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Experimental Restoration of Pine Flatwoods Wetlands

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EXECUTIVE SUMMARY

Fire is recognized as an important disturbance in longleaf pine uplands of the southeastern US, but less is known about the role of fire or other disturbances in the wetlands embedded within this ecosystem. The reticulated flatwoods salamander (*Ambystoma bishopi*), a federally endangered species, and other rare and declining amphibians, are less likely to breed in wetlands with high canopy cover and low herbaceous groundcover (low-quality wetlands) that typically result from fire exclusion. Fire rarely carries through these wetlands during winter because of the presence of standing water at this time of year. Our objective was to evaluate whether mechanical removal of the woody midstory could serve as a surrogate for fire, and create high-quality wetlands with moderate canopy cover and high herbaceous groundcover. We chose a series of high-quality and low-quality ephemeral wetlands for study. A subset of the low-quality wetlands were then treated mechanically and with herbicide, burned, or retained in a low quality state. Additionally, at a subset of these sites we established drift fences and collected data on the breeding migration for reticulated flatwoods salamanders. Mechanical treatments reduced canopy cover to similar levels as high-quality sites; however, herbaceous groundcover did not increase by one year post-treatment. Fire reduced the canopy cover, and herbaceous groundcover was similar to high-quality sites as of 4-months post burn. More time will be required to assess the response of herbaceous groundcover and whether mechanical methods can be used as a surrogate for fire to restore amphibian breeding habitat. If longer-term monitoring shows that herbaceous vegetation and salamander populations do increase in response to mechanical treatments, this surrogate for fire would add an important technique to our management toolbox. Based on the current information, however, it appears that fire will be necessary to retain high-quality habitat. Continuing to monitor the response of endangered species to alternative management actions will improve our ability to recover populations.

INTRODUCTION

The southeastern United States, historically dominated by longleaf pine savannas, was a landscape shaped by frequent fire (Frost 1995, Stout and Marion 1993). After over a century of fire suppression, land managers currently recognize the importance of fire to longleaf pine uplands (e.g., Provencher *et al*. 2001), but there are still problems with implementing fires in wetlands within this ecosystem (Bishop and Haas 2005). Our research focuses on restoration of wetlands within longleaf pine savannas for rare and declining amphibians in the Gulf Coastal Plain of the Florida Panhandle. In particular, our goal is to evaluate and identify management practices that will improve breeding habitat for the reticulated flatwoods salamander (*Ambystoma bishopi*), a federally endangered species that breeds in ephemeral wetlands within the longleaf pine ecosystem (USDI Fish and Wildlife Service 2009, Palis 1996).

Fire has played an important role in wetlands in the longleaf pine ecosystem, but historic fire regimes of these wetlands are not as well understood as those of the surrounding uplands (Frost 1995, Kirkman 1995, Hinman and Brewer 2007). Longleaf pine uplands are thought to have been subject to low-intensity fires every 1-4 years (Platt *et al.* 1988, Martin and Kirkman 2009). Ephemeral ponds that are embedded in a fire-dominated landscape are likely to have burