailed description of the vegetation quality index is given in Chapter 2, and treatment groups are described above. .... 118

Table A2. Vegetation measurements in 21 ephemeral wetlands on Eglin Air Force Base, Florida. Dissolved oxygen, depth, and canopy cover measurements were recorded during 2014. Herbaceous vegetation cover and shrub density were measured during fall of 2013, and hydroperiod was calculated by averaging the longest yearly hydroperiod from 2010 – 2014 that occurred at least partially between October and April..... 119

Table A3. Vegetation structure in 21 ephemeral wetlands on Eglin Air Force Base was measured during the fall of 2013. Tree and shrub species were measured using the point-centered quarter method, and herbaceous vegetation species were recorded using a Daubenmire frame. .... 120

## Appendix B. Pooled amphibian dipnetting captures

Table B1. Total amphibian dipnetting captures from surveys of 21 ephemeral wetlands on Eglin Air Force Base, Florida. Wetlands were dipnetted up to 10 times per season from December – April in 2012 – 2014 when there was water in the wetlands. Sampling was reduced from twice a month to once a month if *A. bishopi* were captured in the wetlands that season, to avoid damage to the vegetation. Additional dipnetting was conducted along a transect set along each wetlands longest axis once a month from January – April 2014. .... 122

## Appendix C. Full AIC modeling results

Table C1. Multiple linear regression modeling results relating invertebrate abundance to vegetation characteristics and wetland hydrology in ephemeral wetlands on Eglin Air Force Base, Florida (K = the number of parameters, AICc = second-order Akaike’s Information Criteria,  $\Delta AICc$  = change in AICc, and  $w_i$  = relative amount of support for each model). .... 123

## LIST OF FIGURES

### **Chapter 1: Hindcasting historical breeding conditions for an endangered salamander in ephemeral wetland habitats of the southeastern USA: Implications of climate change since 1896**

Figure 1.1. Average monthly wetland depth from 2006 – 2013 for 17 *Ambystoma bishopi* breeding wetlands plotted alongside the average monthly precipitation over the same time period. Depth measurements were recorded bimonthly from October to April and once a month the rest of the year. Precipitation totals were downloaded for a single geographic point near the centroid of the study area from the PRISM climate group. .... 39

Figure 1.2. Measured hydroperiods from 2007 – 2012 for five characteristic *Ambystoma bishopi* breeding wetlands. Measurements were recorded on a monthly (May – September) and bimonthly (October – April) basis. Years are arranged from September – August, and grey lines are used to separate years. .... 40

Figure 1.3. Predicted hydroperiod for a ‘wet’ and ‘dry’ *Ambystoma bishopi* breeding wetland from 1896 – 2013 based on logistic regression model classifications of wetland conditions. Change points in average hydroperiod occurred during the 1970s and 1990s and are indicated by gaps in the solid lines above the graph. The numbers along the lines are the average hydroperiod for the ‘wet’ and ‘dry’ wetland respectively. The continuous horizontal line represents the hydroperiod below which *A. bishopi* cannot complete metamorphosis. .... 41

Figure 1.4. Percentage of years from 1896 – 2013 in which hydroperiod was likely insufficient for *Ambystoma bishopi* reproduction. Each bar represents a different breeding wetland arranged in order of increasing wetted area. Larval *A. bishopi* require between 3 – 5 months to successfully metamorphose from aquatic larvae into terrestrial adults. .... 42

### **Chapter 2: The effects of changing habitat structure in pine flatwoods wetlands on aquatic invertebrate and amphibian communities**

Figure 2.1. Schematic of sampling transect set along 21 ephemeral wetland’s longest axis. Points were spaced every 20 m in a majority of wetlands and every 40 m in five wetlands larger than 1.5 ha. .... 79

Figure 2.2. Percent canopy cover and herbaceous vegetation cover in wetlands occupied and unoccupied (sample sizes in parentheses) by A) Ornate Chorus Frogs (*Pseudacris ornata*) and B) Reticulated Flatwoods Salamanders (*Ambystoma bishopi*). Data were collected during 2013 – 2014 in 20 ephemeral wetlands on Eglin Air Force Base, Florida. Error bars represent standard error. .... 80

Figure 2.3. Aquatic invertebrate abundance recorded from January – April 2014 in high canopy and low canopy sections of six ephemeral wetlands on Eglin Air Force Base, Florida. Total invertebrate abundance for all wetlands (n = 21) sampled during the period is also included (light gray dots). Error bars represent standard error. .... 81

**Chapter 3: The effects of crayfish predation and vegetation cover on tadpole growth, survival, and nonlethal injury**

Figure 3.1. Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpole survival (A) and growth rates (B) in a mesocosm experiment with six treatments: high vegetation (68% coverage) with and without a predator, low vegetation (34% coverage) with and without a predator, and no vegetation with and without a predator. A single Panhandle Crayfish (*Procambarus evermanni*) was present in the predator treatment groups. .... 105

Figure 3.2. The frequency of Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpoles with tail injury in mesocosms containing a single Panhandle Crayfish (*Procambarus evermanni*). Mesocosms had three different amounts of herbaceous vegetation cover: high (68% coverage), low (34% coverage), and no vegetation. .... 106

Figure 3.3. Ornate Chorus Frog (*Pseudacris ornata*) tadpole survival rates in four treatments including high vegetation (80% coverage) with and without a predator and low vegetation (40% coverage) with and without a predator. A single Panhandle Crayfish (*Procambarus evermanni*) was present in the predator treatments. .... 107

**Appendix A. Study site information**

Figure A1. Photo of a pine flatwoods ephemeral wetland that has a fire-maintained vegetation structure (top), and a pine flatwoods ephemeral wetland that has a vegetation structure characteristic of long-term fire suppression (bottom). .... 121

## Appendix D. Photos of tail injury caused by crayfish

Figure D1. Non-lethal tail injury inflicted on a Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpole (top) by Panhandle Crayfish (*Procambarus evermanni*) in a mesocosm experiment..... 126

Figure D2. Non-lethal tail injury inflicted on an Ornate Chorus Frog (*Pseudacris ornata*) tadpole by Panhandle Crayfish (*Procambarus evermanni*) in an aquaria experiment. .... 127

## PREFACE

The Coastal Plain of the southeastern United States was historically dominated by longleaf pine (*Pinus palustris*) ecosystems. After European settlement these ecosystems experienced a dramatic decline due to widespread anthropogenic disturbance. Today, only around 776,000 ha (2% of the original coverage) of longleaf pine forests remain (Means 1996). The remaining forests are often highly fragmented and degraded to varying degrees because of the large human population in the southeastern U.S. Further, longleaf pine forests were historically maintained by regular growing-season fires that had a return interval of 1 – 3 years (Frost 1995). Regular growing-season fires clear the midstory of oaks and other hardwoods and increase the recruitment of longleaf pines and fire-dependent grasses (Brockway and Lewis 1997). During the 1920s and 1930s government agencies and local landowners began to work extensively to suppress wildfires in many places (Southard 2011). Although fire suppression is no longer as aggressive, roads and clearings now act as natural fire breaks, and reduce the extent of fires (Ware et al. 1993). This history of fire suppression has further degraded many longleaf pine forests by removing the natural disturbance regime and leading to changes in vegetation structure. Today, many longleaf pine forests are regularly burned using prescribed fires, but these fires are usually conducted during the winter and early spring, outside the natural lightning-ignition season and at a time when wetlands are typically inundated and will not burn successfully.

Pine flatwoods are a type of longleaf pine forest found in low-lying, poorly drained areas of the Coastal Plain. These lowland forests are characterized by a higher abundance of slash pines (*Pinus elliottii*) and the presence of ephemeral wetlands that can cover a large percentage of the landscape (Riekerk and Korhnak 2000). Pine flatwoods wetlands generally fill with water

during fall and winter rains, but then experience a dry period during spring and summer. A regular drying period makes ephemeral wetlands ideal breeding habitat for many species, especially amphibians. Large predatory fish are unable to persist through drying events, which lowers the predation pressure on amphibian larvae when compared to permanent water bodies (Skelly 1997). Pine flatwoods wetlands support a diverse amphibian assemblage including several rare species (Means et al. 2004).

My thesis research focuses on identifying how habitat changes affect ephemeral wetland communities along the Northern Gulf Coastal Plain. The wetlands included in the following study were located on Eglin Air Force Base in the Florida panhandle, and these wetlands were embedded in actively managed longleaf pine stands that regularly receive prescribed burns. In addition to prescribed burns, these wetlands have received various treatments as part of an ongoing adaptive management project aimed at reducing the impacts of long-term fire suppression on wetland vegetation structure (see Appendix A for a detailed description of treatments and other habitat information; Gorman et al. 2013). Adaptive management projects and a varied burn history have created wetlands with a wide range of habitat characteristics that are ideal for identifying relationships between aquatic communities and wetland habitat characteristics.

I focused parts of my study on two rare and declining amphibians, Reticulated Flatwoods Salamanders (*Ambystoma bishopi*) and Ornate Chorus Frogs (*Pseudacris ornata*). *Ambystoma bishopi* were listed as federally endangered in 2009 when *A. cingulatum* was split into two distinct species (Pauly et al. 2007; United States Department of the Interior, Fish and Wildlife Service (USFWS) 2009). The USFWS (2009) estimated that there are only 22 surviving populations and many of these populations were based on the capture of a single individual.

These small ambystomatid salamanders breed exclusively in ephemeral wetlands during late fall and early winter. Females deposit eggs in dry wetland basins, and eggs hatch once they are inundated by winter rains (Anderson and Williamson 1976). Larval *A. bishopi* require between 11-18 weeks to develop and metamorphose into terrestrial adults, which is a longer larval period than many ephemeral pond-breeding amphibians (Palis 1995). *Pseudacris ornata* are more common and widespread than *A. bishopi*, but populations are believed to be declining over much of their range. *Pseudacris ornata* often breed in habitats similar to *A. bishopi* and appear to be sensitive to some of the same habitat changes (Gorman et al. 2013).

For species that breed in ephemeral wetlands, the hydroperiod or length of inundation is one of the most important wetland characteristics determining breeding success, especially for species with long development times (e.g. *A. bishopi*). During years with a shortened hydroperiod it is possible for amphibians to experience complete reproductive failure with no recruitment to the population (Gorman et al. 2009). For the already rare *A. bishopi* these events could have severe negative impacts on the viability of a population. Therefore, I modeled the hydroperiod of historic *A. bishopi* breeding wetlands using climate data that could be projected back in time (Chapter 1). I then examined how changing hydroperiod could relate to a recent decline in the number of occupied breeding wetlands and assessed how future climate changes are likely to affect wetland hydroperiods. Future climate change models predict that the southeastern United States will experience an increase in average temperature leading to an increased frequency of drought, but will also experience an increase in the total amount of precipitation (Karl et al. 2009; Strzepek et al. 2010; Ingram et al. 2013; Romero-Lankao et al. 2014). However, precipitation patterns are likely to become more unpredictable and come in

more severe weather events. These changes will likely have a direct impact on ephemeral wetland hydrology (Brooks 2009).

In addition to changing hydrology, vegetation shifts caused by long-term fire suppression are likely to impact ephemeral wetland communities, especially sensitive species. Prescribed burns often do not penetrate wetland basins because wetlands are typically inundated at the time of year when prescribed fires are set (Bishop and Haas 2005). Absence of fire in pine wetlands leads to the development of a thick woody vegetation midstory and a reduction in the amount of herbaceous vegetation in the wetland basin (Hinman and Brewer 2007; Martin and Kirkman 2009). The effects of habitat change on ephemeral wetland communities is poorly studied in pine wetlands (Jones et al. 2010). Therefore, I quantified the habitat structure and biotic communities (amphibian and invertebrate) in a variety of ephemeral wetlands (Chapter 2). My goals were to identify the habitat characteristics that had a strong effect on invertebrate abundance and identify how decreased herbaceous vegetation and increased canopy cover influenced *A. bishopi* and *P. ornata*. Finally, I examined how a shift in habitat structure, in this case a decrease in herbaceous vegetation, can influence tadpole growth rate and survival in the presence of a predator (Chapter 3). I tested the vegetation effects on two species of tadpole, Southern Leopard Frogs (*Lithobates sphenoccephalus*) and *P. ornata*.

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## CHAPTER 1

### **Hindcasting historical breeding conditions for an endangered salamander in ephemeral wetland habitats of the southeastern USA: Implications of climate change since 1896**

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#### **ABSTRACT**

The hydroperiod of ephemeral wetlands is often the most important characteristic determining amphibian breeding success, especially for species with long development times. In mesic and wet pine flatwoods of the southeastern United States, ephemeral wetlands are a common landscape feature supporting high amphibian diversity. Reticulated Flatwoods Salamanders (*Ambystoma bishopi*), a federally endangered species, depend exclusively on ephemeral wetlands and require at least 11 weeks to successfully metamorphose into terrestrial adults. We empirically modeled hydroperiod of 17 *A. bishopi* breeding wetlands by combining historical climate model data with a recent 7-year record (2006 – 2012) of observed water levels. Empirical models were subsequently used to reconstruct wetland hydrologic conditions from 1896 – 2013 using the historical climate datasets. Reconstructed hydroperiods for the 17 wetlands were highly variable through time, but were frequently unfavorable for *A. bishopi* reproduction (e.g. only 53% of years, using the most conservative estimate of development time, were conducive to larval development). Using change point analysis, we identified significant shifts in average hydroperiod over the last century for 15 of 17 wetlands that generally indicate a trend towards shorter hydroperiods in recent years, and thus less suitable reproductive conditions. We suggest that climate change will play an increasing role in future reproductive

success of *A. bishopi* by altering the number of years with suitable hydroperiods. Consequently, we emphasize the importance of conservation and management for reducing other forms of habitat degradation, especially maintenance of networks of high quality breeding wetlands where reproduction can occur during appropriate environmental conditions.

**Keywords:** ephemeral wetlands, *Ambystoma bishopi*, change point analysis, hydroperiod, amphibians, reticulated flatwoods salamander

## INTRODUCTION

Small ephemeral wetlands are ecologically important systems that characterize a variety of landscapes worldwide (Skelly 1996; Finlayson and Spiers 1999; Johnson et al. 2005). These wetlands often support unique taxa, especially as sites for reproduction, and are thus important for linking aquatic and terrestrial environments (Snodgrass et al. 2000; Russell et al. 2002). Because of their ephemeral nature, hydroperiod (i.e., length of inundation between drying events) plays a key role in driving species composition and biological success in most ephemeral wetlands (Schriever and Williams 2013). Species inhabiting ephemeral wetlands frequently experience tradeoffs between reduced predation pressure and increased risks of mortality from desiccation (Skelly 1997). Regular drying events extirpate predatory fish populations and reduce the rate at which slow-developing predatory invertebrates become established in ephemeral wetlands, which lowers the predation pressure on other aquatic organisms (Turner and Montgomery 2009; Walls et al. 2013a). However, short hydroperiods also increase the risk for direct mortality caused by drying events (Semlitsch and Wilbur 1988). Larval amphibians require minimum hydroperiods in order to successfully metamorphose into terrestrial adults

(Pechmann et al. 1989), and aquatic invertebrates require a hydroperiod long enough to reproduce and reach a desiccation-resistant stage (Sim et al. 2013).

Hydroperiods in small, isolated ephemeral wetlands are predominantly influenced by precipitation and evapotranspiration (Lide et al. 1995; Mansell et al. 2000; Brooks 2004). For example, in the southeastern United States coastal plain physiographic province, a majority of the annual precipitation feeding into an ephemeral wetland can be lost through evapotranspiration (Lu et al. 2009). Evapotranspiration rates are directly influenced by air temperature, with higher temperatures normally leading to higher evapotranspiration rates (Leibowitz and Brooks 2008). Ephemeral wetlands in pine flatwoods can also serve as sources or sinks for groundwater flow depending on geologic conditions of the surrounding landscape, but this usually has a smaller influence on hydroperiod (Crownover et al. 1995; Lu et al. 2009). The strong influence of short-term weather events and lack of a consistent groundwater component in ephemeral wetland hydrology makes these systems susceptible to changes in temperature and precipitation patterns that result from climate change (Brooks 2009).

Pine flatwoods, a common forest type, are found along the coastal plain of the southeastern United States in low-lying, poorly drained areas (Means 1996). Embedded within this ecosystem are shallow ephemeral wetlands that have relatively open canopies and abundant herbaceous vegetation (Kirkman 1995; Gorman et al. 2013). These wetlands typically fill with water during fall or winter rains when evapotranspiration rates are low but normally experience a dry period during the spring or summer when evapotranspiration rates are high (Riekerk and Korhnak 2000; Sun et al. 2002). Climate change models predict that the southeastern United States will likely face longer periods of drought, a more unpredictable rainfall pattern, and higher evapotranspiration rates (Mulholland et al. 1997; Karl et al. 2009; Strzepek et al. 2010; Ingram et

al. 2013; Romero-Lankao et al. 2014). Climatic changes will undoubtedly alter hydrologic processes in these ecosystems (Brooks 2009) and thus represent a significant challenge to species endemic to these environments.

Ephemeral wetlands in pine flatwoods provide crucial breeding habitat for a diverse amphibian assemblage (Dodd and Cade 1998; Means et al. 2004). This includes the federally endangered Reticulated Flatwoods Salamander (*Ambystoma bishopi*), which was officially listed in 2009, after it was recognized as a separate species from the closely related Frosted Flatwoods Salamander (*Ambystoma cingulatum*; Pauly et al. 2007; U.S. Fish and Wildlife Service 2009). Adult flatwoods salamanders migrate from the surrounding uplands to breeding wetlands on rainy nights during October – December (Anderson and Williamson 1976; Palis 1997). Females lay eggs in dry wetland basins, and the eggs hatch after they are inundated by winter rains (Anderson and Williamson 1976; Hill 2013). Larval salamanders then require between 11 – 18 weeks to fully develop and metamorphose into adults (Palis 1995a). This relatively long larval period makes flatwoods salamander populations susceptible to complete reproductive failure not only during severe droughts, but also during years where wetlands have a shortened or variable hydroperiod.

Well-documented worldwide amphibian declines have been attributed to many factors including climate change (Carey and Alexander 2003; Stuart et al. 2004). In ambystomatid salamanders, population declines and reproductive failures can be caused by shortened breeding wetland hydroperiods during drought years (Daszak et al. 2005; Taylor et al. 2006; Church et al. 2007; Walls et al. 2013b). For example, recent population declines in *A. cingulatum* were linked to complete reproductive failure during consecutive drought years (Palis et al. 2006; Westervelt et al. 2013). Furthermore, declines in the adult breeding population can continue to negatively

impact reproductive success even during years with favorable breeding conditions (McMenamin et al. 2008), and large percentages of some ambystomatid salamander species appear to skip migrations to breeding wetlands even during years with adequate rainfall (Kinkead and Otis 2007).

The goals of this study were to: 1) characterize the hydroperiod of *A. bishopi* breeding wetlands during recent years (2006-2013); 2) generate empirical models capable of predicting historical hydroperiods of *A. bishopi* breeding wetlands; 3) identify temporal changes in wetland hydroperiods over the last century, and 4) identify any correlation between change points in hydroperiod and recent *A. bishopi* population declines. We predicted that historical weather conditions, and therefore historical hydroperiods, were once more favorable (i.e. longer) for successful flatwoods salamander reproduction and that the frequency and severity of drought periods has increased since flatwoods salamanders were first documented on Eglin Air Force Base in the early 1990s.

## **MATERIALS AND METHODS**

### *Study Site*

Ephemeral wetlands were identified as *A. bishopi* breeding wetlands during surveys conducted in the 1990s on Eglin Air Force Base in Okaloosa and Santa Rosa counties, Florida (Palis 1995b; Palis 1997). Eglin AFB is a large U.S. military installation covering over 186,000 ha, of which 146,000 ha are actively managed longleaf pine forests. Beginning in the early 2000s, we have continuously monitored 17 of the *A. bishopi* breeding wetlands (Bishop 2005; Gorman et al. 2009). The most recent surveys (2003-2013) have documented *A. bishopi* larvae in only 10 of the original 17 wetlands despite increased survey effort, 11 years of survey data,

and habitat protection on Eglin AFB (Gorman et al. 2009). Study wetlands typically had a longleaf pine (*Pinus palustris*) and slash pine (*Pinus elliottii*) overstory with an understory of herbaceous plants, but some wetlands had developed a woody midstory, as a result of historic fire suppression (Kirkman 1995; Gorman et al. 2013; Appendix A).

### *Data Collection*

Based on accumulated experience from sampling the 17 wetlands for *A. bishopi* larvae, a metal stake was anchored in place at the approximate centroid of each wetland. We measured water depth at this point in each wetland from November 2005 – May 2013. Initially, we recorded measurements monthly, but in November 2007, measurement frequency was increased to twice a month. For each wetland, we calculated a maximum observed depth (based on our measurements) and maximum wetted area. Wetted areas were calculated by walking the border of each wetland with a handheld GPS. GPS tracks were imported into ArcGIS 9 (Environmental Systems Research Institute, Redlands, CA) where they were converted to polygon files, which were used to calculate the area of each wetland.

We used the recorded depth measurements to describe hydroperiods for each wetland. We identified all instances of wetland filling and drying during our study period and calculated the longest hydroperiod in each breeding season (i.e., from November – May). However, dates outside of this monthly range were included if there was a continuous hydroperiod that started before or ended after the breeding season (dates were truncated to begin on September 1 if wetlands were wet before then). We used these dates to calculate mean and median filling and drying dates and an average observed hydroperiod for each wetland from 2006 – 2013. Depth measurements were converted into a binary code where months in which wetlands held water

were coded as 1, and months where wetlands had no water at the center stake were coded as 0 (months with two measurements were coded as 0 if there was no water at the center stake during either measurement because a single drying event would likely cause mortality for larval salamanders).

We downloaded modeled, historic climate data for our study wetlands from the PRISM climate database (Oregon State University, <http://prism.oregonstate.edu>, created 10 February 2013). Briefly, PRISM utilizes climatologically-aided interpolation to reconstruct long-term (1895-present) climate normals for a geographic point of interest. Ultimately modeled climate conditions for a geographic point of interest are based on interpolations of climate data from whatever weather stations and data sources are available for the period of interest. We used PRISM climate data for a single geographic coordinate (30.439°, -86.793°), representing the centroid of our study area. All 17 wetlands used in our analysis were within 12.5 km of this point. The PRISM dataset contained monthly estimates of precipitation totals, monthly maximum temperature, and monthly minimum temperature. We also included all three climate variables lagged by one month so that the previous month's climate data aligned with the water-level data (six climate observations per month). Only data from November through May were included in the final analyses because by late November most adult *A. bishopi* would have migrated to breeding wetlands, and larvae occur in wetlands from December through April or May (Haas and Gorman, unpublished data).

### *Statistical Analyses*

We used a principal component analysis (PCA) to reduce climate variables to principal components with an eigenvalue > 0.9. We constructed 17 logistic regression models, one for

each of the 17 *A. bishopi* breeding wetlands, using principal component scores derived from the PCA and the water-level data for each wetland from the 2006 – 2012 breeding seasons (the year refers to the January – May portion of the breeding season). Models classified each wetland as being wet or dry on a monthly basis during the *A. bishopi* breeding period. Water-level data from the November 2012 – May 2013 breeding season were used as a validation dataset and were therefore not used in model construction. We further validated models with another dataset of water-level data independently collected during *A. bishopi* surveys from 1993 – 1994 (Palis 1995b) and 2003-2005 (Bishop 2005).

We used modeled wet/dry classifications from each logistic regression to calculate hydroperiods (defined as the longest period of consecutive wet months) for each wetland in every breeding season (November – May) from 1896 – 2013. We performed a bootstrapping analysis to generate 95% confidence intervals for hydroperiod predictions in each year. The initial dataset (PCA components and wet/dry data) was sampled with replacement 5,000 times, and a new logistic regression model was created with each replacement sample. We then used the 5,000 bootstrap iterations to generate confidence intervals around logistic regression parameter estimates. Pairwise correlations were used to make comparisons between wetland hydroperiods, and simple linear regressions were used to compare average length of a wetland's hydroperiod against wetted area of that wetland and the wetland's maximum measured depth. We also used modeled hydroperiods to determine the number of years that would be unsuitable for flatwoods salamander reproduction. An unsuitable breeding year was defined as a year where the hydroperiod was too short for successful reproduction based on three different lengths of time: 3 months, 4 months, and 5 months (Palis 1995a).

Lastly, we performed a change-point analysis (CPA) on the calculated hydroperiod data for each wetland. Briefly, change-point analysis identifies points in time-ordered data where the statistical properties of those data have changed (Basseville and Nikiforov 1993). We used methods described by Taylor (2000) to identify points where the mean hydroperiod of studied wetlands may have shifted. This approach uses cumulative sum charts to estimate when a change in mean has occurred by identifying abrupt changes in cumulative sum values. To generate a confidence estimate for identified change points, the original dataset is resampled, creating a random reordering of the data. This process is repeated using a large number of bootstrap samples that are then used to calculate a confidence estimate for the identified change point. If a change is identified, the time series is split in half and the analysis is repeated to identify additional change points. For our analyses, we used 100,000 bootstrap iterations to generate confidence estimates and only reported changes that had a 90% or greater probability of occurrence. All change point analyses were conducted using Change Point Analyzer (Taylor Enterprises Inc., Libertyville, IL, USA). Confidence intervals for hydroperiod estimates were generated in R (R Core Team 2014), and all other analyses were performed in SAS JMP (SAS Institute Inc., Cary, NC, USA).

## RESULTS

Empirically-measured water levels were available to characterize wetland hydrology from 2006 – 2013. Over this eight-year period, wetlands were usually deeper during the *A. bishopi* breeding season (November – May) despite an increase in precipitation during summer months (Figure 1.1). Wetland fill dates varied from year to year. However, average fill dates usually occurred during January, while average dry dates generally occurred during April or May

(Table 1.1). Hydroperiods at the 17 study wetlands also varied among wetlands and across years (Figure 1.2). Multiple salamander breeding seasons were characterized by severe drought with the worst occurring from 2006 – 2007 (all wetlands dry for 18 months). However, we also observed years where wetlands were full for the entire salamander breeding season (Figure 1.2).

Local climate data were summarized into three principal components that had eigenvalues of  $> 0.9$  and together explained 85.3% of the variation in the data (Table 1.2). These three principal components were therefore selected to be included in our logistic regression models (PC1 = 3.04, 50.7%; PC2 = 1.14, 19.0%; PC3 = 0.93; 15.6%). The first PC was positively correlated with maximum and minimum monthly temperatures and with the previous month's maximum and minimum temperatures (Table 1.2). PC2 was positively correlated with the previous month's precipitation and less strongly correlated with monthly precipitation and minimum temperature. Finally, PC3 was positively correlated with the monthly precipitation. Logistic regression models for all 17 wetlands were statistically significant and, on average, correctly classified 79.3% of months from January 2006 to May 2012 correctly (based on training data; Table 1.3). Using independent water-level data as validation datasets, models correctly classified 180 out of 253 (71.2%) months including 102 out of 155 (65.8%) wet months, and 78 out of 98 (79.6%) dry months.

Modeled hydroperiods over the past seven years were not significantly different from measured hydroperiods over the same time period (2.04 (SE = 0.15) months modeled compared to 2.35 (SE = 0.21) months actual;  $t_{248} = 1.22$ ;  $P = 0.222$ ). Average predicted wetland hydroperiod from 1896 – 2013 ranged from 1.49 to 3.95 months (reflecting the variation among wetlands). The 95% confidence intervals for hydroperiod predictions ranged from 2.03 to 2.97 months with an overall average of 2.61 months. In addition to the variation among wetlands,

there was also substantial variation in modeled hydroperiod through time (Figure 1.3). Furthermore, modeled hydroperiods for 16 of the 17 wetlands were significantly correlated with each other ( $P < 0.0001$ ). Wetted areas ranged from 0.4 ha to 20.9 ha, and wetlands with larger areas tended to have longer average hydroperiods ( $R^2 = 0.27$ ;  $P = 0.031$ ). However, there was no relationship between a wetland's maximum recorded depth and modeled hydroperiods ( $P = 0.857$ ).

Overall, a majority of years from 1896 – 2013 were unsuitable for *A. bishopi* reproduction based on modeled hydroperiods (Figure 1.4). In addition to the overall variability in hydroperiod, we detected a change in average hydroperiod for 15 of 16 wetlands (data from one wetland did not fit a mean shift model, and these data were excluded from the analysis; Table 1.4). Four wetlands shifted to a longer average hydroperiod around 1958, and one wetland shifted to a longer hydroperiod around 2008. The remaining 10 wetlands shifted to a longer hydroperiod during the 1970s, but then experienced a second shift to a much shorter hydroperiod during the late 1990s (Figure 1.3). Average hydroperiods after this second shift were shorter than the hydroperiods before 1970 in all 10 wetlands (i.e., averages after the last shift were, on average, 0.39 months shorter than at any time during the last 118 years).

## DISCUSSION

This is the first study to examine hydroperiod fluctuations in ephemeral wetlands of the southeastern United States over the past century. Our model predictions indicate that a majority of wetlands (59%) experienced multiple shifts in the average hydroperiod during the past 118 years (Table 1.4; Figure 1.3), and all ten wetlands with multiple change points have experienced the shortest average hydroperiod from the late 1990s – 2013. Furthermore, other wetlands

involved in the study were also drier over the past 15 years than from the 1970s – 1990s, but to a lesser extent. Changes in hydroperiod were likely driven by similar changes in average monthly precipitation during the *A. bishopi* breeding period (109 mm per month from 1896 – 1976, 132 mm per month from 1977 – 1999, and 103 mm per month from 2000 – 2013). Shifts in ephemeral wetland hydroperiods are important because these wetlands provide critical breeding habitat to numerous plant and animal species (Snodgrass et al. 2000; Russell et al. 2002). Hydroperiod is often the most important characteristic driving species composition and breeding success, especially for slow-developing amphibian species like *A. bishopi* (Semlitsch and Wilbur 1988; Pechmann et al. 1989; Schriever and Williams 2013).

The shortened hydroperiods coincide with a recent decline in the number of wetlands occupied by *A. bishopi*, despite increased survey effort (Bishop 2005; Gorman et al. 2009). Average hydroperiod across all wetlands during the initial *A. bishopi* surveys (1993-1994) was 3.6 months compared to 2.3 months from 2003 – 2013 (when current surveys were conducted). We also documented one breeding season (2006 – 2007) where none of the study wetlands filled and several others where hydroperiods in most wetlands were unsuitable for *A. bishopi* reproduction. Others have documented complete reproductive failure and decreases in the adult population size of *A. cingulatum* populations after consecutive drought years during the early 2000s (Palis et al. 2006; Westervelt 2013). Similarly, severe droughts leading to pond drying increased extinction probabilities for Mole Salamanders (*Ambystoma talpoideum*) in northern Florida during the same time period (Walls et al. 2013b). These results therefore support the hypothesis that an increased frequency and severity of drought has contributed to Reticulated Flatwoods Salamander population declines in the Florida Panhandle.

Despite shorter hydroperiods and apparent population declines in recent years, modeled reconstructions of wetland hydroperiods indicated that approximately 53% of breeding seasons over the past 118 years have likely been insufficient for flatwoods salamander reproduction (Figure 1.4). Even if all larval salamanders were able to successfully metamorphose in only three months (the shortest time required to complete metamorphosis; Palis 1995a), only seven wetlands would have had a suitable hydroperiod in > 50% of breeding seasons. This demonstrates that *A. bishopi* populations are naturally adapted to persist through drought years when complete reproductive failure often occurs, which has been documented during recent years (Figure 1.2; Gorman et al. 2009). During years with little or no recruitment, any reduction in adult survival rates would have severe negative impacts on the ability of populations to persist (Palis et al. 2006; Taylor et al. 2006). Little is known about adult *A. bishopi* after they emigrate from breeding wetlands, and information on habitat requirements, foraging success, and survival in the uplands (especially with rising temperatures) deserves further study. However, in captivity, adult flatwoods salamanders lived for no longer than four years (Palis and Means 2005). Even though other species of ambystomatid salamanders can live longer (Petranka 1998), some studies suggest that many adult ambystomatids may only breed once (Trenham et al. 2000; Church et al. 2007). A short adult life span, potential negative effects of increased temperature on growth and survival (Rohr and Palmer 2013), and generally low reproductive output across ambystomatid salamanders increases the susceptibility of flatwoods salamander populations to rising drought severity and habitat changes that negatively affect larval, juvenile, or adult survival.

The frequency and severity of drought across the southeastern United States is projected to increase over the next century due to rising temperatures (Karl et al. 2009; Romero-Lankao et

al. 2014). In northern Florida, both minimum and maximum temperatures are projected to increase by around 1.7° to 2°C over the next century, which will increase evapotranspiration rates in pine flatwoods wetlands (Sun et al. 2009; Ingram et al. 2013; U.S. Geological Survey 2013). This region is also projected to experience an increase in total rainfall over the next century, but this increased rainfall is likely to come more unpredictably in severe weather events (Mulholland et al. 1997; Karl et al. 2009; Ingram et al. 2013; Romero-Lankao et al. 2014). Given the flat topography, flash-flooding in the Florida Panhandle can introduce fish populations to ephemeral wetlands, and increased connectivity with permanent water bodies may also increase the risk of disease. Walls et al. (2013a; 2013b) constructed a conceptual model outlining the potential effects that increased rainfall and more frequent drought could have on *A. talpoideum*. These effects would likely be similar for *A. bishopi*, although *A. bishopi* are less likely to be able to colonize new wetlands because of their reproductive strategy (i.e. terrestrial egg laying). Overall, given the percentage of dry years since 1896 and the shortening of hydroperiods after the late-1990s, it is likely that any benefits of increased precipitation will be offset by less predictable hydroperiods and a higher frequency of years with reproductive failure. The predictability of hydroperiods is more important for flatwoods salamanders than other ambystomatid salamanders that rely on the presence or depth of water to identify breeding sites.

Precipitation and evapotranspiration rates are often the two most important factors influencing the hydrology of small, isolated ephemeral wetlands (Lide et al. 1995; Brooks 2004). The importance of evapotranspiration rates is reflected in the summer drying period despite an increase in precipitation over the same time period (Figure 1.1; Table 1.1). Temperature also had a significant effect in 11 of 17 logistic regression models (PC 1 was strongly correlated with the maximum and minimum temperature in the current and previous months; Table 1.2). Monthly

precipitation was actually less important in determining whether or not a wetland held water than previous month's precipitation (PC2 significant effect in all 17 models, PC3 never significant). This effect is perhaps unsurprising given that current water table heights are often integrating climatological and environmental processes occurring over periods greater than one month's time. Although precipitation and evapotranspiration are the primary determinants of hydroperiod in pine flatwoods wetlands (Lu et al. 2009, Martin and Kirkman 2009), it is also possible that groundwater flow patterns could have some influence on wetland hydroperiods in the study area. However, the influence of groundwater flow on pine flatwoods wetland hydrology can be variable (Lu et al. 2009), and further research would be needed to identify the role of groundwater flow in these wetlands. Overall, using climate data as predictor variables allowed us to accurately classify when wetlands would hold water and generate long-term hydroperiod predictions that would not have been possible using other data sources.

There are several factors that could have impacted the accuracy of our models. First, water levels were measured at only a single approximate center point in each wetland, which did not necessarily correspond to the deepest point in the wetland. It is possible that water was present in small amounts in other parts of the wetland but not at the water level stake. Second, the data collected for our models were only collected twice a month at most, and monthly climate data were used to build models. The resolution of the climate data and the measured water levels means that our models cannot make predictions on finer spatiotemporal scales. For example, ephemeral wetlands do not necessarily fill or dry at the beginning or end of the month even though our predictions suggest this dynamic. Third, it is possible that larval salamanders are able to prolong the effective hydroperiod of these wetlands by retreating into crayfish burrows or other similar holes that provide access to the groundwater (Ashton 1992). This

would represent an important behavioral adaptation that could allow salamanders to successfully reproduce even in years with an intermittent hydroperiod. Finally, there are other factors that can influence the hydroperiod of ephemeral wetlands that were not included in our models. For example, vegetation structure can influence evapotranspiration rates, and a history of fire suppression has changed the vegetation structure in many of the study wetlands (Gorman et al. 2013). There is no record of vegetation structure in the study wetlands during the early 1900s, but extensive fire suppression was uncommon until the 1930s and fire was still a common tool used to manage forests during the early 1900s (Southard 2011), so it is likely that wetlands had more suitable vegetation during the early 1900s. Therefore, actual changes in hydrology from the early 1900s to the early 2000s are, if anything, likely to have been greater than those shown by the climate-based model.

The modeling technique that we applied in this paper has applications for a wide variety of other systems around the globe. Ephemeral wetlands or ecologically similar habitats (e.g., ephemeral streams and floodplains) occur in many ecosystems (Finlayson and Spiers 1999), and these systems are often directly influenced by climatic variation (Brooks 2009). Thus, there are many organisms including other salamanders breeding in ephemeral wetlands (Russell et al. 2002; Church et al. 2007; McMenemy et al. 2008), fish inhabiting floodplain lakes (Rypel et al. 2012), aquatic invertebrates in ephemeral habitats (Sim et al. 2013), and even wetland plants (Miller and Zedler 2003) that face similar ecological challenges to the ones described in this paper. More generally, suitable breeding conditions could be modeled using similar techniques for any species that depends, at least in part, on some optimum set of climatic circumstances. Linking historical climatological variations to periods of time when a species experiences

suitable breeding conditions will allow managers to better understand how future climate changes are likely to impact populations.

The frequency of years with an unsuitable hydroperiod for *A. bishopi* reproduction implies that populations are susceptible to habitat changes that reduce recruitment or adult survival (e.g., Palis et al. 2006). The continued and increasing influences of climate change across the southeastern United States will likely intensify this effect. Longleaf pine forests are one of the most endangered ecosystems in the world, and there are numerous anthropogenic changes that can negatively impact breeding and upland habitat including fire suppression, logging, sedimentation, altered hydrology from ditching or well-drilling, and road construction or other increases in impervious surfaces (Means et al. 1996; Palis 1996; Gorman et al. 2013). The long history of fire suppression and the inability of dormant-season fires to burn through wetland basins has altered the vegetation structure in many of the remaining pine flatwoods wetlands (Kirkman 1995; Bishop and Haas 2005). Altered vegetation structures could exacerbate the shortened hydroperiods observed during recent years by reducing habitat quality, and potentially altering wetland hydrology by affecting evapotranspiration rates (Bishop and Haas 2005). Even though prescribed burns are common on Eglin Air Force Base, these burns are typically conducted during dormant-season when wetland basins are inundated. Growing-season burns may be possible in some cases, especially if wetland basins are surrounded by previously burned areas. However, there are often constraints on growing-season burns because of challenges controlling the fire, air quality issues in the case of a “muck fire” (when organic matter in dry wetland basins burns), and weather related constraints (i.e. the wind direction must be suitable to blow smoke away from human development). A priority management goal should be to focus on supporting natural vegetation structure in wetlands by insuring that wetland basins

are regularly burned and potentially removing woody vegetation from wetlands that are already overgrown (Martin and Kirkman 2009; Gorman et al. 2013; Skelly et al. 2013).

Increasing spatial and temporal variation in ephemeral wetland hydroperiods is also generally considered to be an important conservation management strategy (Semlitsch and Bodie 1998; Snodgrass et al. 2000). Breeding in multiple wetlands with variable hydroperiods may increase the chances of some successful reproduction for a species even during years with moderate hydroperiods. It is unlikely that even wetlands experiencing the best management practices will have a suitable hydroperiod during severe drought years. Thus, we recommend that managers focus on maintaining or restoring breeding wetlands that have high quality vegetation structures, unaltered hydrology, and support the longest possible hydroperiod during moderate years. We found that larger wetlands (> 3.5 ha) tended to have longer hydroperiods, although there were exceptions and our sample size was small. Larger wetlands may be more likely to support flatwoods salamander reproduction in moderate years, and even low recruitment in a few wetlands during moderate years reduces the potential for prolonged droughts to result in local extirpations. Finally, managing pine flatwoods landscapes to support a diversity of wetlands that are not isolated from each other increases the chances that a portion of the population can persist through severe drought to recolonize wetlands that experience local extirpations.

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Table 1.1. Mean and median filling and drying dates for 17 *Ambystoma bishopi* breeding wetlands in northwest Florida. Water levels were measured at an approximate wetland centroid from 2006 – 2013. The range of fill dates was September 1 – April 8, and the range of dry dates was January 21 – October 21 of the following fall.

Wetland I.D.	Wetland Area (ha)	Mean Hydroperiod (Months)	Date of Filling		Date of Drying	
			Mean	Median	Mean	Median
3	4.6	1.7	Jan. 26	Feb. 9	Apr. 8	Apr. 8
4	0.4	1.8	Jan. 22	Feb. 9	Apr. 2	Apr. 5
13	0.2	2.0	Jan. 25	Jan. 19	Apr. 7	Mar. 28
33	1.5	2.1	Jan. 26	Feb. 26	Apr. 10	Mar. 28
5	0.4	2.2	Jan. 28	Feb. 8	Apr. 15	Apr. 8
53	4.9	2.7	Jan. 21	Feb. 8	Apr. 25	Apr. 21
41	0.9	2.9	Jan. 10	Jan. 22	May 10	May 1
36	1.4	3.0	Dec. 30	Jan. 12	Apr. 10	Apr. 11
12	1.7	3.1	Jan. 26	Feb. 26	May 16	Apr. 8
19	5.9	3.1	Jan. 21	Feb. 22	May 5	Apr. 21
14	1.0	3.2	Jan. 10	Jan. 29	May 20	May 3
16	1.7	3.2	Jan. 19	Feb. 8	May 8	Apr. 8
2	1.1	3.3	Jan. 12	Feb. 8	May 4	Apr. 25
30	5.3	3.6	Jan. 10	Feb. 8	May 11	May 10
21	20.9	4.3	Dec. 31	Jan. 19	May 25	May 2
15	3.6	4.4	Dec. 24	Jan. 12	May 27	May 10
18	10.8	5.1	Dec. 24	Dec. 18	Jun. 17	Jun. 3

Table 1.2. Factor loadings for three principal components from the PCA of six climate variables used in modeling wetland hydroperiod. Eigenvalues were 3.0, 1.1, and 0.9 for principal components 1, 2, and 3, respectively. Other principal components were not included because their eigenvalues were less than 0.9.

	PC1	PC2	PC3
Max Temp (°C)	0.88	0.30	0.06
Min Temp (°C)	0.84	0.37	0.01
Precipitation (mm)	-0.25	0.39	0.89
Lagged Max Temp (°C)	0.86	-0.31	0.17
Lagged Min Temp (°C)	0.87	-0.20	0.13
Lagged Precipitation (mm)	-0.04	0.79	-0.32

Table 1.3. Model results for 17 logistic regression models that predicted whether or not historically active *Ambystoma bishopi* breeding wetlands were wet or dry on a monthly basis based on historic climate data. PC1, PC2, and PC3 were the three principal components that were used to construct models. The percent correct column indicates the number of months the models classified correctly from the training data.

Wetland I.D.	LL	P-value	R <sup>2</sup>	PC1 P	PC2 P	PC3 P	% Correct
2	18.0	< 0.0001	0.60	0.02	0.001	0.20	83
3	15.8	< 0.0001	0.55	0.03	0.004	0.06	83
4	14.9	0.0003	0.51	0.01	0.01	0.33	85
5	22.4	0.0058	0.33	0.03	0.02	0.63	75
12	22.6	0.0017	0.38	0.16	0.003	0.30	75
13	21.5	0.0012	0.40	0.05	0.01	0.23	75
14	22.5	0.0005	0.42	0.05	0.002	0.71	77
15	18.9	< 0.0001	0.58	0.05	0.001	0.36	85
16	20.7	0.0001	0.49	0.07	0.001	0.36	81
18	16.3	< 0.0001	0.65	0.01	0.002	0.70	85
19	20.8	0.0001	0.49	0.13	0.001	0.22	79
21	18.0	< 0.0001	0.61	0.01	0.002	0.47	83
30	21.7	< 0.0001	0.49	0.04	0.002	0.76	77
33	17.8	0.0002	0.50	0.06	0.003	0.10	83
36	19.8	< 0.0001	0.55	0.01	0.002	0.31	85
41	23.0	0.0057	0.33	0.11	0.01	0.98	67
53	22.6	0.0018	0.38	0.08	0.004	0.31	72

Table 1.4. Results of change-point analyses of modeled hydroperiods for 15 *Ambystoma bishopi* breeding wetlands. Change points indicate periods where the average hydroperiod has significantly shifted.

Wetland I.D.	Year	Confidence Interval	Confidence Level	Average Hydroperiod Before Shift	Average Hydroperiod After Shift
2	1973	(1959, 1984)	99%	2.6	4.0
	1999	(1986, 2008)	93%	4.0	2.5
3	1978	(1961, 1993)	97%	1.6	2.7
	1999	(1985, 2005)	94%	2.7	1.1
4	1958	(1940, 2002)	100%	1.1	1.9
5	1977	(1970, 1986)	100%	1.6	3.2
	1999	(1992, 2000)	100%	3.2	1.1
12	1978	(1961, 1988)	96%	2.0	3.3
	1999	(1992, 2004)	98%	3.3	1.5
13	1978	(1964, 1987)	94%	1.9	3.2
	1999	(1991, 2004)	97%	3.2	1.5
14	1958	(1920, 2005)	98%	2.3	3.0
16	1978	(1960, 1987)	97%	2.5	3.9
	1999	(1993, 2003)	99%	3.9	1.9
18	2008	(1993, 2012)	91%	2.8	4.7
19	1978	(1958, 1987)	96%	2.4	3.7
	1999	(1992, 2005)	99%	3.7	1.9
21	1958	(1932, 1987)	99%	3.3	4.3
33	1978	(1961, 1991)	92%	1.7	3.0
	1999	(1989, 2004)	95%	3.0	1.3
36	1958	(1921, 1998)	96%	2.7	3.6
41	1978	(1970, 1982)	100%	2.0	3.6
	1999	(1995, 2002)	100%	3.6	1.7
53	1978	(1960, 1988)	96%	2.0	3.3
	1999	(1988, 2006)	92%	3.3	1.8

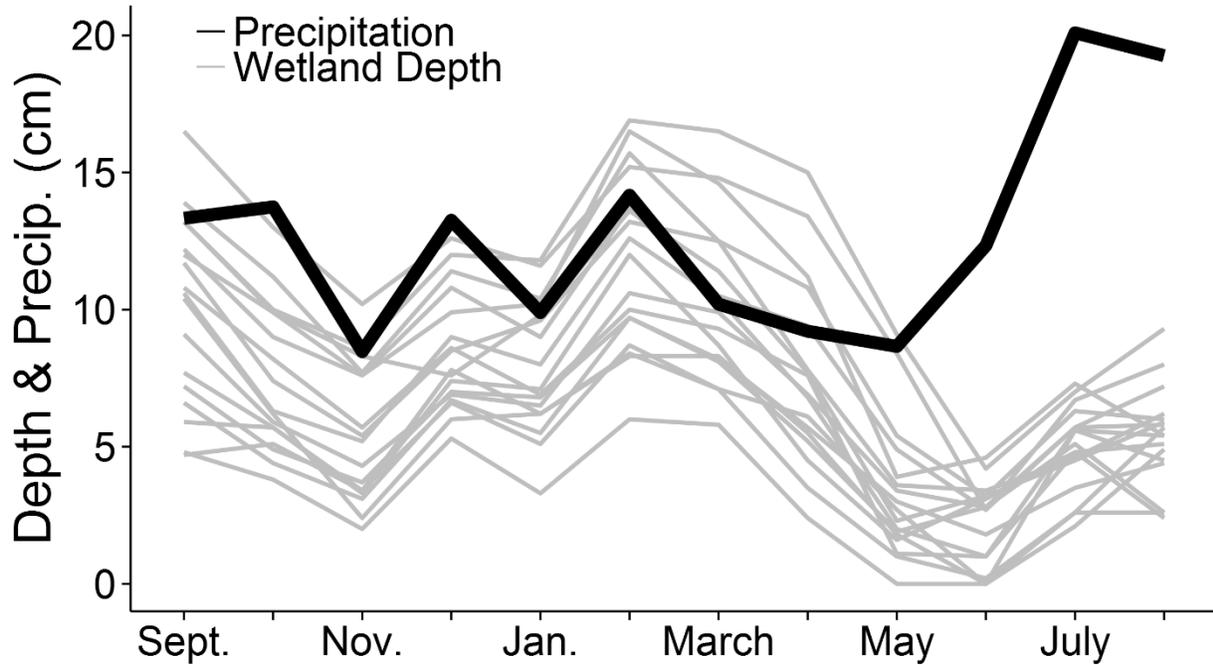


Figure 1.1. Average monthly wetland depth from 2006 – 2013 for 17 *Ambystoma bishopi* breeding wetlands plotted alongside the average monthly precipitation over the same time period. Depth measurements were recorded bimonthly from October to April and once a month the rest of the year. Precipitation totals were downloaded for a single geographic point near the centroid of the study area from the PRISM climate group.

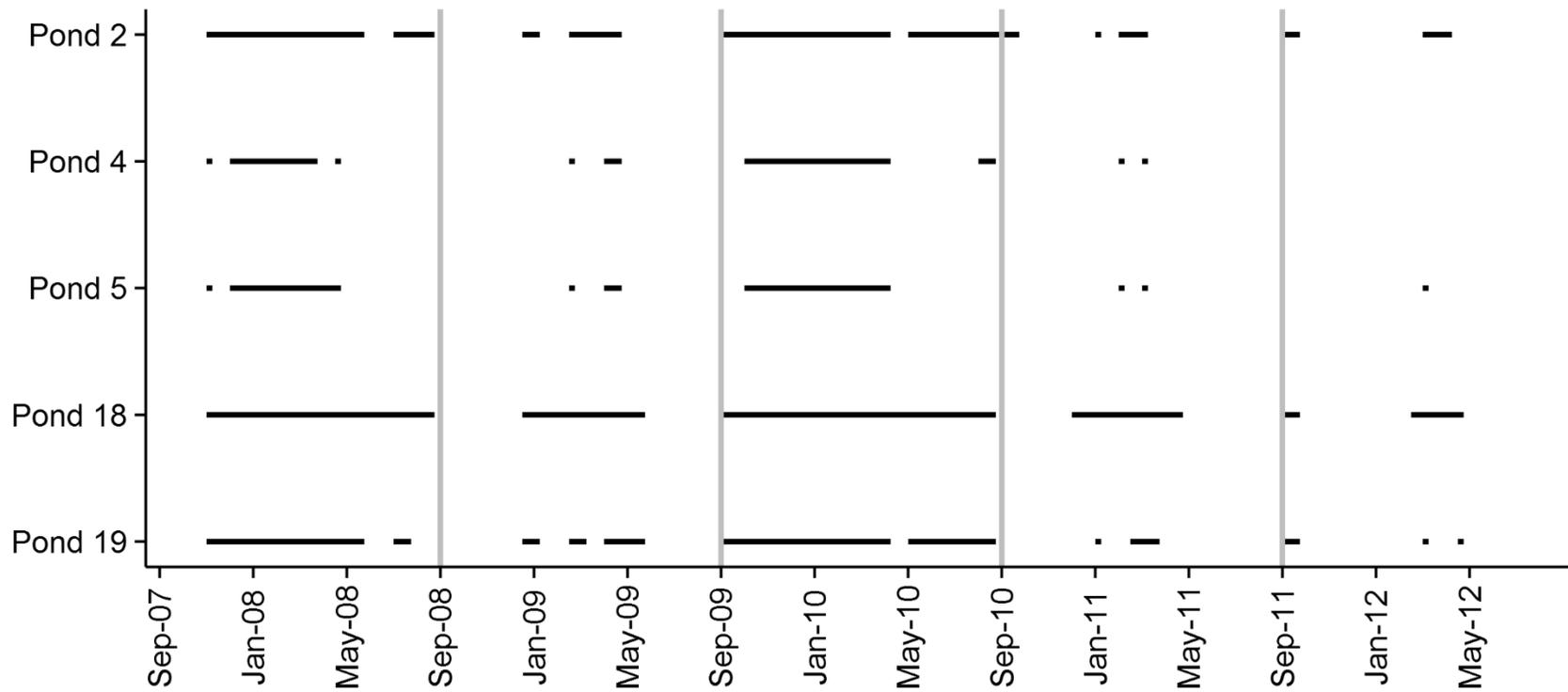


Figure 1.2. Measured hydroperiods from 2007 – 2012 for five characteristic *Ambystoma bishopi* breeding wetlands. Measurements were recorded on a monthly (May – September) and bimonthly (October – April) basis. Years are arranged from September – August, and grey lines are used to separate years.

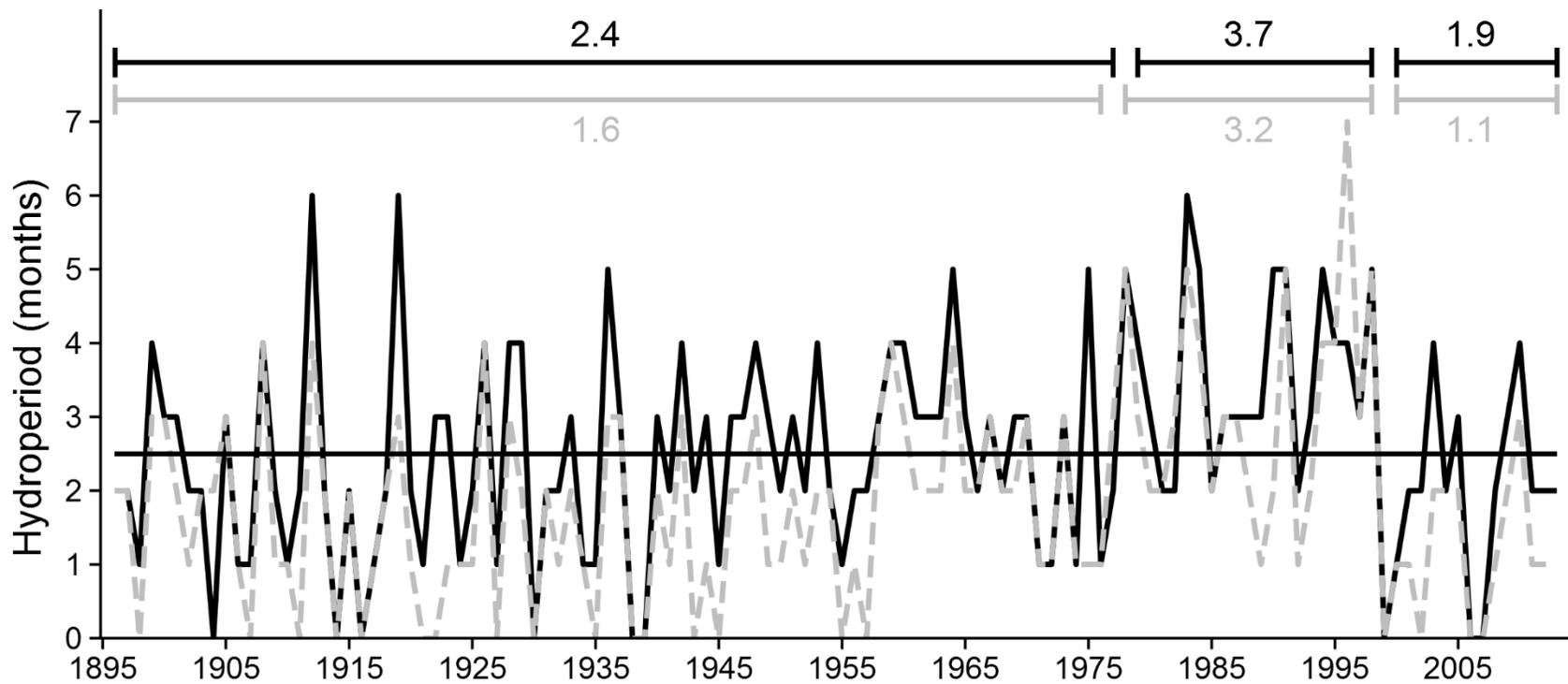


Figure 1.3. Predicted hydroperiod for a ‘wet’ and ‘dry’ *Ambystoma bishopi* breeding wetland from 1896 – 2013 based on logistic regression model classifications of wetland conditions. Change points in average hydroperiod occurred during the 1970s and 1990s and are indicated by gaps in the solid lines above the graph. The numbers along the lines are the average hydroperiod for the ‘wet’ and ‘dry’ wetland respectively. The continuous horizontal line represents the hydroperiod below which *A. bishopi* cannot complete metamorphosis.

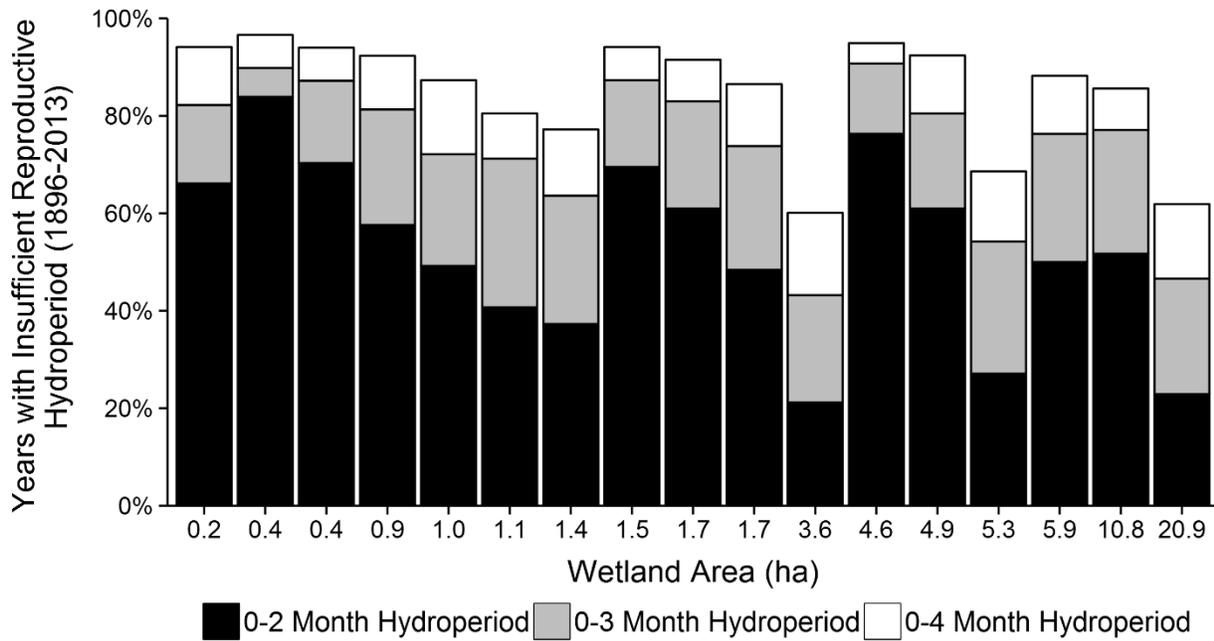


Figure 1.4. Percentage of years from 1896 – 2013 in which hydroperiod was likely insufficient for *Ambystoma bishopi* reproduction. Each bar represents a different breeding wetland arranged in order of increasing wetted area. Larval *A. bishopi* require between 3 – 5 months to successfully metamorphose from aquatic larvae into terrestrial adults.

## CHAPTER 2

### **The effects of changing habitat structure in pine flatwoods wetlands on aquatic invertebrate and amphibian communities**

Houston C. Chandler, Carola A. Haas, and Thomas A. Gorman

#### **ABSTRACT**

Natural disturbances play a critical role in structuring many ecosystems. In the southeastern United States, a long history of fire suppression has removed the natural disturbance regime from many environments, which leads to changes in vegetation characteristics. We sampled aquatic invertebrate and amphibian communities in 21 pine flatwoods wetlands in northwest Florida. Our objectives were to quantify amphibian and invertebrate community structure, identify similarity in amphibian communities across an environmental gradient, identify how two rare amphibian species responded to habitat change (Reticulated Flatwoods Salamanders [*Ambystoma bishopi*] and Ornate Chorus Frogs [*Pseudacris ornata*]), and identify how invertebrate communities responded to different habitat characteristics. Amphibian communities were similar across a range of vegetation characteristics but varied over a range of wetland hydroperiods. Both *A. bishopi* and *P. ornata* tended to occupy wetlands with high herbaceous vegetation cover and low canopy cover. For invertebrates, we created a set of a priori models and used multiple linear regression models to relate the abundance of isopods, chironomid midges, and damselflies to wetland vegetation and hydrology. The best-approximating models indicated that invertebrate groups responded to different habitat variables and that both vegetation and hydrology were important. There was also significantly higher total

invertebrate abundance in sections of wetlands with low canopy cover when compared to sections of the same wetlands with high canopy cover. To maintain aquatic invertebrate and amphibian communities, managers should focus on restoring appropriate vegetation structure to wetlands that have experienced long-term fire suppression or exclusion.

## INTRODUCTION

Habitat loss, fragmentation, and degradation are considered the primary threats facing many ecosystems (Fahrig 1997; Cushman 2006). Over time, habitat degradation can change ecosystem functioning and lead to shifts in communities as sensitive species often lack the ability to adapt to changes in habitat structure (Casatti 2006; Ernst et al. 2006). Limited dispersal abilities or environmental barriers may prevent some populations from shifting their distribution away from degraded habitat leading to local extirpations (Reinhardt et al. 2005). In lentic freshwater systems, many species lack the ability to actively disperse from one body of water to another, except during extreme events (e.g. floods). Thus, habitat degradation that affects an entire wetland has the potential to dramatically alter the aquatic community in that wetland and affect landscape connectivity by creating wetlands that possess inhospitable habitat characteristics for sensitive species (Amezaga et al. 2002).

Small ephemeral wetlands are common landscape features in many ecosystems. In these ephemeral wetlands, aquatic organisms must be adapted to persist through regular drying events by leaving the wetland, retreating below ground, or reaching a desiccation-resistant life-stage. Organisms that cannot survive through drying events or that are slow to recolonize newly-filled wetlands are generally excluded from ephemeral wetland systems. This often includes large slow-developing predatory fish species, thereby reducing the predation pressures on other members of the aquatic community (Skelly 1997). Despite the ecological challenges associated

with living in a dynamic environment, many ephemeral wetlands are characterized by highly diverse amphibian (Russell et al. 2002) and aquatic invertebrate communities (Wiggins et al. 1980; Batzer and Wissinger 1996).

The southeastern United States supports a high diversity of ephemeral wetlands (Sutter and Kral 1994; Russell et al. 2002), but this region has experienced severe loss and degradation of wetlands because of human disturbance (Hefner and Brown 1984). Pine flatwoods, a type of pine forest found in low-lying, poorly drained areas of the coastal plain, are characterized by the presence of shallow ephemeral wetlands (Means 1996). These forests are similar to other pine forests of the southeastern U.S. in that they were historically fire-maintained systems (Frost 1995; Glitzenstein et al. 1995). However, a long history of fire suppression and an inability of dormant-season fires (the typical season for setting prescribed burns) to burn through wetland basins has altered the vegetation structure in many pine flatwoods wetlands (Bishop and Haas 2005). Historically, these wetlands had a relatively open canopy with thick herbaceous vegetation covering wetland basins, but a lack of regular fire creates a dense woody midstory (Hinman and Brewer 2007; Martin and Kirkman 2009). The development of this midstory leads to increases in canopy cover, decreases the amount of herbaceous vegetation present in wetland basins, alters leaf litter inputs, and decreases the amount of solar radiation (Hinman and Brewer 2007). The effects of changing vegetation structure on the composition of aquatic communities in pine wetlands have not been well studied (Jones et al. 2010).

Complex aquatic invertebrate communities in pine flatwoods wetlands are critical to maintaining normal ecosystem functioning. Many invertebrate groups are primary consumers that feed on algae and submerged vegetation, which can lead to competition with anuran larvae (Morin et al. 1988). Some of these invertebrate groups also provide important food sources to

predatory species including salamander larvae (Whiles et al. 2004). However, some aquatic invertebrates such as beetles and dragonfly larvae also act as primary predators in fishless wetlands (Batzler and Wissinger 1996; Cobbaert et al. 2010). Depending on the vegetation type and litter quality, some invertebrates also affect decomposition rates (Kok and Van Der Velde 1994). Crayfish are a particularly important group because they can act as a keystone species in aquatic food webs (Rabeni 1992; Creed 1994) and link aquatic and terrestrial environments (Parkyn et al. 2001). Furthermore, crayfish species in ephemeral wetlands are capable of digging burrows that reach the water table, allowing them to persist through regular drying periods. Crayfish are poorly studied in many aquatic systems, and this is especially true in pine flatwoods wetlands (Franz and Franz 1990). The importance of invertebrate communities on aquatic ecosystem function makes understanding the effects of habitat changes on invertebrate populations a key research question.

The goals of our study were to characterize the habitat and aquatic communities in pine flatwoods wetlands that have experienced varying degrees of habitat alteration and are part of an ongoing adaptive management project (Appendix A; Gorman et al. 2013). First, we describe the vegetation structure of the study wetlands and quantify the amphibian and invertebrate communities in these wetlands. Second, we examine the relationship between wetland vegetation and the presence of two rare amphibian species: Reticulated Flatwoods Salamanders (*Ambystoma bishopi*) and Ornate Chorus Frogs (*Pseudacris ornata*). Third, we use the substantial variation in habitat characteristics between and within wetlands to examine how habitat variation affects invertebrate abundance. We predicted that factors influencing the invertebrate abundance would be 1) variables related to the vegetation characteristics altered by long-term fire suppression and 2) variables related to wetland hydrology.

## MATERIALS AND METHODS

### *Study Site*

We sampled 21 ephemeral wetlands located on Eglin Air Force Base in Okaloosa County, Florida from 2012 – 2014. Eglin AFB is a large military installation covering over 186,000 ha, of which 146,000 ha are actively managed longleaf pine forests. We focused our sampling efforts from late fall to early spring (December – April) as this is the typical period when these wetlands hold water. We sampled 21 wetlands that were part of ongoing adaptive management projects designed to lower canopy cover and increase herbaceous vegetation coverage by eliminating the shrub layer present in many wetlands (Appendix A). All treatments were applied prior to the beginning of the 2012 sampling season (detailed description of treatments in Appendix A but see Gorman et al. (2013) for a complete description of the management prescriptions). We established a transect in each of the 21 wetlands along the wetland's longest axis. Transects were divided into basin (B) and ecotone (E) points. Basin points fell on the long transect, and ecotone points were placed perpendicular to the basin transect at each end of a wetland (Figure 2.1). We anchored metal stakes in place to mark sampling points along the transect. We placed stakes every 20 m in small wetlands and every 40 m in five wetlands larger than 1.5 hectares. These transects were used as a basis to perform a majority of the sampling that was conducted during our study (see below).

### *Aquatic Invertebrate Sampling*

We sampled aquatic invertebrates along the pre-established transect in each wetland once a month from January – April 2014. We used a 1 mm mesh dipnet (SH-2D, Mid-Lakes Corporation, Knoxville, Tennessee) to sample aquatic invertebrates along the entire wetted

length of the transect (this length changed from month to month and did not include the ecotone points). We performed five approximately 1 m dipnet sweeps every 20 m (sections were split in half in the larger wetlands with longer distances between stakes). Dipnet sweeps were approximately evenly spaced along the 20 m between each stake. We identified and counted all aquatic invertebrates to the lowest taxonomic level possible in the field prior to releasing them. Amphibians caught during these sampling events were also recorded.

In addition to performing dipnet surveys, we also used sampling techniques designed to target crayfish. We conducted monthly spotlighting surveys in all wetlands containing water from March – April 2013 (2 sampling events) and February – April 2014 (3 sampling events). We walked the transect in each wetland at night using headlamps to spot crayfish. Crayfish are generally more active at night and can often be seen by their characteristic eyeshine. We captured crayfish using dipnets. We also conducted limited minnow trapping for crayfish in a subset of seven wetlands during April 2014. This sampling was done primarily to confirm that the species encountered during spotlighting surveys were the only species present in study wetlands. We baited half of the minnow traps placed in a wetland with dog food and half with glow sticks on every trap night. We placed 4 – 6 traps in one wetland in the evening, and we checked traps the following morning. All adult crayfish caught during dipnetting, spotlighting, and minnow trapping were identified to species and sexed. We also measured crayfish carapace length (CL), post-orbital carapace length (POCL), and carapace width (CW) using calipers.

### *Amphibian Sampling*

We sampled amphibians in the 21 study wetlands using multiple techniques during both years. All sampling was dependent on the presence of water in each wetland, so the number of

sampling events varied among wetlands and between years. We conducted nighttime call surveys twice a month at each wetland that had water from December – April. Each survey consisted of a 5 minute listening period where we recorded all calling anurans along with an approximate number of calling individuals (Gorman et al. 2013). On each occasion, we conducted call surveys at each end of the sampling transect (two sampling events per wetland). We also performed surveys designed to target *A. bishopi* larvae. We used a 3 mm mesh SH-2 or SH-2D dipnet (Mid-Lakes Corporation, Knoxville, Tennessee) to sample high quality larval flatwoods salamander habitat (areas with dense herbaceous vegetation; Gorman et al. 2009b). Surveys were performed for 30 minutes in each wetland, once or twice a month from December – April, depending on sufficient water. We identified all captured amphibians to species prior to release.

### *Habitat Surveys*

We sampled multiple habitat characteristics along the pre-existing transect in each wetland. We used a combination of the point-centered quarter method (Cottam and Curtis 1956) and a Daubenmire frame (Daubenmire 1959) to quantify the vegetation characteristics around each stake along the transect (basin and ecotone stakes). From each point, we measured the distance to the nearest tree (DBH > 7.6 cm) in each of the four cardinal directions and measured the DBH. We also recorded the distance to the nearest shrub (DBH < 7.6 cm) in each of the four cardinal directions, and the height of each shrub was estimated to the nearest half meter. We identified all trees and shrubs to species, which allowed us to describe the dominant species in each wetland (Mitchell 2007). The total distance to the nearest shrub was used to estimate a shrub density for the entire wetland (Mitchell 2007). A Daubenmire frame (0.5 m X 0.2 m

rectangular plot marked in quarters; Daubenmire 1959) was placed around the sampling stake to estimate the herbaceous vegetation cover. We used the Daubenmire (1959) cover class scale to estimate the percent herbaceous groundcover, and the total cover class of all herbaceous vegetation in each quarter. The total cover class for each sampling point in a wetland was averaged to generate an estimate of percent herbaceous cover for the entire wetland. We used a convex spherical densiometer to measure canopy cover along each wetland transect. Canopy cover measurements were recorded in each of the four cardinal directions at each sampling stake and halfway in between each pair of stakes (ecotone points were not included). Canopy cover measurements were recorded during spring 2014, and all other vegetation measurements were recorded during fall 2013.

Additionally, we sampled the water depth and hydroperiod in each wetland. Depth measurements were recorded at an approximate center point in each wetland (located along the pre-existing transect). Depth measurements were recorded twice a month from October – April and once a month the rest of the year. We calculated the average depth in each wetland from January – April 2014 because this was the time period when all invertebrate sampling was conducted. Then we calculated an average hydroperiod from 2010 – 2014 (a longer period should generate a more accurate representation of the hydroperiod through time) for each wetland using the same depth measurements from the approximate center point. We identified the longest hydroperiod that occurred at least partially from October – April for each year and used these yearly hydroperiods to calculate an average for each wetland. Finally, we measured dissolved oxygen (DO) in a subset of 12 wetlands. We conducted DO sampling once a month from February – April 2014 on three consecutive days (four wetlands per day). We measured DO at each stake where there was sufficient water for measurements to be recorded. In wetlands

with less than 10 sampling stakes, we recorded a DO measurement on each side of the sampling stake (2-3 m apart). All DO sampling occurred between 0900 and 1200 to reduce the amount of temperature fluctuation during the three-day sampling period. We calculated Pearson's correlation coefficients for DO, canopy cover, and herbaceous vegetation cover.

### *Statistical Analysis*

We examined the effects of habitat on amphibian communities by calculating binary similarity indices for study wetlands. We created two separate groupings of study wetlands based on 1) herbaceous vegetation and canopy cover and 2) on the average hydroperiod (each grouping contained all 21 wetlands). First, we categorized wetlands as having either high herbaceous vegetation (> 30%), low canopy cover (< 50%) or low herbaceous vegetation cover (< 20%), high canopy cover (> 50%). Wetlands that did not fit into either category were grouped into a third category (i.e. these wetlands only met one of the criteria for the other two categories). Second, we categorized wetlands as having a long hydroperiod (> 5 months), a mid-length hydroperiod (3 – 5 months), or a short hydroperiod (< 3 months). All wetlands were included in both groupings, and we did not compare between vegetation and hydroperiod categories. We then calculated similarity indices for each grouping using Jaccard's method, as described by Krebs (1998). We chose this method because the anuran call surveys did not provide adequate abundance data to use a metric that includes abundance in the calculation. Values for Jaccard's similarity index range from 0 (no similarity) to 1 (complete similarity).

To examine how *A. bishopi* and *P. ornata* responded to vegetation characteristics, we used two-sample t-tests to compare the mean herbaceous vegetation cover and canopy cover in occupied vs. unoccupied wetlands. Wetlands were classified as occupied if the species was

detected in that wetland using any sampling method in 2013 or 2014. We omitted one wetland from this analysis because after extremely high rainfall in 2014 the wetland expanded into an area not included in vegetation measurements. *Ambystoma bishopi* larvae were detected in this area in 2014. We chose to analyze amphibian communities using these two methods because amphibian captures were generally low across both years (Appendix B) and call surveys did not provide adequate abundance estimates.

Invertebrate abundance was calculated as the number of invertebrate captures per wetland standardized by the number of dipnet sweeps conducted in that wetland, unless otherwise specified. We used multiple linear regression models to examine the effects of habitat on the three most abundant invertebrate groups (isopods, damselflies, and chironomid midges). Our predictor variable set included percent canopy cover, percent herbaceous vegetation cover, shrub density, average water depth, and average hydroperiod, which are all biologically relevant factors for invertebrate communities (Carpenter and Lodge 1986; Batzer and Resh 1991; Batzer and Wissinger 1996; Zimmer et al. 2000). We removed the ecotone points from the herbaceous vegetation and shrub density calculations because invertebrate sampling only occurred along the basin points of the transect (Figure 2.1). We checked for multicollinearity by calculating the variance inflation factors (VIFs) for the global model for each invertebrate group. VIFs of  $> 5$  indicate problems with multicollinearity in the predictor variables (Montgomery et al. 2012).

We used an information theoretic approach and Akaike's Information Criterion corrected for small sample sizes (AICc) to evaluate the strength of a priori hypotheses (Burnham and Anderson 2002). We considered the model with the lowest AICc to have the best balance of statistical parsimony and goodness of fit for the data. We also calculated AICc weights ( $w$ ), which range from 0 to 1, with the best fitting model having the highest weight. Using our five

predictor variables we created 31 models for each of the three invertebrate groups. We considered models with a  $\Delta\text{AICc}$  value of  $< 2$  to be equally supported by the data and models with a  $\Delta\text{AICc}$  value  $> 2$  and  $< 4$  to be moderately supported (Burnham and Anderson 2002). We compared the best-approximating model for each invertebrate group to evaluate habitat preferences among these groups. We also calculated the relative importance of each predictor variable in a model set by summing the AICc weights for all models containing that variable (Burnham and Anderson 2002). All AIC values were calculated using the R package `AICcmodavg` (Mazerolle 2014).

To further examine the effects of habitat on invertebrate communities, we identified six wetlands that had sections of high and low canopy cover within the same wetland. In this case, low canopy sections had an average canopy cover of  $< 40\%$ , and high canopy sections had an average canopy cover of  $> 65\%$ . We used two-sample t-tests to compare the total invertebrate abundance in high and low canopy sections of these six wetlands on a monthly basis. All analyses were performed in R (R Development Core Team 2014).

## RESULTS

### *Habitat Characteristics*

As expected, we observed substantial variation in habitat characteristics among the 21 wetlands, ranging from wetlands with open canopies and almost continuous herbaceous vegetation cover to wetlands with dense shrubs, thick canopies, and almost no herbaceous vegetation (Table 2.1; Appendix A). We recorded variation in vegetation characteristics within some wetlands that allowed us to further investigate the effects of habitat on aquatic invertebrates (see below). Wetland depths and hydroperiods were also variable among wetlands

and through time (2013 characterized by drought and 2014 characterized by well-above average precipitation). DO measurements were highly variable between wetlands, but, in general, were highest in March and lowest in April (Appendix A). The lowest average DO over the three month period was 2.34 (SE = 0.17), and the highest average was 7.61 (SE = 0.31). DO was negatively correlated with canopy cover (PCC = -0.40,  $P = 0.20$ ) and positively correlated with herbaceous vegetation cover (PCC = 0.18,  $P = 0.58$ ), but neither relationship was significant.

Slash pine (*Pinus elliottii*) was the most abundant tree species in 19 of the 21 wetlands (see Appendix A for more detail on wetland vegetation characteristics). Longleaf pine (*Pinus palustris*) was the most abundant tree species in the other two wetlands and was the second most abundant tree in six more wetlands. Galberry (*Ilex glabra*) and myrtle dahoon (*Ilex myrtifolia*) were the most abundant shrub species encountered during vegetation surveys. Overall, shrub diversity was high throughout our study wetlands with an average of 8.9 shrub species encountered per wetland. There was also a high diversity of herbaceous vegetation species encountered during vegetation surveys, but wiregrass (*Aristida stricta*) was the most abundant species in 13 wetlands and the second most abundant species in three more wetlands. Other commonly encountered species included flattened pipewort (*Eriocaulon compressum*) and members of the genus *Dichanthelium*. However, there were also nine wetlands where thick leaf litter and bare ground dominated the majority of the sampling points (i.e. no herbaceous vegetation).

### *Amphibian Communities*

From 2012 – 2014, we identified 17 amphibian species (14 anurans and 3 caudates) in the 21 study wetlands (Table 2.2). The number of species per wetland ranged from 2 – 11 with an

average of six species per wetland. Southern Cricket Frogs (*Acris gryllus*) and Southern Leopard Frogs (*Lithobates spinocephalus*) were the most common amphibian species encountered during amphibian surveys (100% and 95% of sites occupied, respectively). Pine Woods Treefrogs (*Hyla femoralis*) were also commonly encountered on call surveys but were never captured while dipnetting (81% of sites occupied). *L. spinocephalus* was the most abundant species, accounting for approximately 63% of the total amphibian captures during dipnetting surveys. Together, *A. gryllus*, *L. spinocephalus*, and *P. ornata* accounted for 89% of the total dipnetting captures from 2012 – 2014 (Appendix B). We also identified a single Florida Bog Frog (*Lithobates okaloosae*), a species endemic to northwestern Florida, calling from one study wetland, which is notable as this species usually breeds in steephead ravines and slow moving sections of streams (but one individual was previously detected calling from an isolated cypress dome; Gorman et al. 2009a).

Binary similarity coefficients indicated that amphibian communities were similar across a range of vegetation types found in the 21 wetlands. Wetlands with high herbaceous vegetation and low canopy cover were similar to wetlands with low herbaceous vegetation, high canopy cover ( $S_J = 0.86$ ) and to wetlands that either had low herbaceous vegetation or high canopy cover ( $S_J = 0.76$ ). Wetlands with a long average hydroperiod were more similar to wetlands with a mid-length hydroperiod ( $S_J = 0.76$ ) than to wetlands with a short hydroperiod ( $S_J = 0.35$ ). Furthermore, wetlands with a long hydroperiod were more species rich (17 species) than wetlands with a short hydroperiod (6 species). Wetlands with a mid-length hydroperiod were also less similar to wetlands with a short hydroperiod ( $S_J = 0.46$ ) than wetlands with a long hydroperiod.

From 2012 – 2014, *A. bishopi* and *P. ornata* tended to occupy wetlands ( $n = 20$ ) with higher herbaceous vegetation cover and lower canopy cover (Figure 2.2). However, only the difference in canopy cover between wetlands occupied by *P. ornata* and wetlands not occupied by *P. ornata* was significant ( $t_{18} = -2.15$ ,  $P = 0.023$ ). Furthermore, *P. ornata* occupied 75% of the eight wetlands that we classified as having high herbaceous vegetation and low canopy cover, but only 37% of the eight wetlands classified as having low herbaceous vegetation and high canopy cover (Table 2.2). Only one high canopy, low herbaceous site was occupied by *A. bishopi*, but *A. bishopi* were only detected in six of the study wetlands despite the above average precipitation during 2014.

### *Invertebrate Communities*

From January – April, we captured a total of 30,073 invertebrates during dipnet surveys (Table 2.3). Isopods (Order Isopoda) accounted for 70.8% of the total invertebrate captures over the four-month sampling period. Non-biting midges (Family Chironomidae) were the second most common invertebrate captured (6.2%) followed closely by damselfly larvae (Suborder Zygoptera) at 5.6%. Other groups were locally abundant in some wetlands but were uncommon at a landscape scale. For example, phantom midges (Family Chaoboridae) accounted for 71% (605 individuals) of the total captures in one wetland, but only 46 individuals were captured in the remaining 20 wetlands. Other groups were commonly encountered in a majority of wetlands including backswimmers (Family Notonectidae), boatmen (Family Corixidae), and mosquito larvae (Family Culicidae; Table 2.3). Large predatory invertebrates were rarely encountered during dipnet surveys but were observed during spotlighting surveys. Of the four months

sampled, February had highest invertebrate abundance, and January had the lowest abundance (Figure 2.3).

Through a combination of dipnetting, spotlighting, and minnow trapping, we captured crayfish in 15 of 21 study wetlands from 2013 – 2014 (Appendix A). We encountered only three species of crayfish inside of study wetlands: *Procambarus evermanni*, *P. hubbelli*, and an unknown *Procambarus* species that most closely resembles *P. apalachicola* (genetic work is currently being completed by Jesse Breinholtto [University of Florida] to identify these specimens). One additional species, *Fallicambarus byersi*, was collected from burrows along the edge of wetland 50, and one dead individual was found at the edge of wetland 4. *Fallicambarus byersi* is likely common throughout the study area, but this species is a primary burrower that spends the majority of time underground. We did not catch any species using minnow traps that were not caught during dipnetting and spotlighting surveys. *P. evermanni* was by far the most abundant crayfish species in the study wetlands, accounting for more than 98% of total crayfish captures (394 adult crayfish captured). Adult male and adult female *P. evermanni* were similar in size (Table 2.4). There was also evidence of *P. evermanni* reproduction in 14 of 15 occupied wetlands (presence of small juveniles and/or first form males). However, during the two years of our study we never encountered a female that was carrying eggs or juveniles. Male *P. evermanni* also exhibited dramatically increased chela size when in 1<sup>st</sup> form compared to non-reproductive 2<sup>nd</sup> form males.

#### *Invertebrate Abundance and Habitat Characteristics*

Our modeling indicated that a mix of vegetation and hydrologic characteristics influenced invertebrate abundance in pine wetlands (for full results see Appendix C). For isopods, the best-

approximating model included variables for canopy cover and shrub density (Table 2.5). Both canopy cover and shrub density had a negative effect on isopod abundance (Table 2.6). No other models had a  $\Delta\text{AICc}$  value  $< 2$ , while six models had a  $\Delta\text{AICc}$  between 2 and 4. These six moderately-supported models mostly contained variables relating to vegetation characteristics. Canopy cover and shrub density were both included in four of six models, while depth and hydroperiod variables were only included in one of the six moderately-supported models. Canopy cover and shrub density also had the highest relative importance among the predictor variables (Canopy Cover = 0.79; Shrub Density = 0.75; Herbaceous Cover = 0.37; Hydroperiod = 0.19; Depth = 0.17).

There were two models with  $\Delta\text{AICc}$  values  $< 2$  in the damselfly (Zygoptera) model set (Table 2.5). The best-approximating model included variables for canopy cover and hydroperiod. Canopy cover had a negative effect on damselfly abundance, but damselfly abundance was positively related to hydroperiod (Table 2.6). The other well-supported model contained variables for canopy cover, shrub density, and hydroperiod (Table 2.5). Furthermore, there were three models that had a  $\Delta\text{AICc}$  value between 2 and 4. All three of these moderately-supported models included the canopy cover variable, and all three models included either the hydroperiod or depth variable. The shrub density variable was not included in any of the moderately-supported models. Canopy cover had by far the highest relative importance among the predictor variables, and shrub density had a relatively low relative importance even though it was included in one of the well supported models (Canopy Cover = 0.97; Hydroperiod = 0.81; Shrub Density = 0.33; Depth = 0.23; Herbaceous Cover = 0.19).

Finally, the effects of habitat characteristics on chironomid midge abundance were less clear. The best-approximating model included variables for depth and hydroperiod but only had

an AICc weight of 0.15 (Table 2.5). Depth had a positive effect on chironomid midge abundance, while the effect of hydroperiod was not significant (Table 2.6). Three other models had a  $\Delta\text{AICc} < 2$ , and 13 models had a  $\Delta\text{AICc}$  between 2 and 4. The remaining well-supported models included only a single factor in each model (depth, herbaceous cover, and canopy cover, respectively; Table 2.5). Moderately-supported models contained all five potential variables. Depth and hydroperiod were included in six moderately-supported models each, and each vegetation variable was included in five of the moderately-supported models. Depth and hydroperiod had the highest relative importance among predictor variables, but the relative importance of all five variables was similar (Depth = 0.59; Hydroperiod = 0.44; Canopy Cover = 0.33; Herbaceous Cover = 0.32; Shrub Density = 0.22).

We also detected differences in total invertebrate abundance within a subset of six wetlands that contained high and low canopy sections. Invertebrate abundance was significantly higher in low canopy sections when compared to high canopy sections (Figure 2.3). The average canopy cover in high canopy sections was 73% compared to just 31% in the low canopy sections. Average invertebrate abundance in high canopy sections was 5.1 (SE = 0.72) captures per dipnet sweep compared to 17.3 (SE = 3.16) captures per sweep in low canopy sections. Over time, invertebrate abundance was significantly higher from February – April (January:  $t_{10} = 1.61$ ,  $P = 0.07$ ; February:  $t_{10} = 2.19$ ,  $P = 0.03$ ; March:  $t_{10} = 2.54$ ,  $P = 0.01$ ; April:  $t_{10} = 2.63$ ,  $P = 0.01$ ). Furthermore, low canopy sections generally had an invertebrate abundance higher than the average abundance for all 21 wetlands, and high canopy sections had a lower invertebrate abundance than the average for all wetlands (Figure 2.3).

## DISCUSSION

We documented a diverse assemblage of amphibians and aquatic invertebrates (when compared with other pine wetlands cf. Golladay et al. 1997; Dietz-Brantly et al. 2002) occupying pine flatwoods wetlands from 2012 – 2014 (Tables 2.2 and 2.3), and our results indicate that both groups were affected by differences in vegetation characteristics and wetland hydrology. Amphibian communities in wetlands with long hydroperiods were more diverse than in wetlands with short hydroperiods, and many amphibian species are more likely to successfully reproduce in wetlands with a longer hydroperiod (see Chapter 1). Species present in wetlands with long hydroperiods that were absent in wetlands with shorter hydroperiods tended to be species with long development times (e.g. *A. bishopi*) or species that breed later in the year when many short hydroperiod wetlands may have already dried (e.g. members of the genus *Hyla* and *Lithobates*). Snodgrass et al. (2000) also found a positive relationship between hydroperiod and amphibian species richness but suggested in some cases hydroperiods were long enough to support fish populations and lower amphibian diversity. In contrast to the observed differences in amphibian communities across a hydroperiod gradient, amphibian communities were similar over a range of canopy and herbaceous vegetation cover. Many of the species identified in our surveys are habitat generalists that likely do not respond rapidly to changes in vegetation structure (e.g. *Acris gryllus* and *Lithobates sphenoccephalus*; Table 2.2). Pine Woods Treefrogs (*Hyla femoralis*) were also identified calling in all wetlands classified as having low herbaceous vegetation cover and high canopy cover and a majority of wetlands in the other two vegetation categories. Despite the overall similarity in amphibian communities across different vegetation characteristics, both *A. bishopi* and *P. ornata* appeared to use wetlands with low canopy cover and high herbaceous vegetation cover (Figure 2.2; Gorman et al. 2013). These species are both

specialists of pine flatwoods wetlands. *Pseudacris ornata* likely benefit from the presence of herbaceous vegetation during the larval period (Alford 1986), and *A. bishopi* may benefit from herbaceous vegetation during all life stages (Gorman et al. 2009b; Jones et al. 2012; Gorman et al. in press).

At a broad spatial scale, our results indicate that aquatic invertebrate abundance responded to a variety of vegetation and hydrology characteristics depending on the invertebrate group. Isopods accounted for the majority of captures during our invertebrate surveys, and the abundance of this group was strongly influenced by vegetation characteristics (primarily lower canopy cover and lower shrub density; Table 2.5). The negative relationship in the best-approximating model between canopy cover and shrub density and isopod abundance suggests that this group prefers pine flatwoods wetlands with a vegetation structure that is consistent with a fire-maintained landscape (Table 2.6). Similarly, damselfly larval abundance was lower when canopy cover was high but was higher with longer wetland hydroperiod, possibly due to a relatively late breeding period. Chironomid midge abundance was primarily associated with wetland hydrology rather than vegetation characteristics. However, our results for this group were less consistent than the other two groups (four models with a  $\Delta AICc < 2$  and similar relative importance values among predictor variables). These results may be caused by high diversity in the family Chironomidae (Stagliano et al. 1998; Voshell 2002), which could result in multiple species with different habitat preferences being lumped into one group. Furthermore, many midges in this family may not be large enough to be consistently collected using a 1 mm mesh dipnet, and this likely affected our abundance estimates.

At finer spatial scales, canopy cover again appeared to be an important factor influencing invertebrate abundance (Figure 2.3). The similarity between high canopy and low canopy

sections during January was probably due to lower invertebrate abundance, especially isopods, across a majority of wetlands during this initial sampling event, which can likely be attributed to temporal factors (i.e. timing of reproduction events and colder temperatures during January). Overall, these results demonstrate a strong effect of shifting vegetation structure on certain aquatic invertebrates groups similar to those identified for several amphibian species (Gorman et al. 2009b; Gorman et al. 2013).

There are multiple pathways through which shifting vegetation structures could affect amphibian and aquatic invertebrate communities. High canopy cover in isolated wetlands can cause decreases in water temperatures, decreases in periphyton growth rates, and decreases in DO levels (Skelly et al. 2002). Increasing canopy cover and shrub density are also likely to shift litter inputs away from grass-dominated to more deciduous plant based litter (this was evident in many of the study wetlands). Overall, little is known about how leaf litter inputs affect invertebrate communities in ephemeral wetlands and some studies have documented differential effects (Magnusson and Williams 2006; Batzer and Palik 2007). Litter quality can affect the success of some species (Palik et al. 2006), but this has not been examined in pine wetlands or in a wide range of invertebrate species. For amphibian larvae, Williams (2008) found that shifting from grass-dominated litter to hardwood tree litter negatively impacted amphibian success, likely due to decreased phytoplankton growth rates. Further, large amounts of organic matter can decrease DO levels during decomposition (Magnusson and Williams 2006). Overgrown ephemeral wetlands often have lower DO levels than restored wetlands with more open canopies, and higher DO levels (greater than 4 mg/L) increased recruitment in Spotted Salamanders (*Ambystoma maculatum*; Sacerdote and King 2009). We documented variable DO levels across study wetlands (Appendix A), and there appeared to be little effect of vegetation structure on DO

levels. This is likely attributable to sampling DO at only a few points in each wetland and possibly due to other factors that we did not quantify such as the amount of leaf litter or algae in a wetland. Finally, it is important to note that increasing canopy cover tends to decrease the amount of herbaceous cover in pine wetlands (this relationship was likely decreased in our study sites due to ongoing adaptive management studies focused on decreasing canopy and increasing herbaceous vegetation; Gorman et al. 2013). The amount of herbaceous cover is often positively associated with invertebrate communities (Zimmer et al. 2000). Herbaceous vegetation can increase surface area for periphyton growth (Cattaneo and Kalff 1979), enhance litter quality (Williams 2008), alter DO levels (Ma et al. 2013), and provide refugia from predators (Batzer and Resh 1991; Kopp et al. 2006).

Despite the apparent negative impacts of shifting vegetation structures (i.e. increased canopy cover and decreased herbaceous cover) on invertebrate and amphibians communities, we documented diverse assemblages in both groups (Tables 2.2 and 2.3). Ephemeral wetlands are known to support high amphibian diversity, especially in the southeastern United States (Means et al. 2004; Russell et al. 2002). Invertebrate communities are poorly studied in this habitat type, but we documented similar communities to those previously reported from pine wetlands (Golladay et al. 1997; Dietz-Brantly et al. 2002). Isopods were the most abundant invertebrate group in our samples, which was unexpected given that this group is susceptible to local extinctions during drying events and does not possess many of the adaptations necessary to persist through drying events (Styron 1968). Isopods may be able to rapidly colonize ephemeral wetlands by moving across the landscape in overwash from other water bodies during precipitation events (this likely represents an important movement vector for many species in this system). The prevalence of isopods in our samples could have important ecological

applications because this group is one of the primary food sources of larval *A. bishopi* (Whiles et al. 2004). Therefore, wetlands with low canopy cover and low shrub density may have increased availability of food resources for larval salamanders. It is important to note that our sampling procedures prevented us from identifying invertebrates to genus or species in most cases, and the mesh size on our dipnets prevented us from collecting micro-crustaceans (e.g. copepods and cladocerans), which can also serve as important food sources for larval *A. bishopi* (Whiles et al. 2004). Over two years of sampling, we documented surprisingly low crayfish diversity. Even though diversity was low, *Procambarus evermanni* were commonly encountered in the majority of wetlands, and adults reached sufficient size to prey on amphibian larvae (Table 2.4). Given the number of small juveniles and first form males that were captured it is also surprising that no females carrying eggs were captured. This suggests that females with eggs took shelter in burrows until the eggs hatched. We also documented an unknown crayfish species that either represents a significant range extension for *P. apalachicola* or a previously undescribed species. The presence of an unknown crayfish and the lack of information about aquatic invertebrate communities in pine flatwoods wetlands along the Gulf Coastal Plain suggests that further study is needed in these types of environments.

Over time, habitat degradation can lead to changes in community structure, which can negatively impact ecosystem function (Casatti 2006; Ernst et al. 2006). Our results demonstrate that aquatic invertebrate communities and some amphibian species in pine flatwoods wetlands do not exploit wetlands with a vegetation structure that has resulted from long-term fire suppression or exclusion. Both amphibians and invertebrates can increase landscape level connectivity by linking aquatic and terrestrial environments (Stagliano et al. 1998; Nakano and Murakami 2001; Whiles et al. 2006), and in some cases emergent aquatic insect inputs into terrestrial systems may

exceed secondary terrestrial production (Bartrons et al. 2013). Finally, to promote high-quality aquatic habitat, managers should focus on ensuring that wetland basins are effectively burned rather than leaving entire wetlands as unburned pockets during burns of larger areas (Bishop and Haas 2005). Severe drought years may provide a unique opportunity to successfully burn wetlands during a time of year when wetlands would not normally burn. Others have demonstrated that combining regular burns with mechanical and herbicide treatments aimed at reducing woody vegetation in overgrown wetlands could increase the amount of suitable wetland habitat at a landscape scale (Martin and Kirkman 2009; Gorman et al. 2013). Focus should be placed on restoring wetlands that support a longer hydroperiod as these wetlands are important for amphibian communities and reproductive failure can be a severe problem for some species (Chapter 1). Active management may be necessary as a first step to restoring degraded wetlands, but returning to a fire return interval that maintains the disturbance dependent ecosystem should be a primary goal for maintaining proper ecosystem function.

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Table 2.1. Mean habitat characteristics for 21 ephemeral wetlands located on Eglin Air Force Base, Florida. Hydroperiod measurements represent an average of the longest hydroperiod occurring at least partially between October and April from 2010 – 2014. Vegetation measurements were recorded once in each wetland during fall 2013 or spring 2014. Depth measurements represent the average depth (measured bimonthly) at an approximate center point from January – April 2014 and are likely higher than average because 2014 was an abnormally wet year.

	Mean	SE	Min	Max
Canopy Cover (%)	48.33	3.93	18.00	81.80
Herbaceous Cover (%)	25.28	4.66	2.50	84.38
Shrub Density (per m)	0.99	0.13	0.40	2.67
Average Depth (cm)	29.07	2.91	4.60	63.60
Hydroperiod (months)	4.29	0.28	2.30	6.60

Table 2.2. Amphibian species recorded in 21 ephemeral wetlands on Eglin Air Force Base, Florida from 2013 – 2014. Wetlands with herbaceous vegetation cover > 30% and canopy cover < 50% were high quality, wetlands with herbaceous vegetation cover < 20% and canopy cover > 50% were low quality, and wetlands that did not meet both criteria for either group were considered intermediate quality. Wetlands were also divided by the length of their average hydroperiod from 2010-2014 (long: > 5 months, medium: 3 – 5 months, and short: < 3 months). The number of wetlands fitting into each category is indicated in parentheses.

Species	Vegetation Quality			Hydroperiod		
	High (8)	Med (5)	Low (8)	Long (7)	Med (10)	Short (4)
Southern Cricket Frog ( <i>Acris gryllus</i> )	8	5	8	7	10	4
Southern Leopard Frog ( <i>Lithobates sphenoccephalus</i> )	7	5	8	7	9	4
Ornate Chorus Frog ( <i>Pseudacris ornata</i> )	6	5	3	6	7	1
Pine Woods Treefrog ( <i>Hyla femoralis</i> )	5	4	8	6	8	3
Southern Chorus Frog ( <i>Pseudacris nigrita</i> )	4	2	2	3	4	1
Oak Toad ( <i>Anaxyrus quercicus</i> )	2	3	3	5	3	0
Reticulated Flatwoods Salamander ( <i>Ambystoma bishopi</i> )	2	3	1	2	4	0
Southern Toad ( <i>Anaxyrus terrestris</i> )	2	2	2	2	4	0
Pig Frog ( <i>Lithobates grylio</i> )	2	2	2	5	1	0
Eastern Newt ( <i>Notophthalmus viridescens</i> )	2	2	2	3	3	0
Dwarf Salamander ( <i>Eurycea quadridigitata</i> )	1	3	1	1	3	1
Barking Treefrog ( <i>Hyla gratiosa</i> )	1	1	1	3	0	0
Eastern Narrow-mouthed Toad ( <i>Gastrophryne carolinensis</i> )	1	1	0	1	1	0
Green Frog ( <i>Lithobates clamitans</i> )	0	1	5	3	3	0
Green Treefrog ( <i>Hyla cinerea</i> )	0	1	0	1	0	0
Squirrel Treefrog ( <i>Hyla squirella</i> )	0	1	0	1	0	0
Bog Frog ( <i>Lithobates okaloosae</i> )	0	1	0	1	0	0

Table 2.3. Total invertebrate captures from dipnetting surveys conducted once a month from January – April 2014 in 21 ephemeral wetlands on Eglin Air Force Base, Florida. Surveys were conducted using a 1mm mesh dipnet, and invertebrates were identified to the lowest possible taxonomic level in the field.

Phylum (Subphylum)	Class	Order (Suborder)	Family	Species	Total	% Captures
Annelida	Oligochaeta				57	0.19
Arthropoda (Chelicerata)	Arachnida	Trombidiformes			52	0.17
Arthropoda (Crustacea)	Malacostraca	Isopoda			21,281	70.76
		Amphipoda			441	1.47
		Decapoda	Cambaridae	<i>Procambarus sp.</i>	27	0.09
				<i>P. evermanni</i>	12	0.04
				<i>P. hubbelli</i>	1	0.003
	Branchiopoda	Anostraca			983	3.27
Arthropoda (Uniramia)	Insecta	Hemiptera (Heteroptera)	Corixidae		501	1.67
			Notonectidae		1165	3.87
			Belostomatidae		6	0.02
			Gerridae		131	0.44
		Coleoptera			432	1.44
			Gyrinidae		33	0.11
			Dytiscidae		32	0.11
			Hydrophilidae		3	0.01
		Odonata (Zygoptera)			1683	5.60
		Odonata (Anisoptera)			61	0.20
			Aeshnidae		35	0.12
			Libellulidae		139	0.46
		Ephemeroptera			15	0.05
		Diptera			9	0.03
			Culicidae		399	1.33
			Chaoboridae		651	2.16
			Chironomidae		1871	6.22
			Tabanidae		1	0.003
			Ceratopogonidae		52	0.17

Table 2.4. Size measurements for adult male and adult female Panhandle Crayfish

(*Procambarus evermanni*) captured in ephemeral wetlands from 2013 – 2014 on Eglin Air

Force Base, Florida.

	Carapace Length (mm)	Post Orbital Carapace Length (mm)	Carapace Width (mm)
<b>Female (n = 154)</b>			
Average ( $\pm$ SE)	30.2 $\pm$ 0.49	21.8 $\pm$ 0.41	13.8 $\pm$ 0.26
Max	45.0	38.5	22.0
<b>Male (n = 160)</b>			
Average ( $\pm$ SE)	30.7 $\pm$ 0.51	22.2 $\pm$ 0.40	13.9 $\pm$ 0.26
Max	45.0	34.0	22.0

Table 2.5. Multiple linear regression modeling results relating invertebrate abundance to vegetation characteristics and wetland hydrology in ephemeral wetlands on Eglin Air Force Base, Florida (K = the number of parameters, AICc = second-order Akaike's Information Criteria,  $\Delta$ AICc = change in AICc, and  $w_i$  = relative amount of support for each model).

Potential variables included in the models are listed in Table 2.1.

Candidate Models	AICc	K	$\Delta$ AICc	$w_i$
<b>Isopoda</b>				
Canopy Cover + Shrub Density	145.9	4	0	0.32
Canopy Cover + Herbaceous Cover + Shrub Density	148.4	5	2.49	0.09
<b>Chironomidae</b>				
Depth + Hydroperiod	61.0	4	0	0.15
Depth	61.6	3	0.58	0.11
Herbaceous Cover	61.8	3	0.80	0.10
Canopy Cover	61.8	3	0.86	0.10
Shrub Density + Depth + Hydroperiod	63.0	5	2.04	0.06
<b>Zygoptera</b>				
Canopy Cover + Hydroperiod	35.6	4	0	0.34
Canopy Cover + Shrub Density + Hydroperiod	36.5	5	0.96	0.21
Canopy Cover + Depth + Hydroperiod	38.6	5	3.0	0.08

Table 2.6. Parameter estimates and 95% confidence intervals for the best-approximating model relating habitat variables to the abundance of three aquatic invertebrate groups. Aquatic invertebrates were captured once a month from January – April 2014 in 21 ephemeral wetlands on Eglin Air Force Base, Florida.

Group	Parameter	Estimate	Standard Error	Lower CL	Upper CL
Isopoda	Intercept	30.38	5.21	19.42	41.31
	Canopy Cover	-0.31	0.08	-0.49	-0.14
	Shrub Density	-0.0006	0.0002	-0.001	-0.00008
Chironomidae	Intercept	0.92	0.66	-0.47	2.30
	Depth	0.05	0.02	0.009	0.08
	Hydroperiod	-0.34	0.18	-0.72	0.05
Zygoptera	Intercept	0.71	0.43	-0.19	1.62
	Canopy Cover	-0.02	0.006	-0.03	-0.009
	Hydroperiod	0.22	0.08	0.05	0.39

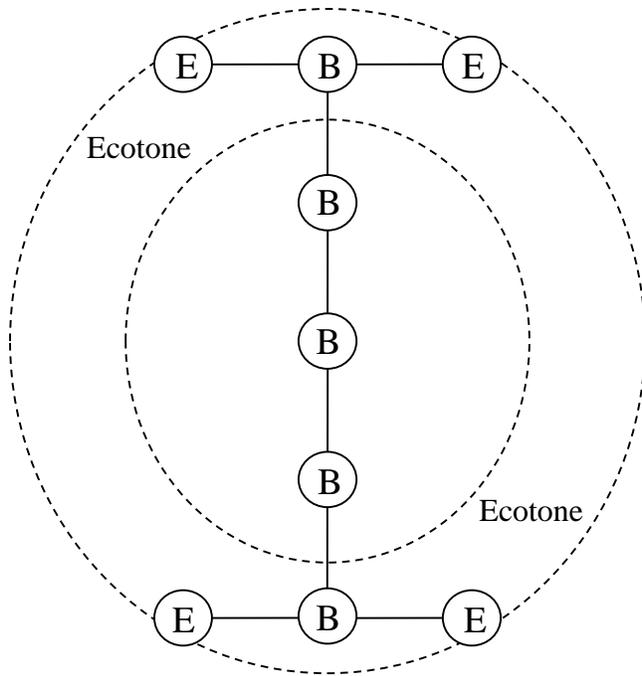


Figure 2.1. Schematic of sampling transect set along 21 ephemeral wetland's longest axis.

Points were spaced every 20 m in a majority of wetlands and every 40 m in five wetlands larger than 1.5 ha.

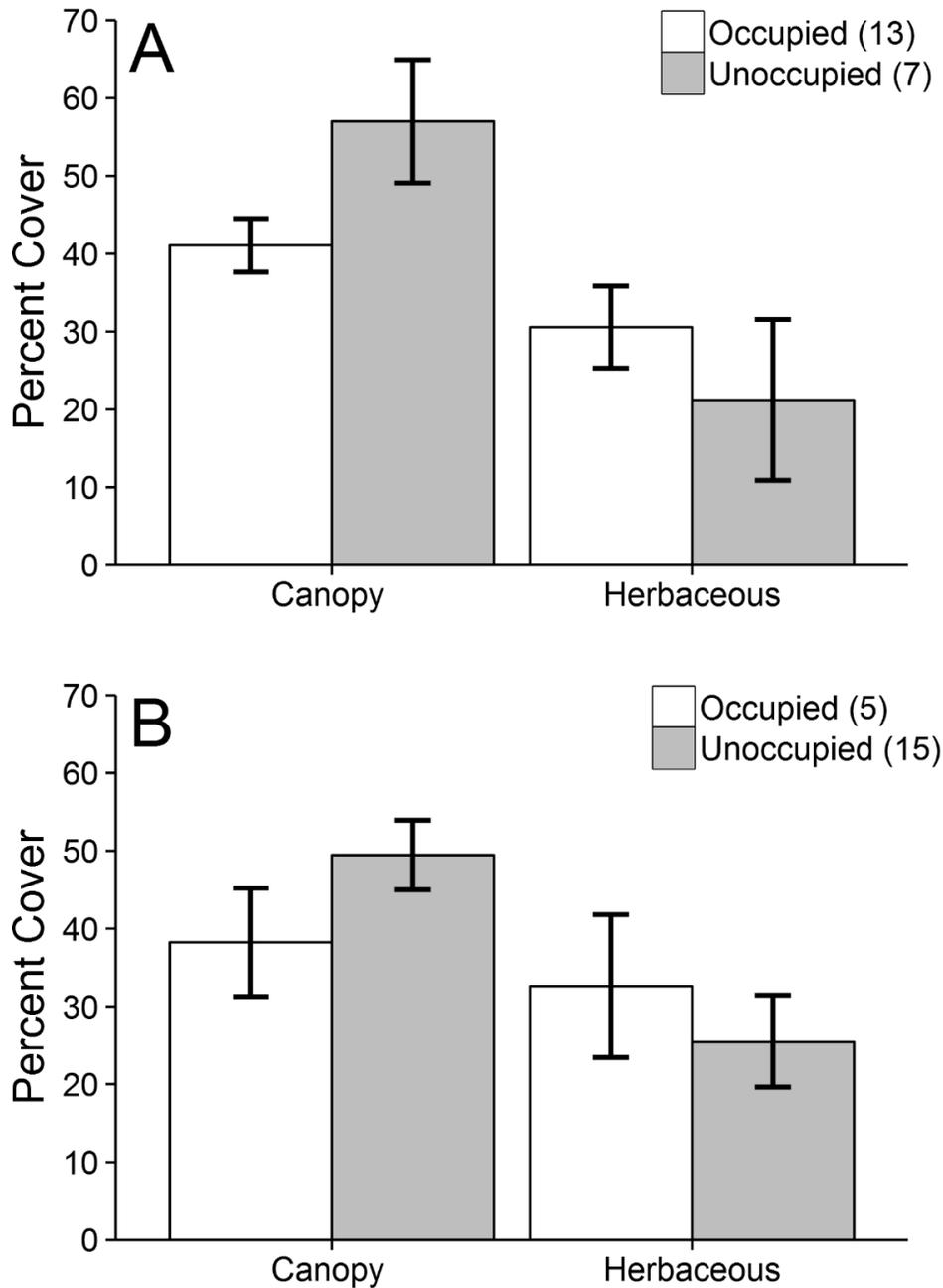


Figure 2.2. Percent canopy cover and herbaceous vegetation cover in wetlands occupied and unoccupied (sample sizes in parentheses) by A) Ornate Chorus Frogs (*Pseudacris ornata*) and B) Reticulated Flatwoods Salamanders (*Ambystoma bishopi*). Data were collected during 2013 – 2014 in 20 ephemeral wetlands on Eglin Air Force Base, Florida. Error bars represent standard error.

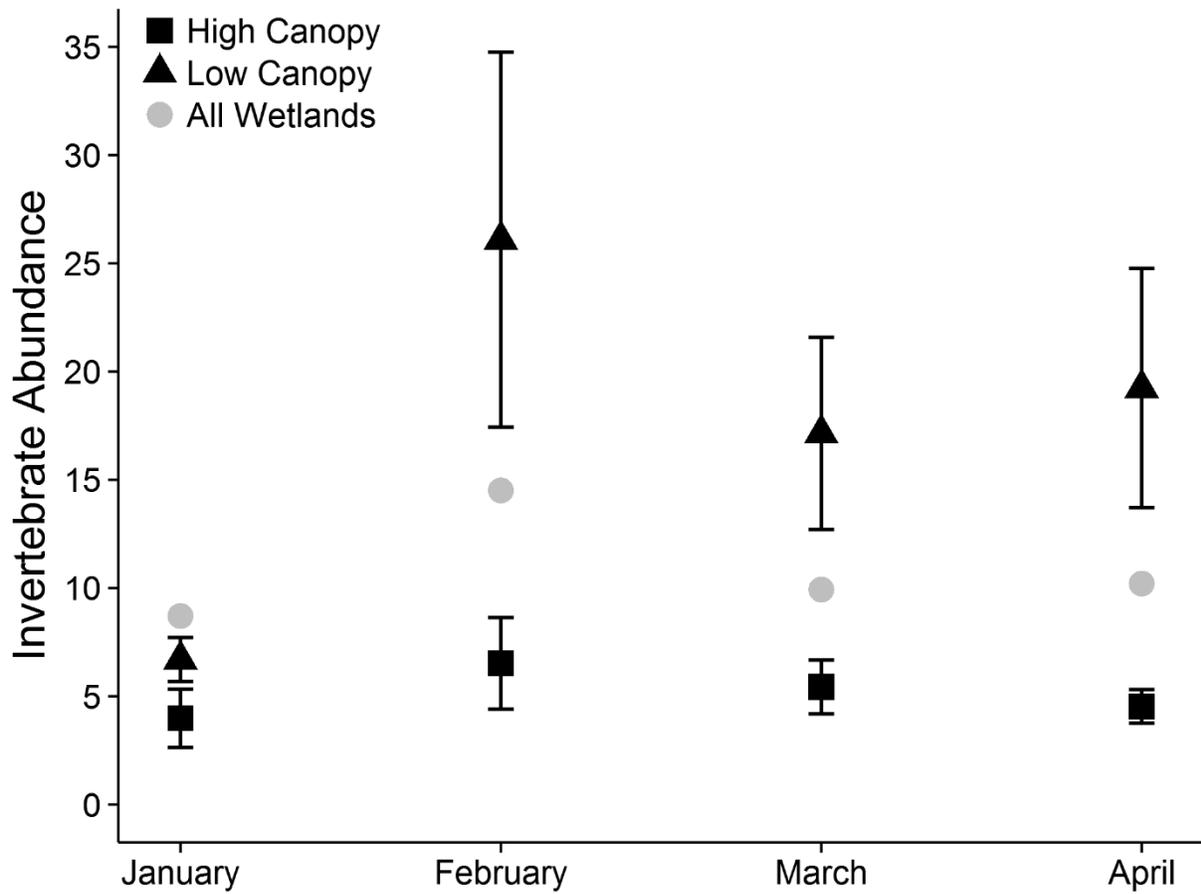


Figure 2.3. Aquatic invertebrate abundance recorded from January – April 2014 in high canopy and low canopy sections of six ephemeral wetlands on Eglin Air Force Base, Florida. Total invertebrate abundance for all wetlands (n = 21) sampled during the period is also included (light gray dots). Error bars represent standard error.

## CHAPTER 3

### **The effects of crayfish predation and vegetation cover on tadpole growth, survival, and nonlethal injury**

Houston C. Chandler, Thomas A. Gorman, and Carola A. Haas

#### **ABSTRACT**

Predator-prey interactions play an important role in structuring larval anuran communities in ephemeral wetlands. The type of interaction often depends on the predator species and the complexity of the aquatic habitat. We experimentally evaluated the effects of Panhandle Crayfish (*Procambarus evermanni*) predation on larval Southern Leopard Frogs (*Lithobates sphenoccephalus*) and larval Ornate Chorus Frogs (*Pseudacris ornata*). We performed separate experiments for each anuran species and used multiple vegetation treatments to examine whether or not vegetation could provide refugia from crayfish predation. Our results indicate that crayfish are effective predators of anuran larva over a range of herbaceous vegetation coverage (0 – 80%). Encounters between tadpoles and crayfish often resulted in nonlethal tail injury for tadpoles. Leopard frog tadpoles in predator treatments also grew larger than their counterparts in nonpredator treatments suggesting a reduction in intraspecific competition. Reduced competition could allow anuran larvae to metamorphose faster and escape aquatic predators and/or wetland drying. Crayfish predation likely plays an important role in ephemeral wetland communities because adult crayfish are able to persist through dry periods by burrowing.

**Key words:** Tail injury; Mesocosms; Ornate Chorus Frogs; Panhandle Crayfish; *Procambarus evermanni*; *Pseudacris ornata*; Southern Leopard Frogs; *Lithobates sphenoccephalus*

## INTRODUCTION

In freshwater ecosystems, community structure is determined by a complex set of biotic and abiotic interactions. Predation is an important biotic interaction in aquatic systems, and its effects on community structure and composition have been well documented (Welbourn et al. 1996; Wilbur 1997; Rudolf and Rasmussen 2013). Predators actively remove prey species from the environment, lowering their abundance and even completely eliminating them from the environment (Smith 1983). However, a decrease in prey abundance from predation can be positive for other members of the community. Reduced competition (both intra- and interspecific) can change community structure and increase the success of other species or individuals (Morin 1983; Wilbur 1997). The presence of predators can also affect behavior and morphology of prey, usually as a response to chemical stimuli (Relyea 2001). For example, many species of tadpoles, including Southern Leopard Frogs (*Lithobates sphenoccephalus* [formerly *Rana sphenoccephala*]), will reduce activity in the presence of a predator, which can slow growth rates and increase time to metamorphosis (Feminella and Hawkins 1994; Bishop et al. 2012). Morphological changes in tail shape and size are also common when predators are present (Relyea 2001).

Vegetation type and structure can also influence aquatic communities. Aquatic vegetation can increase the surface area on which biofilm and periphyton can grow (Cattaneo and Kalff 1979; Morris and Monier 2003), alter nutrient cycling (Granéli and Solander 1988), modify dissolved oxygen (DO) levels, and create refugia for many species, which can alter

predator-prey interactions. Many prey species are able to avoid predation attempts by using dense vegetation as refugia (Lefcort and Eiger 1993; Kopp et al. 2006), but some predators will alter their hunting strategies depending on the habitat complexity (Savino and Stein 1989; Davis et al. 2012). Furthermore, litter inputs from aquatic vegetation can also strongly influence species success (Williams et al. 2008). The potential for vegetation to affect predator-prey interactions suggests that changes to vegetation structure as a result of anthropogenic disturbances are likely to impact biotic interactions within aquatic communities.

Ephemeral wetlands often support a diverse amphibian assemblage (Dodd and Cade 1998). Typically, many anuran species breed in the same wetland with at least partial overlap in their breeding phenology, resulting in a complex tadpole community. The predation pressure in ephemeral wetlands is generally considered to be lower than in more permanent water bodies because of the regular drying events that exclude slow-developing predators, such as fish (Skelly 1996; Skelly 1997). However, some predators (e.g. salamanders and crayfish) are actually adapted to persist through drying events or colonize wetlands shortly after they fill (Wilbur 1997). Several studies have demonstrated that these predators can directly influence anuran breeding success and the structure of tadpole communities in ephemeral wetlands (Morin 1983; Buskirk 1988; Alford 1989).

Even though predation is an important ecological process, direct measures of predation are often difficult to obtain because predation events (successful or unsuccessful) are rarely observed (Morin 1985). Tail injury of larval anurans commonly results from unsuccessful predation attempts by many predators including newts, turtles, crayfish, and dragonfly larva (Morin 1985; Semlitsch 1990; Wilbur and Semlitsch 1990). Unsuccessful predation attempts tend to increase as predator density increases (Morin 1985), and tail injury can make larval

anurans more susceptible to future predation events (Semlitsch 1990; Figiel and Semlitsch 1991). An increased susceptibility to future predation events allows nonlethal injury to act as a regulating mechanism within a population because predation rates will increase along with prey populations and then fall when prey populations decrease (Harris 1989). Thus, tail injury has important ecological consequences for individuals and is a useful metric for measuring predation intensity on tadpoles, especially when survival until metamorphosis cannot be recorded.

Pine flatwoods are found in low-lying poorly drained areas of the southeastern United States (Means 1996). Ephemeral wetlands embedded in pine flatwoods are typically characterized by an open canopy and abundant herbaceous vegetation (Kirkman 1995; Gorman et al. 2013). Pine flatwoods wetlands provide important breeding habitat for multiple anuran species, including Ornate Chorus Frogs (*Pseudacris ornata*), now declining across much of their range. These wetlands are fire dependent and often lose their characteristic vegetation structure in the absence of regular growing-season fires (Gorman et al. 2013). Fire suppression causes wetlands to shift from open, herbaceous vegetation dominated systems to systems with high canopy cover and low amounts of herbaceous vegetation (Appendix A; Kirkman 1995). This loss of herbaceous vegetation is thought to contribute to the declines of native amphibians (Bishop and Haas 2005; Gorman et al. 2009), including Ornate Chorus Frogs, which are more commonly found in sites with high herbaceous vegetation cover (Chapter 2; Alford 1986; Gorman et al. 2013). As part of an ongoing adaptive management project to restore fire-maintained vegetation structure in these wetlands, we were interested in understanding the response of rare amphibians to increased herbaceous vegetation. The goals of our study were to examine the effects of a shifting habitat structure on tadpole predation by a common crayfish species. We experimentally tested whether a higher percent cover of herbaceous vegetation

increased tadpole survival or increased tadpole growth rates for two anuran species both in the presence of a crayfish.

## MATERIALS AND METHODS

### *Experiment 1*

We used Southern Leopard Frogs as our focal tadpole species. We originally intended to study Ornate Chorus Frogs, but chose leopard frogs as a replacement because sufficient numbers of Ornate Chorus Frog eggs/tadpoles could not be located. Southern Leopard Frogs are common throughout the southeastern United States and lay eggs in large egg masses, which makes them a convenient species for large-scale experiments. We collected four leopard frog egg masses from a borrow pit on Eglin Air Force Base. We housed egg masses individually in 10-gallon aquaria filled with approximately 6 cm of water from the borrow pit until tadpoles emerged. The water in each aquarium was aerated with a small aerator, and we fed tadpoles a small amount of fish food approximately 24 hours prior to starting the experiment.

We selected the Panhandle Crayfish (*Procambarus evermanni*) as the predator for our experiment. Panhandle Crayfish are the most common crayfish species in pine flatwoods ephemeral wetlands of northwestern Florida (Chandler, unpubl. data, Chapter 2), and they reach sufficient size to serve as a predator of tadpoles. We collected 15 male *P. evermanni* from inundated areas on Eglin AFB. All crayfish appeared to be healthy, had both chelae intact, and had a carapace length between 25 and 30 mm. We housed crayfish in small plastic containers (approximately 20 x 20 cm) prior to adding them to the experiment (all crayfish were fed tadpoles before they were placed in experimental tanks).

We used a completely randomized design in which our experimental units were 30 150-liter oval plastic mesocosms (82 x 54 x 32 cm; Tuff Stuff Products, Terra Bella, California). We arranged experimental mesocosms in a 5 x 6 array in an open field at Eglin AFB, Niceville, Florida. Mesocosms were randomly assigned to one of six possible treatment combinations that were each replicated five times. We manipulated the amount of herbaceous vegetation cover (0%, 34%, and 68% coverage) in each mesocosm and the presence of a predator (in this case a single crayfish). The percent herbaceous cover used in this experiment was representative of the herbaceous cover documented in natural wetlands (Appendix A). To examine the effect of vegetation structure, without the confounding effects of litter production or effects on DO, we simulated the herbaceous vegetation commonly found in ephemeral wetlands in northwestern Florida using plastic grass mats designed for aquaria. Each mat was 25 x 25 cm with 100 clumps of plastic vegetation that were approximately 15 cm tall. Mesocosms assigned to the low vegetation treatment received two mats placed in the center of the mesocosm (34% coverage), and mesocosms assigned to the high vegetation treatment received four mats placed in the center (68% coverage). We placed the vegetation mats together in the center of the mesocosm to form one continuous patch of vegetation.

We added approximately 2 cm of sand to the bottom of each mesocosm to act as substrate. Also, we added 30 g of dried grass litter collected from the edge of a natural wetland and filled each mesocosm to a depth of 20 cm using tap water. In addition to the dried grass litter, we added 10 g of crushed rabbit chow to each mesocosm to promote algal and bacterial growth. We inoculated each mesocosm with 200 ml of filtered water taken from three natural wetlands (combined sample) on the day mesocosms were filled and one week later. We also added two iButton temperature data loggers (one on each end of the mesocosm; Maxim

Integrated, San Jose, California) sealed inside small plastic bags filled with sand. We drilled small holes at one end of each mesocosm to ensure water levels remained close to 20 cm even during precipitation events. Finally, each mesocosm was covered with a fiberglass window screen lid to eliminate the possibility of colonization by unwanted predatory invertebrates or other amphibians. We allowed mesocosms to acclimate for 2.5 weeks before adding animals.

We combined the newly hatched leopard frog tadpoles into a combined sample to reduce the possibility of genetic influences. We added 20 tadpoles to each mesocosm on 19 February 2014. We measured the snout to vent (SVL) and total length (TL) of five tadpoles from each mesocosm prior to adding them to the tanks. When tadpoles were added to treatments they were at stage 25 (Gosner 1960). We randomly assigned one Panhandle Crayfish to each mesocosm in the predator treatment on the same day as the tadpoles.

We sampled the tadpoles in each mesocosm once a week. Before sampling, we removed the artificial vegetation from each tub while being careful to ensure that tadpoles and crayfish were not in the vegetation. If the mesocosm had a crayfish present, we removed the crayfish before catching any tadpoles to reduce the chances of inadvertently causing a predation event. Then we attempted to catch all of the tadpoles alive in each mesocosm. We randomly selected 10 tadpoles from each mesocosm and measured their SVL and TL. Tail injury (an indicator of failed predation attempts; Morin 1985; Semlitsch 1990; Wilbur and Semlitsch 1990) was recorded in all surviving tadpoles from weeks 6 – 10. We returned artificial vegetation and crayfish to their respective mesocosm prior to returning tadpoles. All surviving tadpoles were returned to the middle of the mesocosm at the same time.

We repeated this sampling protocol once a week for 10 weeks. During these 10 weeks, several crayfish were found dead during mesocosm sampling. All dead crayfish were replaced

either on the day they were found or the next day, and no mesocosm had a dead crayfish on more than one occasion. We believe that these deaths were likely related to the cold weather in late February and early March (e.g. water temperatures in most mesocosms fell to approximately 5°C on February 27 and 28) and the initial stress of being kept in the lab prior to being added to mesocosms. On 30 April 2014 we destructively sampled all mesocosms, and final measurements for tadpoles and crayfish were obtained. We euthanized all surviving tadpoles in a buffered solution of tricaine methane sulfonate (MS-222).

### *Experiment 2*

We used Ornate Chorus Frog tadpoles collected from three wetlands on Eglin AFB. We collected 75 tadpoles and housed them overnight in a large aquarium filled with water from one of the capture sites. Six Panhandle Crayfish were collected using the same methods described above. We housed crayfish individually, overnight in small plastic containers.

We arranged 12 10-gallon glass aquaria in a 4 x 3 array in the same field where the large scale experiment took place. Tanks were randomly assigned to one of four treatment combinations, which were replicated three times. We manipulated the amount of vegetation (high or low) and the presence of a single crayfish. We omitted the 'no vegetation' level for logistical reasons (insufficient number of tadpoles) and because it is unlikely that Ornate Chorus Frogs would use habitat with no herbaceous vegetation (Gorman et al. 2013). We used the same type of artificial vegetation for this experiment, but vegetation was cut into smaller pieces so that it fit into the smaller tanks. The 'high vegetation' treatments received 80% vegetation coverage, and the 'low vegetation' treatments received 40% vegetation coverage.

We filled the 10-gallon aquaria to a depth of 15 cm with a 40/60 mixture of filtered pond water and dechlorinated tap water. Each aquarium had 2 cm of sand in the bottom, and 1.5 g of ground rabbit chow to promote algal growth. We covered all of the aquaria with a large tent, which had mesh sides to allow sunlight to reach the aquaria. On 23 March 2014, we added six Ornate Chorus Frog tadpoles to each aquarium from a combined sample of all tadpoles that were collected. A single Panhandle Crayfish was randomly assigned to the predator aquaria and added to each tank.

We sampled tadpoles in each aquarium twice a week. Artificial vegetation and crayfish were removed prior to sampling to reduce the chances of influencing the predation rate. Tadpole SVL, TL, and instances of tail injury were measured once a week, and the number of surviving tadpoles in each aquarium was recorded twice a week. We also conducted 50% water changes using dechlorinated tap water once a week, while study animals were removed from the aquaria. There was no crayfish mortality in this experiment. On 20 April 2014, all surviving tadpoles were euthanized in a buffered solution of tricaine methane sulfonate (MS-222).

### *Statistical Analysis*

We used linear mixed-effects models to analyze the effects of vegetation and crayfish on tadpole survival and SVL. We fit two models for each experiment (one for survival and one for SVL). We used SVL instead of TL because the tail injury inflicted by crayfish caused TL averages to vary substantially from week to week. Linear mixed-effects models also allowed us to include data from mesocosms that had data from the beginning of the experiment, but later in the experiment had no measurements because there were no surviving tadpoles. We included the week of measurement, presence of a predator, vegetation level, and all two-way interactions as

fixed effects. The mesocosm was treated as a random effect to account for differences between mesocosms. All models were fitted with random intercepts only. Model likelihoods were calculated using restricted maximum likelihood (REML), and Wald tests were used to calculate *P*-values for the fixed effects.

We performed contrasts on significant two-way interactions using *Z*-tests on the calculated least squared means. For interactions involving time, we did not examine differences between weeks as we expected measurements to change over time. We did test for differences between the different levels of vegetation and predator within the same week. We adjusted *P*-values for contrasts using the Holm-Bonferroni correction (Holm 1979). We adjusted the *P*-values for each mixed-model separately because models were generated using different datasets. We used an ANOVA to examine whether the vegetation treatments affected the percentage of surviving tadpoles with tail injury at the end of the experiment (week 10 data). We performed post hoc multiple comparisons (Tukey HSD) on the means at different vegetation levels. All analyses were performed in R (R Development Core Team 2014). Mixed-effects models were fit using the nlme package (Pinheiro et al. 2014), and least squared means and contrasts were performed using the lsmeans package (Lenth 2014).

## RESULTS

### *Experiment 1*

Mean survival of Southern Leopard Frogs was 74% lower in crayfish treatments than in treatments without crayfish (Figure 3.1). Over time, there was a significant effect of crayfish presence on tadpole survival ( $F_{9,234} = 51.1$ ,  $P < 0.001$ ; Table 3.1). There was no interaction between vegetation and crayfish presence on tadpole survival ( $F_{2,24} = 0.4$ ,  $P = 0.71$ ) and no

effect of vegetation over time on survival ( $F_{2,24} = 0.6, P = 0.88$ ). The number of surviving tadpoles in the predator treatments (averaged across vegetation levels) was lower than the number in the nonpredator treatments by week 3 ( $Z = 3.6, P = 0.001$ ), and this effect continued through the end of the experiment ( $P < 0.001$  for weeks 4 – 10). The substantial decline in tadpole survival was observed despite 67% (10 of 15) of crayfish being replaced due to mortality. However, eight of the dead crayfish were replaced by week 4 and no mesocosm had a crayfish replaced more than once. In addition to the decline in survival, tail injury was observed in all 15 mesocosms with a crayfish (Appendix D; only three instances of tail injury occurred in non-crayfish mesocosms). By week 10, there was a significant difference between the percentage of surviving tadpoles with tail injury in the different vegetation treatments ( $F_{2,10} = 6.8, P = 0.013$ ). The high vegetation treatment had a significantly lower percentage of tail injury compared to the no vegetation treatment (Tukey HSD,  $P = 0.014$ ; Figure 3.2). However, this trend was not consistent over weeks 6 – 10 of the experiment (tail injury data for all surviving tadpoles were only available over this time period; Figure 3.2).

Over the course of the experiment crayfish presence had a significant effect on leopard frog tadpole SVL ( $F_{9,229} = 12.3, P = < 0.001$ ; Table 1). Surviving tadpoles in the crayfish treatments were 6.9% larger than tadpoles in nonpredator treatments by week seven ( $Z = 3.3, P = 0.032$ ; Figure 3.1). This trend continued through the end of the experiment, and by week 10 tadpoles in predator treatments were 13.0% larger than tadpoles in nonpredator treatments. Over time, the amount of vegetation also had an effect on tadpole SVL ( $F_{18,229} = 3.3, P < 0.001$ ). Tadpoles in both the high and low vegetation treatments tended to be larger than tadpoles in the no vegetation treatment (Figure 3.1). Tadpoles in the low vegetation treatment were the largest and were significantly larger than tadpoles from the no vegetation treatment during five of the

last six weeks. The largest difference occurred during week 7 when tadpoles were, on average, 1.86 mm longer in the low vegetation treatments than the no vegetation treatments ( $Z = 5.2$ ,  $P < 0.001$ ). Finally, there was no significant interaction between vegetation and predator on tadpole SVL ( $F_{2,24} = 0.4$ ,  $P = 0.67$ ; Table 3.1). However, it does appear that tadpoles in the crayfish treatments survived slightly better when high amounts of vegetation were present (Figure 3.1). The high vegetation treatments had, on average, 1.7 more tadpoles alive from weeks 5 – 10 than the no vegetation treatments and 2.1 more tadpoles than the low vegetation treatments.

### *Experiment 2*

Ornate Chorus Frog tadpole survival was significantly affected by the presence of crayfish over time ( $F_{7,63} = 6.5$ ,  $P < 0.001$ ; Table 3.1). This effect was evident throughout the experiment and by week four, 66% of aquaria with a crayfish had no surviving tadpoles (Figure 3.3). On average, there were 4.3 more surviving tadpoles in the nonpredator aquaria by week 4 ( $Z = 7.6$ ,  $P < 0.001$ ). We also observed tail injury in 5 of 6 aquaria with a crayfish but none of the aquaria without a crayfish. Tadpole survival was not significantly affected by the amount of vegetation present in the tanks ( $F_{7,63} = 0.3$ ,  $P = 0.59$ ). Furthermore, neither crayfish nor vegetation had an effect on *P. ornata* SVL ( $F_{3,22} = 0.7$ ,  $P = 0.42$ ;  $F_{3,22} = 0.1$ ,  $P = 0.81$ ; Table 3.1).

## **DISCUSSION**

Overall, the effects of crayfish predation on larval anurans in ephemeral wetlands are poorly studied compared to other common predators. Previous studies have indicated that crayfish can exert strong predation pressure on larval anurans in a variety of habitats, especially if the species is invasive (Figiel and Semlitsch 1991; Gherardi et al. 2001; Cruz et al. 2006;

Davis et al. 2012). In both experiments, we observed a rapid decline in tadpole survival when a crayfish was present (Figures 3.1 and 3.3). The strong effect of predation was evident in all three vegetation treatments indicating that crayfish can be effective predators even in environments with a high amount of vegetation structure. Contrary to our expectations, we did not detect an interaction effect between crayfish presence and vegetation on tadpole survival in either experiment. Leopard frog tadpoles did survive at a slightly higher rate (~10%) in the high vegetation treatment, but this effect was not significant. Figiel and Semlitsch (1991) also found that crayfish predation was not affected by the presence of structure in the environment. Furthermore, in certain situations crayfish may actually become more successful predators by using vertical structure to reach the water's surface where tadpoles can congregate when a predator is present (Davis et al. 2012). We did not observe crayfish exhibiting this behavior during our experiment, but *P. evermanni* have been observed climbing on vegetation near the water's surface in natural wetlands (Chandler, pers. obs.). Overall, increasing habitat complexity appears to have little effect on the ability of crayfish to capture prey items, and some crayfish may even prefer vegetated habitats as a means to reduce their own predation risk (Jordan et al. 1996).

In addition to the rapid decline in tadpole survival, we also observed a high percentage of tail injury in both experiments (Appendix D). The percentage of surviving tadpoles with tail injury increased over the course of experiment 1, and by week 10 significantly more leopard frog tadpoles in the no vegetation treatment had tail injury than tadpoles in the high vegetation treatment (Figure 3.2). This suggests that even though survival was low across all treatments predator-prey encounters may have been less frequent when higher amounts of vegetation were present (Babbit and Tanner 1998; Nunes et al. 2010). To our knowledge this is the first study to

experimentally demonstrate that crayfish frequently cause nonlethal tail injury in larval anurans. However, crayfish presence has been shown to increase tail injury rates in natural wetlands (Nunes et al. 2010). Tail injury in larval anurans reduces swimming velocity and travel distances, which increases an injured tadpole's susceptibility to future predation attempts by crayfish (Figiel and Semlitsch 1991) and other predators (Semlitsch 1990).

Despite the negative impacts of crayfish on overall tadpole survival, leopard frog tadpoles grew larger when a crayfish was present. This is likely due to reduced intraspecific competition for food resources, which has been shown to increase tadpole growth rates when predators are present (Morin 1983; Morin 1987; Babbit and Tanner 1998). We also observed a general trend of increasing leopard frog tadpole size when vegetation was present, although this relationship was less consistent (Figure 3.1). Even though both increases in SVL were small, it suggests that more food resources were available to the surviving individuals in predator treatments (due to reduced competition) and when herbaceous vegetation was present (possibly because of increased surface area for algal growth). Increases in growth rate can provide important ecological advantages, especially in ephemeral wetlands where metamorphosing faster decreases the potential for mortality because of drying events (Skelly 1997).

In both experiments, we failed to detect any interactions between crayfish presence and the amount of vegetation. This was somewhat expected for leopard frogs because they are a generalist species that can breed in a variety of wetland types ranging from heavily vegetated wetlands to unvegetated borrow pits. Leopard frogs may be poorly suited to taking advantage of potential refugia offered by aquatic vegetation. However, it was unexpected that there were no interaction effects for Ornate Chorus Frogs given that this species breeds almost exclusively in wetlands dominated by herbaceous vegetation. In this case our sample size was likely too small

to detect any differences that may have existed, given the complete mortality in most tanks with crayfish by week 4. Furthermore, starting with larger tadpoles (because of an inability to locate *P. ornata* egg masses) limited the effects on growth that could be observed. In this case, increasing the aquaria size, the number of replicates, and number of Ornate Chorus Frog tadpoles may provide clearer results. Growth rates also slowed dramatically over the course of both experiments, and it is likely that tadpoles in both experiments were food-limited (Figure 3.1). Wilbur and Fauth (1990) indicated that leopard frog tadpoles can become crowded in mesocosm experiments at around one tadpole per two liters, which is similar to the initial densities in our experiment. Adding supplemental food to mesocosm experiments with a high density of tadpoles may be necessary to limit competition for food over a long-term experiment.

Aquatic vegetation and habitat complexity can have differing effects on predator-prey interactions depending on the type of predator involved in the interaction (Figiel and Semlitsch 1991; Lefcort and Eiger 1993; Davis et al. 2012). Crayfish generally employ a sit-and-wait strategy that allows them to effectively capture prey in diverse environments (Gherardi et al. 2001). Our results demonstrated that crayfish are equally effective predators regardless of the amount of herbaceous vegetation present. This indicates that crayfish likely exert similar predation pressure on larval amphibians in pine flatwoods wetlands with a fire maintained vegetation structure and in pine flatwoods wetlands that have experienced fire suppression or exclusion (both vegetation structures are present on Eglin Air Force Base; Appendix A). In pine flatwoods wetlands, the ability of crayfish to act as significant predators is important because their burrowing ability allows them to persist through dry periods. Further, we demonstrated that encounters between crayfish and tadpoles often result in nonlethal tail injury, which may increase susceptibility to future predation events (Figiel and Semlitsch 1991; Semlitsch 1990).

Our results also indicate that the negative effects of predation also had a positive effect on tadpole growth by reducing intraspecific competition. Herbaceous vegetation tended to increase growth rates but this effect was less than expected and using artificial vegetation eliminated any potential benefits from increased DO or primary production. Increased growth rates are important in ephemeral wetland systems because rapid growth allows developing amphibians to metamorphose faster and at a larger size before wetland drying. Metamorphosing at a larger size may increase fitness by allowing earlier reproduction, higher survival (Smith 1987), or larger adult body size, which is tied to higher reproductive output in anurans (Howard 1978).

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Table 3.1. Fixed effects Wald tests for linear mixed effects models examining the effects of vegetation and presence of a predator on tadpole growth and survival. Experiment 1 was conducted using Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpoles and contained three vegetation treatments (68%, 34%, and 0% coverage). Experiment 2 was conducted with Ornate Chorus Frog (*Pseudacris ornata*) tadpoles and contained two vegetation treatments (80% and 40% coverage). A single Panhandle Crayfish (*Procambarus evermanni*) was used as a predator treatment in both experiments.

	Numerator DF	Denominator DF	F-statistic	P-value
<i>Lithobates sphenoccephalus</i> Survival (Exp. 1)				
(Intercept)	1	234	1033.9	<0.001
Week	9	234	100.7	<0.001
Vegetation	2	24	0.4	0.67
Predator	1	24	75.0	<0.001
Vegetation*Predator	2	24	0.4	0.71
Week*Predator	9	234	51.0	<0.001
Week*Vegetation	18	234	0.6	0.88
<i>Lithobates sphenoccephalus</i> Growth (Exp. 1)				
(Intercept)	1	229	16961.4	<0.001
Week	9	229	793.7	<0.001
Vegetation	2	24	7.3	0.003
Predator	1	24	9.5	0.005
Vegetation*Predator	2	24	0.4	0.67
Week*Vegetation	18	229	3.3	<0.001
Week*Predator	9	229	12.3	<0.001
<i>Pseudacris ornata</i> Survival (Exp. 2)				
(Intercept)	1	63	274.4	<0.001
Week	7	63	21.0	<0.001
Vegetation	1	8	0.3	0.59
Predator	1	8	56.2	<0.001
Week:Predator	7	63	6.5	<0.001
Week:Vegetation	7	63	1.9	0.089
Vegetation:Predator	1	8	0.5	0.48

	Numerator DF	Denominator DF	F-statistic	P-value
<i>Pseudacris ornata</i> Growth (Exp. 2)				
(Intercept)	1	22	7094.5	<0.001
Week	3	22	5.2	0.0074
Vegetation	1	8	0.1	0.81
Predator	1	8	0.7	0.42
Week:Predator	3	22	0.3	0.81
Week:Vegetation	3	22	0.1	0.93
Vegetation:Predator	1	8	0.5	0.50

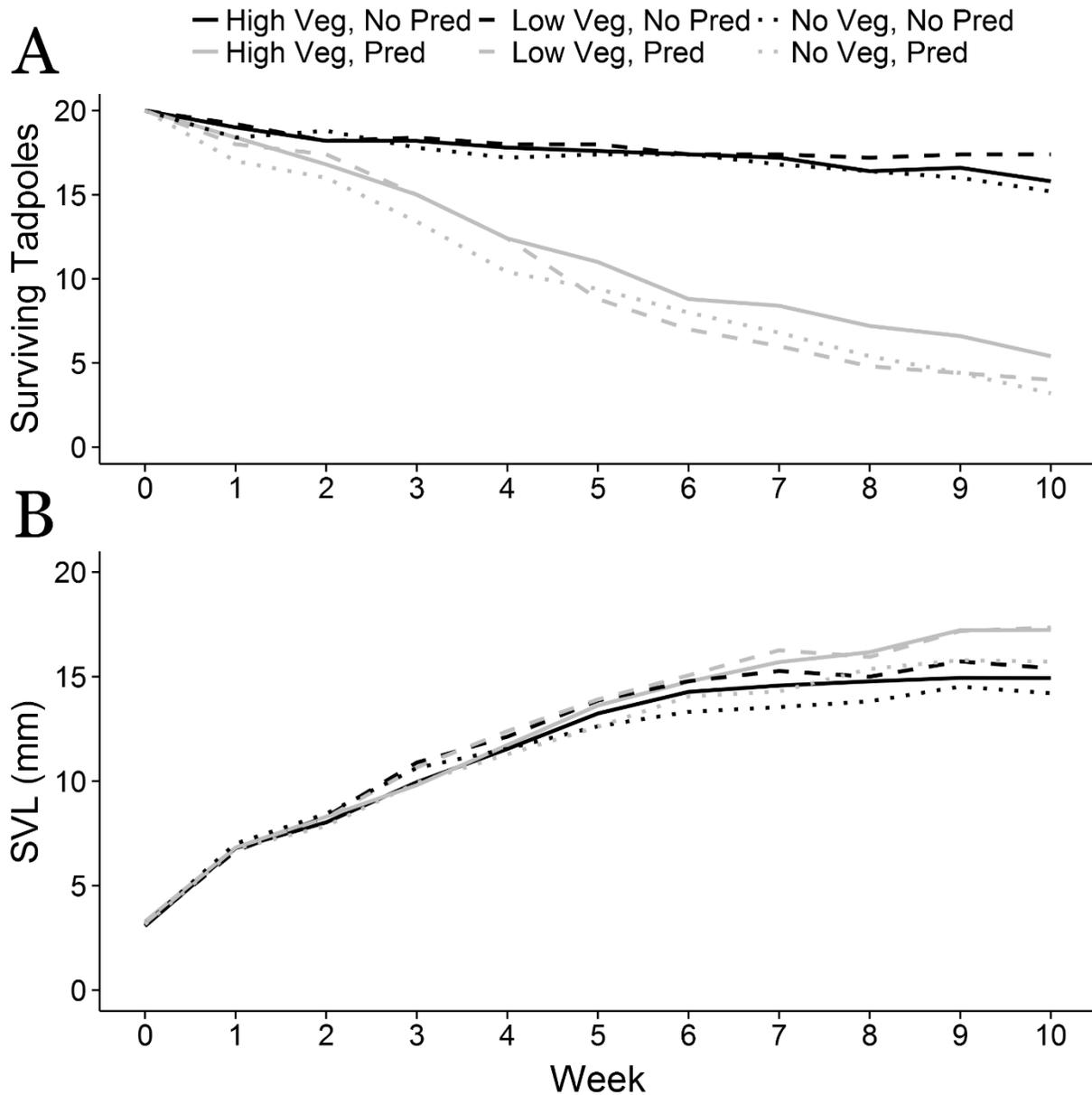


Figure 3.1. Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpole survival (A) and growth rates (B) in a mesocosm experiment with six treatments: high vegetation (68% coverage) with and without a predator, low vegetation (34% coverage) with and without a predator, and no vegetation with and without a predator. A single Panhandle Crayfish (*Procambarus evermanni*) was present in the predator treatment groups.

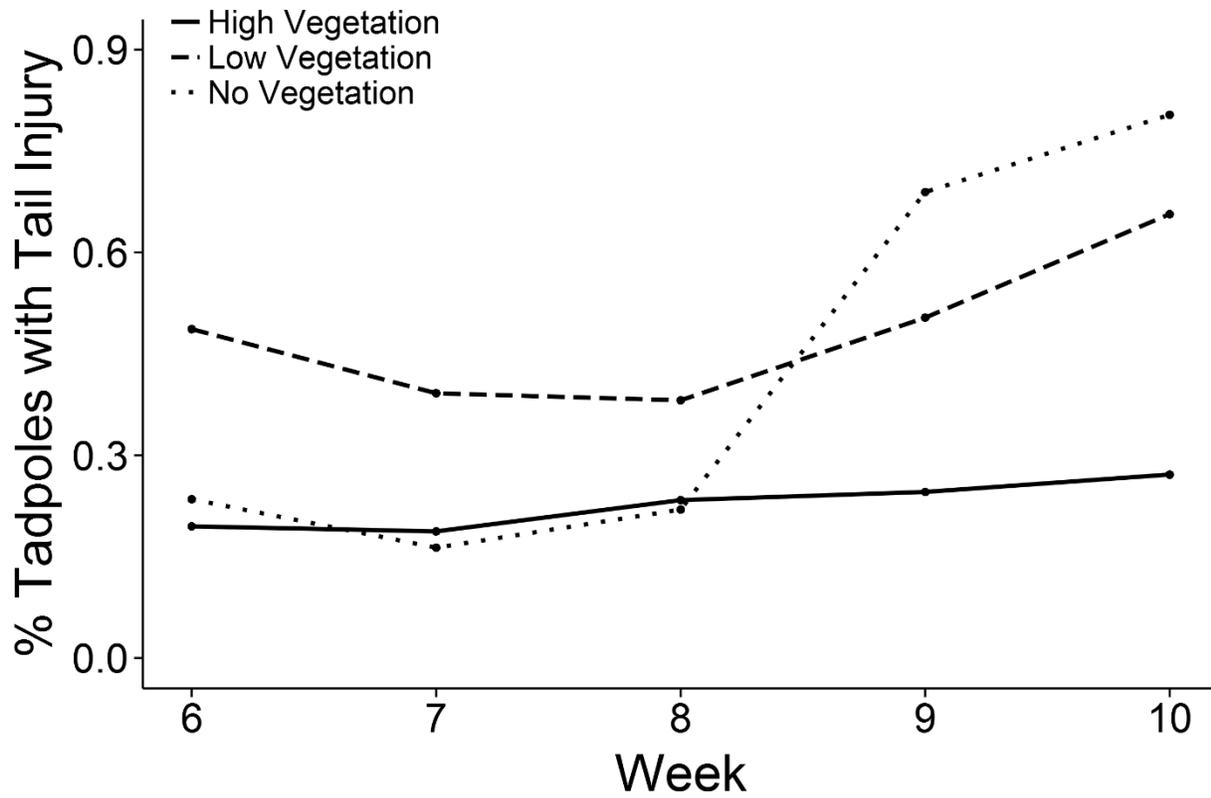


Figure 3.2. The frequency of Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpoles with tail injury in mesocosms containing a single Panhandle Crayfish (*Procambarus evermanni*).

Mesocosms had three different amounts of herbaceous vegetation cover: high (68% coverage), low (34% coverage), and no vegetation.

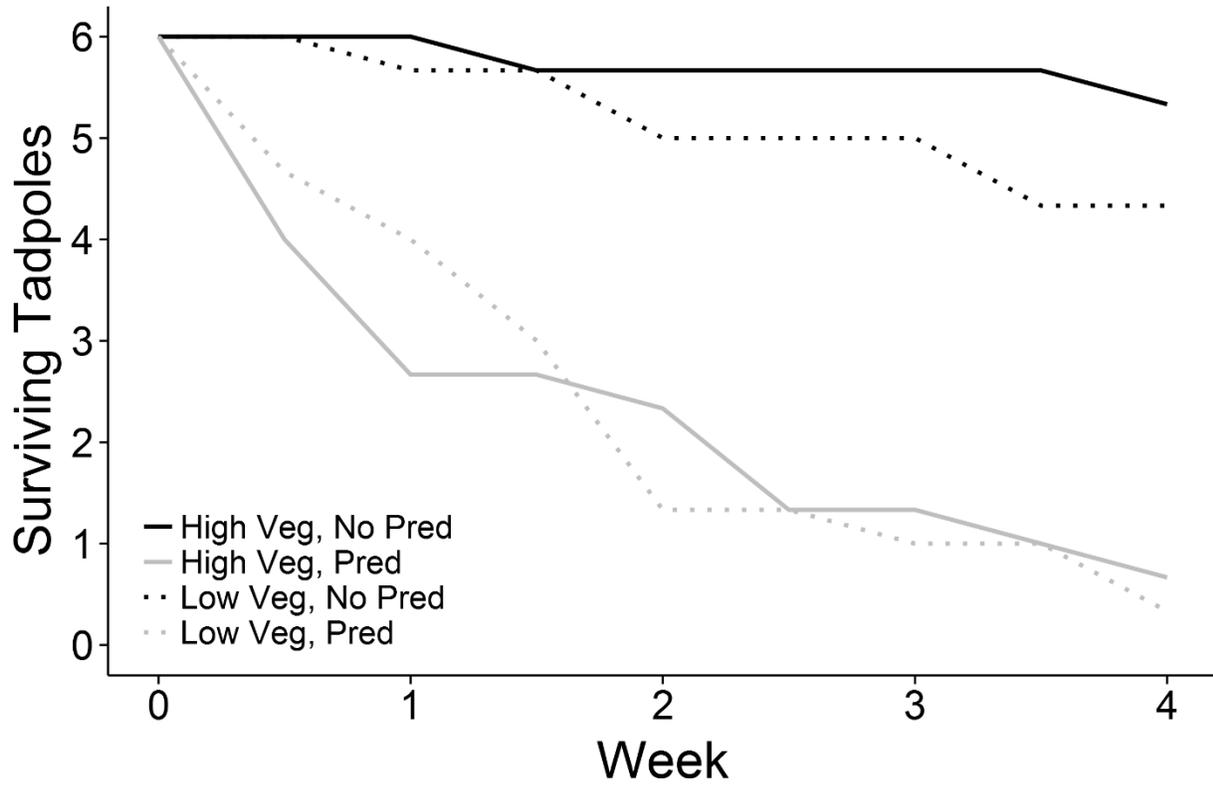


Figure 3.3. Ornate Chorus Frog (*Pseudacris ornata*) tadpole survival rates in four treatments including high vegetation (80% coverage) with and without a predator and low vegetation (40% coverage) with and without a predator. A single Panhandle Crayfish (*Procambarus evermannii*) was present in the predator treatments.

## CONCLUSIONS

Ephemeral wetlands support highly diverse aquatic communities that provide annual inputs of energy into the surrounding landscape through amphibian and insect emergence. The southeastern United States is characterized by a diverse assemblage of ephemeral wetlands but has also experienced severe habitat degradation and destruction since European colonization. Many longleaf pine systems have lost their historic disturbance regime because of fire suppression and a shift from growing-season to dormant-season fires (the normal time when prescribed fires are set). Further, climate changes are likely to impact ephemeral wetland hydrology, which is directly related to precipitation and temperature. Therefore, it is important to understand how past habitat changes and future climate changes will affect ephemeral wetlands and the organisms that depend on them. In this thesis, I have described historic hydroperiods in previously identified *A. bishopi* breeding wetlands using a unique modeling approach (Chapter 1), described amphibian and invertebrate communities and habitat characteristics in pine flatwoods wetlands (Chapter 2), and identified how vegetation changes caused by long-term fire suppression affect amphibian and invertebrate communities (Chapters 2 and 3).

My results suggest that over time hydroperiods in pine flatwoods wetlands were generally unfavorable for *A. bishopi* reproduction because of this species' long development time when compared to other amphibians. These results indicate that *A. bishopi* populations are able to persist despite years with little to no reproductive output in a majority of breeding wetlands. However, regular reproductive failures likely make flatwoods salamander populations more susceptible to other types of habitat change that degrade wetland quality (i.e. changing vegetation structures). A change-point analysis also indicated that a majority of wetlands have

had shorter hydroperiods over the last 15 years than at any point since 1896. The climate in the southeastern United States is predicted to experience an increased frequency of drought, rising temperatures, and more unpredictable precipitation patterns (Mulholland et al. 1997; Karl et al. 2009; Ingram et al. 2013; Romero-Lankao et al. 2014). These changes will likely increase the frequency of years where *A. bishopi* reproduction is unsuccessful. Therefore, focusing on maintaining wetlands with high habitat quality that sustain the longest possible hydroperiod during years with marginal precipitation (i.e. not severe drought years) is a key management goal.

From 2013 – 2014, I documented diverse assemblages of amphibians and aquatic invertebrates occupying pine flatwoods wetlands. This included two amphibian species that are rare and declining over much of their range (*A. bishopi* and *P. ornata*) and a previously undocumented crayfish species. Wetlands also possessed a range of habitat characteristics, in part due to ongoing adaptive management strategies (Appendix A; Gorman et al. 2013), which makes these sites ideal for identifying relationships between habitat quality and wetland communities. Amphibian communities were similar across a range of canopy and herbaceous vegetation coverage but were more diverse in wetlands with longer hydroperiods than in wetlands with short hydroperiods. Wetlands with a hydroperiod that extends into late spring or early summer may provide breeding habitat for a wider range of species that includes both winter and summer breeders. My results also indicate that some invertebrate groups respond directly to changes in wetland vegetation structure. Isopods accounted for a majority of invertebrate captures and were more abundant in wetlands with lower shrub density and lower canopy cover, characteristics associated with frequent fire (Bishop and Haas 2005). Maintaining the historic

fire return interval would likely benefit amphibian and invertebrate communities, especially for species that are specialists in pine wetlands.

Finally, changing wetland vegetation structures are likely to affect important species interactions. I tested this hypothesis using a large scale mesocosm experiment. My results indicate that crayfish pose a significant predation threat to larval anurans and often cause nonlethal tail injury, which can negatively affect tadpole swimming ability (Semlitsch 1990; Figiel and Semlitsch 1991). There was little effect of different amounts of herbaceous vegetation on *L. sphenoccephalus* tadpoles, which is not completely unsurprising given that this species is a habitat generalist that often breeds in wetlands with little or no herbaceous vegetation. It would have been preferable to conduct this experiment with *P. ornata* (a species typically found in wetlands with dense herbaceous vegetation), as originally planned, to increase sample sizes to better detect a response, and to provide supplemental food in order to better assess growth rates. Intraspecific competition did appear to limit tadpole growth in treatments without predators, and decreasing survival caused by predation actually led to higher growth rates. This experiment suggests that crayfish can be important predators in ephemeral systems, especially since they possess adaptations to persist through dry phases as large adults. Further research is needed to identify whether or not herbaceous vegetation amounts affect habitat specialists or provide refugia from other predator species. In addition, this study focused only on the effects of herbaceous vegetation as a refuge from predation. There is still a need for research on how herbaceous vegetation provides litter input to aquatic herbivores, whether it is grazed directly, and its role in dissolved oxygen production (especially during winter when many species are dormant).

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## MANAGEMENT IMPLICATIONS

Eglin Air Force Base has an award-winning natural resource management program including extensive upland fire management for endangered species. Fire management of wetlands, however, has been less of a priority. Wetland species that require open-canopy and dense herbaceous vegetation are declining in the region, and on Eglin AFB. The following recommendations are targeted to improve management of pine flatwoods wetlands in general (but may have applications for other wetlands on the installation) and for Reticulated Flatwoods Salamanders (*Ambystoma bishopi*) in particular.

### Objectives and Strategies

1. Managing wetlands that sustain the longest possible hydroperiod (but still remain ephemeral) is key for successful Reticulated Flatwoods Salamander reproduction. Effort should be focused on lengthening hydroperiods during marginal years where hydroperiods are around 2-3 months, as these are the years when good management could lead to successful reproduction that may not otherwise be possible.
  - Maintain low-stocking density of trees in uplands.
  - Reduce woody midstory in wetlands.
  - Larger wetlands may, on average, be more likely to sustain longer hydroperiods and these wetlands could be targeted for initial management strategies.
  - Wetlands in close proximity have high conservation value (see item 6).
2. For flatwoods salamanders, restoration techniques should focus on wetlands with some high quality habitat where salamanders are actively breeding. Further efforts should be

concentrated on wetlands that may have low quality vegetation structure but are located close to active flatwoods salamander breeding wetlands.

3. Wetlands should be managed to support vegetation structures that were historically maintained by a regular fire return interval. This includes a high abundance of herbaceous vegetation with a pine overstory and few shrubs in the midstory.
  - Focus on burning ephemeral wetland basins during prescribed burns.
  - These wetlands are usually inundated during the normal season for prescribed burns, but wetlands could be burned later in the year if the surrounding areas were burned by an earlier prescribed fire.
  - Severe drought years may also create a unique opportunity to burn wetland basins at a time of year when it would not otherwise be possible.
4. There is a potential to cause mortality for flatwoods salamander adults or eggs when burning during the winter months (November – February). In successful breeding years, there is also a potential to cause mortality for metamorphs leaving breeding wetlands in spring (late March – May). This mortality risk is likely low, but should be considered when planning prescribed burns in pine flatwoods.
  - When possible burn wetlands in June or July.
  - Consult with research crew when planning burns outside the growing-season.
5. Wetlands that have developed a dense shrub midstory due to fire suppression or exclusion will require additional management, because wetlands with a shrub midstory are unlikely to burn even when dry.
  - Remove shrub midstory through mechanical and herbicide treatment (with consultation on appropriate herbicides and timing).

- Follow with growing-season prescribed fire to restore herbaceous vegetation.
  - If regular fire return interval is not restored after treatment, repeated mechanical and herbicide work is likely to be required.
6. Landscape level connectivity is likely important in pine flatwoods wetlands as some species actively colonize wetlands through overwash during precipitation events. This often includes fish species but also may be important for some invertebrates that compose a large percentage of larval flatwoods salamander diets.
- Restore wetlands in close proximity to one another to increase connectivity and benefit aquatic communities.
7. Maintaining multiple separate populations (e.g. East Bay and Oglesby) reduces risk of catastrophic mortality from disease or other factors. External threats should be monitored.
- Development resulting in increased impervious surfaces and/or groundwater withdrawal.
  - Salinization (sea level rise).
  - Climate change (increased ET, more frequent drought, and/or more unpredictable precipitation patterns)

## **FUTURE RESEARCH QUESTIONS**

- How does groundwater influence wetland hydroperiod in the study wetlands?
- How do the changes in vegetation structure affect ephemeral wetland hydrology, especially evapotranspiration rates?
- How long do flatwoods salamanders live in the wild? On average, how many years are adult flatwoods salamanders reproductively active?
- Are species, especially flatwoods salamanders, able to use crayfish burrows (or other holes) to extend wetland hydroperiod by surviving underground?
- What role does overwash from other waterbodies play in allowing species to rapidly colonize ephemeral wetlands?
- Are dissolved oxygen levels related to the changes in wetland vegetation structure? Can DO be a limiting factor for certain species in pine flatwoods wetlands?
- What are the population densities for crayfish in pine flatwoods wetlands? Are there other species that have not been documented in these wetlands?
- Can dormant-season fires effectively replace growing-season fires?
- Do dormant-season fires pose a threat to flatwoods salamander eggs or migrating adults?

## APPENDIX A. STUDY SITE INFORMATION

**Description of Previously Applied Treatments:** Treatments were applied to the 21 wetlands surveyed for Chapter 2 as part of two ongoing active management projects designed to reduce the impacts of fire suppression on wetland vegetation structures (see Gorman et al. 2013 for a detailed description of these projects). Wetlands included in this study were initially categorized as high-quality or low-quality. Low-quality wetlands were divided into multiple treatments resulting in four treatment groups:

- 1) Wetlands were classified as high quality if they were occupied by Reticulated Flatwoods Salamanders between 2006 and 2008 and had high amounts of herbaceous vegetation with low to moderate canopy cover.
- 2) Some wetlands with an overgrown midstory received a mechanical and herbicide treatment to reduce shrubs in the midstory. Shrubs with a DBH < 12.7 cm were mechanically removed, and herbicide was applied to the cut stumps.
- 3) Some wetlands that received the mechanical and herbicide treatment also received a prescribed fire in the surrounding area after the treatment was applied. Burn quality was often inconsistent in the wetland basins.
- 4) Wetlands were classified as low quality if they had a thick shrub midstory, received no treatment, and were not recently occupied by Reticulated Flatwoods Salamanders.

These treatments are listed in Table A1 with the corresponding wetland I.D. number. The year in parentheses indicates when the treatments were applied.

Table A1. Habitat characterizations, sampling data, and the presence of target species in 21 ephemeral wetlands on Eglin Air Force Base, Florida. Minnow trapping was conducted during 2014, and the presence of Reticulated Flatwoods Salamander (*Ambystoma bishopi*), Ornate Chorus Frogs (*Pseudacris ornata*), and Panhandle Crayfish (*Procambarus evermanni*) was identified during 2013 and 2014. A detailed description of the vegetation quality index is given in Chapter 2, and treatment groups are described above.

Site I.D.	Treatment Group (date applied)	Minnow Trapping	Vegetation Quality	Hydroperiod Length	<i>Ambystoma bishopi</i>	<i>Pseudacris ornata</i>	<i>Procambarus evermanni</i>
1	Mech + Burn (2012)		High	Medium			*
2	Mech + Burn (2012)	*	High	Long		*	*
3	Mech + Burn (2012)	*	High	Medium		*	*
4	Mech (2010) + Burn (2012)		High	Medium	*	*	*
5	High Quality		High	Medium	*	*	*
6	Mech (2010)		Low	Medium			*
7	Mech (2010)		Low	Medium		*	
11	Low Quality		Low	Short			
12	Mech (2010) + Burn (2012)	*	Medium	Long		*	*
13	Mech + Burn (2012)	*	High	Short		*	*
15	High Quality		Medium	Long	*	*	*
16	High Quality		High	Medium		*	*
33	Mech (2010)		Medium	Medium	*	*	*
34	Low Quality		Low	Long	*	*	*
50	Low Quality		Low	Short			*
53	High Quality		Medium	Medium	*	*	*
103	Mech (2010)		Low	Long			
107	Mech (2010)	*	Medium	Long		*	
126	Mech + Burn (2010)	*	High	Short			*
200	Low Quality		Low	Medium			
201	Low Quality	*	Low	Long		*	

Table A2. Vegetation measurements in 21 ephemeral wetlands on Eglin Air Force Base, Florida. Dissolved oxygen, depth, and canopy cover measurements were recorded during 2014. Herbaceous vegetation cover and shrub density were measured during fall of 2013, and hydroperiod was calculated by averaging the longest yearly hydroperiod from 2010 – 2014 that occurred at least partially between October and April.

Site I.D.	Dissolved Oxygen (mg/L)			Canopy Cover (%)	Herbaceous Cover (%)	Shrub Density (per m <sup>2</sup> )	Average Depth (cm)	Average Hydroperiod (months)
	February	March	April					
1				18.00	75.09	1.59	15.40	3.5
2	6.94	8.27	7.61	22.60	49.89	0.36	38.20	5.5
3	4.31	5.24	4.19	46.55	32.24	0.56	25.28	3.8
4	2.17	3.14	3.23	28.44	60.30	0.93	15.53	3.5
5	2.61	4.24	3.63	21.37	47.53	1.54	21.88	3.6
6				68.42	3.89	1.02	40.90	4.9
7				52.98	11.50	0.50	39.00	3.4
11				70.29	2.50	1.12	38.30	2.3
12	6.85	7.80	6.38	44.15	28.99	0.72	24.61	6.0
13				37.93	55.55	0.56	25.34	2.8
15	3.96	5.65	3.62	38.97	16.34	2.50	32.49	6.2
16	6.03	6.25	4.82	37.93	42.38	0.93	32.00	4.9
33	3.99	4.97	4.57	39.85	12.84	0.68	17.93	3.3
34	4.57	4.10	2.38	81.80	10.07	0.74	38.76	5.5
50	2.22	2.79	2.45	65.83	15.25	2.08	4.60	2.5
53	1.97	2.70	2.34	62.52	26.06	1.50	17.89	4.8
103				72.53	2.50	1.63	35.10	5.4
107				42.47	6.98	1.97	33.10	5.2
126				37.23	40.86	1.05	7.60	2.3
200				66.79	8.50	1.43	43.00	4.0
201	5.13	4.72	4.25	58.26	6.93	0.69	63.60	6.6

Table A3. Vegetation structure in 21 ephemeral wetlands on Eglin Air Force Base was measured during the fall of 2013. Tree and shrub species were measured using the point-centered quarter method, and herbaceous vegetation species were recorded using a Daubenmire frame.

Site I.D.	# of Sampling Points	Dominant Tree Species	# of Tree Species	Dominant Shrub Species	# of Shrub Species	Dominant Herb Species	# of Herbaceous Species
1	7	<i>Pinus elliottii</i>	2	<i>Ilex glabra</i>	5	<i>Aristida stricta</i>	4
2	11	<i>Pinus elliottii</i>	4	<i>Hypericum chapmanii</i>	8	<i>Aristida stricta</i>	7
3	17	<i>Pinus elliottii</i>	4	<i>Ilex myrtifolia</i>	11	<i>Dichanthelium sp.</i>	12
4	11	<i>Pinus elliottii</i>	3	<i>Ilex glabra</i>	11	<i>Aristida stricta</i>	10
5	8	<i>Pinus palustris</i>	4	<i>Ilex glabra</i>	7	<i>Aristida stricta</i>	7
6	9	<i>Pinus elliottii</i>	5	<i>Cyrilla racemiflora</i>	5	<i>Aristida stricta</i>	2
7	15	<i>Pinus elliottii</i>	5	<i>Ilex myrtifolia</i>	10	<i>Amphicarpum muhlenbergianum</i>	6
11	10	<i>Pinus palustris</i>	6	<i>Cyrilla racemiflora</i>	6	No Herbaceous Vegetation	0
12	14	<i>Pinus elliottii</i>	4	<i>Hypericum chapmanii</i> and <i>Ilex glabra</i>	13	<i>Aristida stricta</i>	8
13	8	<i>Pinus elliottii</i>	3	<i>Cyrilla racemiflora</i>	10	<i>Aristida stricta</i>	6
15	12	<i>Pinus elliottii</i>	7	<i>Cliftonia monophylla</i>	13	<i>Eriocaulon compressum</i>	11
16	16	<i>Pinus elliottii</i>	4	<i>Hypericum chapmanii</i>	6	<i>Eriocaulon compressum</i>	14
33	13	<i>Pinus elliottii</i>	4	<i>Ilex glabra</i>	10	<i>Aristida stricta</i>	10
34	14	<i>Pinus elliottii</i>	5	<i>Ilex myrtifolia</i>	9	<i>Sporobolus floridanus</i>	6
50	15	<i>Pinus elliottii</i>	7	<i>Cliftonia monophylla</i> and <i>Lyonia lucida</i>	13	<i>Arundinaria gigantea</i>	10
53	14	<i>Pinus elliottii</i>	6	<i>Ilex glabra</i>	14	<i>Aristida stricta</i>	8
103	10	<i>Pinus elliottii</i>	5	<i>Lyonia lucida</i>	5	<i>Aristida stricta</i>	2
107	12	<i>Pinus elliottii</i>	6	<i>Lyonia lucida</i>	8	<i>Aristida stricta</i>	5
126	8	<i>Pinus elliottii</i>	2	<i>Ilex glabra</i>	4	<i>Aristida stricta</i>	6
200	10	<i>Pinus elliottii</i>	4	<i>Ilex glabra</i>	7	<i>Calamovilfa curtissii</i>	5
201	11	<i>Pinus elliottii</i>	8	<i>Ilex myrtifolia</i>	11	<i>Aristida stricta</i>	2



Figure A1. Photo of a pine flatwoods ephemeral wetland that has a fire-maintained vegetation structure (top), and a pine flatwoods ephemeral wetland that has a vegetation structure characteristic of long-term fire suppression (bottom).

**APPENDIX B. POOLED AMPHIBIAN DIPNETTING CAPTURES**

Table B1. Total amphibian dipnetting captures from surveys of 21 ephemeral wetlands on Eglin Air Force Base, Florida. Wetlands were dipnetted up to 10 times per season from December – April in 2012 – 2014 when there was water in the wetlands. Sampling was reduced from twice a month to once a month if *A. bishopi* were captured in the wetlands that season, to avoid damage to the vegetation. Additional dipnetting was conducted along a transect set along each wetlands longest axis once a month from January – April 2014.

Species	Wetland I.D. Number																				
	1	2	3	4	5	6	7	11	12	13	15	16	33	34	50	53	103	107	126	200	201
<i>Ambystoma bishopi</i>	0	0	0	5	9	0	0	0	0	0	10	0	6	5	0	38	0	0	0	0	0
<i>Acris gryllus</i>	21	8	13	0	10	3	2	1	9	9	2	19	8	3	14	2	4	6	17	5	8
<i>Anaxyrus quercicus</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Eurycea quadridigitata</i>	1	0	0	0	0	0	0	0	0	0	11	0	7	0	2	1	0	0	0	0	0
<i>Lithobates grylio</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Lithobates sphenoccephalus</i>	0	29	3	15	94	3	89	0	38	0	49	37	11	181	8	36	0	50	0	13	77
<i>Notophthalmus viridescens</i>	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	10
<i>Pseudacris nigrita</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0
<i>Pseudacris ornata</i>	0	1	7	22	32	0	8	0	0	0	0	4	0	1	0	51	0	11	0	0	0
<i>Pseudacris sp.</i>	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0

## APPENDIX C. FULL AIC MODELING RESULTS

Table C1. Multiple linear regression modeling results relating invertebrate abundance to vegetation characteristics and wetland hydrology in ephemeral wetlands on Eglin Air Force Base, Florida (K = the number of parameters, AICc = second-order Akaike's Information Criteria,  $\Delta$ AICc = change in AICc, and  $w_i$  = relative amount of support for each model).

Candidate Model	AICc	K	$\Delta$ AICc	$w_i$
<b>Isopoda</b>				
Canopy + Shrubs	145.9	4	0	0.32
Canopy + Herbaceous + Shrubs	148.4	5	2.49	0.09
Herbaceous + Shrubs	148.5	4	2.58	0.09
Canopy + Shrubs + Hydroperiod	148.5	5	2.66	0.09
Canopy	148.7	3	2.82	0.08
Canopy + Shrubs + Depth	148.9	5	2.97	0.07
Herbaceous	149.5	3	3.62	0.05
Canopy + Hydroperiod	150.8	4	4.87	0.03
Canopy + Herbaceous	150.8	4	4.96	0.03
Canopy + Depth	151.7	4	5.87	0.02
Herbaceous + Shrubs + Hydroperiod	151.9	5	6.00	0.02
Herbaceous + Shrubs + Depth	152.0	5	6.07	0.02
Canopy + Herbaceous + Shrubs + Hydroperiod	152.0	6	6.09	0.02
Canopy + Herbaceous + Shrubs + Depth	152.3	6	6.39	0.01
Herbaceous + Hydroperiod	152.4	4	6.48	0.01
Herbaceous + Depth	152.5	4	6.59	0.01
Canopy + Shrubs + Depth + Hydroperiod	152.5	6	6.62	0.01
Canopy + Herbaceous + Hydroperiod	153.8	5	7.90	0.01
Canopy + Depth + Hydroperiod	154.0	5	8.10	0.01
Canopy + Herbaceous + Depth	154.3	5	8.40	0.00
Herbaceous + Depth + Hydroperiod	155.3	5	9.46	0.00
Shrubs	155.5	3	9.62	0.00
Hydroperiod	155.8	3	9.89	0.00
Depth	155.8	3	9.90	0.00
Herbaceous + Shrubs + Depth + Hydroperiod	155.9	6	9.99	0.00
Shrubs + Depth	156.0	4	10.09	0.00
Canopy + Herbaceous + Shrubs + Depth + Hydroperiod	156.6	7	10.70	0.00
Canopy + Herbaceous + Depth + Hydroperiod	157.2	6	11.28	0.00
Shrubs + Hydroperiod	157.3	4	11.44	0.00
Depth + Hydroperiod	158.5	4	12.62	0.00
Shrubs + Depth + Hydroperiod	159.4	5	13.56	0.00

Candidate Model	AICc	K	$\Delta$ AICc	$w_i$
<b>Chironomidae</b>				
Depth + Hydroperiod	61.0	4	0	0.15
Depth	61.6	3	0.58	0.11
Herbaceous	61.8	3	0.80	0.10
Canopy	61.8	3	0.86	0.10
Shrubs + Depth + Hydroperiod	63.0	5	2.04	0.06
Canopy + Depth	63.27	4	2.29	0.32
Canopy + Depth + Hydroperiod	63.45	5	2.46	0.29
Herbaceous + Depth + Hydroperiod	63.55	5	2.57	0.28
Herbaceous + Depth	63.77	4	2.79	0.25
Herbaceous + Hydroperiod	63.93	4	2.95	0.23
Shrubs + Depth	64.36	4	3.38	0.18
Canopy + Herbaceous	64.49	4	3.51	0.17
Canopy + Hydroperiod	64.57	4	3.59	0.17
Hydroperiod	64.63	3	3.64	0.16
Canopy + Shrubs	64.74	4	3.76	0.15
Shrubs	64.74	3	3.76	0.15
Herbaceous + Shrubs	64.78	4	3.80	0.15
Canopy + Shrubs + Depth + Hydroperiod	65.62	6	4.63	0.10
Herbaceous + Shrubs + Depth + Hydroperiod	66.05	6	5.07	0.08
Canopy + Shrubs + Depth	66.25	5	5.27	0.07
Canopy + Herbaceous + Depth	66.75	5	5.77	0.06
Herbaceous + Shrubs + Depth	66.97	5	5.99	0.05
Canopy + Herbaceous + Hydroperiod	67.21	5	6.23	0.04
Herbaceous + Shrubs + Hydroperiod	67.25	5	6.27	0.04
Canopy + Herbaceous + Depth + Hydroperiod	67.34	6	6.35	0.04
Shrubs + Hydroperiod	67.71	4	6.73	0.03
Canopy + Shrubs + Hydroperiod	67.82	5	6.83	0.03
Canopy + Herbaceous + Shrubs	67.82	5	6.84	0.03
Canopy + Herbaceous + Shrubs + Depth + Hydroperiod	70.20	7	9.22	0.01
Canopy + Herbaceous + Shrubs + Depth	70.25	6	9.27	0.01
Canopy + Herbaceous + Shrubs + Hydroperiod	70.96	6	9.98	0.01
<b>Zygoptera</b>				
Canopy + Hydroperiod	35.6	4	0	0.34
Canopy + Shrubs + Hydroperiod	36.5	5	0.96	0.21
Canopy + Depth + Hydroperiod	38.6	5	3.0	0.08
Canopy + Herbaceous + Hydroperiod	38.7	5	3.12	0.21
Canopy + Depth	38.8	4	3.20	0.20
Canopy	39.9	3	4.38	0.11
Canopy + Herbaceous + Shrubs + Hydroperiod	40.1	6	4.50	0.11

Candidate Model	AICc	K	$\Delta$ AICc	$w_i$
Canopy + Shrubs + Depth + Hydroperiod	40.5	6	4.90	0.09
Canopy + Herbaceous	41.3	4	5.70	0.06
Canopy + Shrubs + Depth	41.7	5	6.12	0.05
Canopy + Shrubs	41.9	4	6.35	0.04
Canopy + Herbaceous + Depth	41.9	5	6.38	0.04
Canopy + Herbaceous + Depth + Hydroperiod	42.4	6	6.82	0.03
Herbaceous + Hydroperiod	42.6	4	7.07	0.03
Canopy + Herbaceous + Shrubs	43.4	5	7.83	0.02
Hydroperiod	44.0	3	8.46	0.01
Canopy + Herbaceous + Shrubs + Depth + Hydroperiod	44.7	7	9.12	0.01
Canopy + Herbaceous + Shrubs + Depth	45.2	6	9.65	0.01
Herbaceous + Shrubs + Hydroperiod	45.3	5	9.71	0.01
Herbaceous	45.8	3	10.26	0.01
Herbaceous + Depth + Hydroperiod	46.0	5	10.41	0.01
Herbaceous + Depth	46.4	4	10.83	0.00
Depth + Hydroperiod	46.7	4	11.13	0.00
Shrubs + Hydroperiod	46.9	4	11.32	0.00
Depth	47.1	3	11.50	0.00
Shrubs	47.3	3	11.72	0.00
Herbaceous + Shrubs	48.6	4	13.08	0.00
Herbaceous + Shrubs + Depth + Hydroperiod	49.3	6	13.70	0.00
Shrubs + Depth + Hydroperiod	49.6	5	14.02	0.00
Herbaceous + Shrubs + Depth	49.8	5	14.29	0.00
Shrubs + Depth	50.1	4	14.55	0.00

**APPENDIX D. PHOTOS OF TAIL INJURY CAUSED BY CRAYFISH**

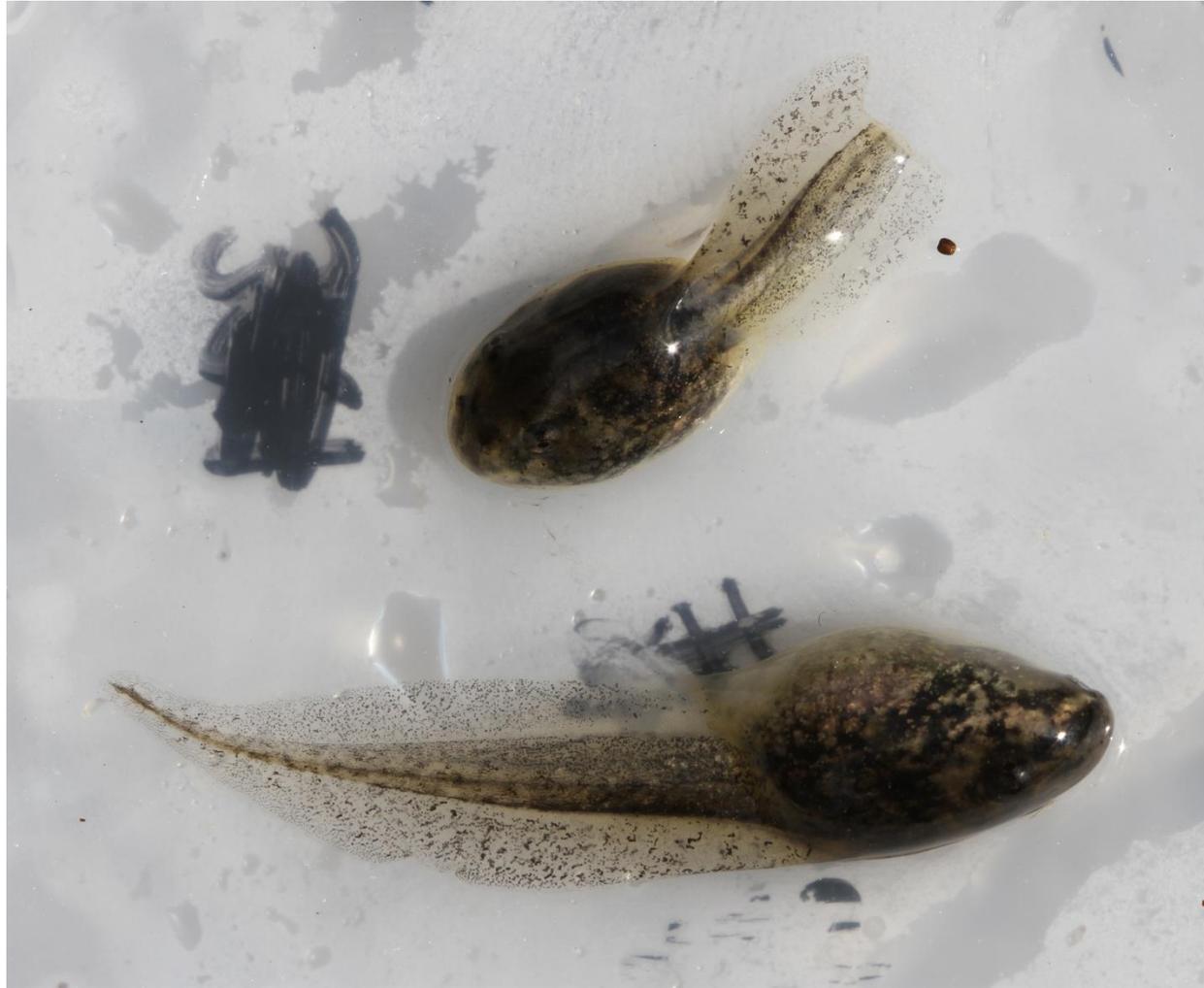


Figure D1. Non-lethal tail injury inflicted on a Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpole (top) by Panhandle Crayfish (*Procambarus evermanni*) in a mesocosm experiment.



Figure D2. Non-lethal tail injury inflicted on an Ornate Chorus Frog (*Pseudacris ornata*) tadpole by Panhandle Crayfish (*Procambarus evermanni*) in an aquaria experiment.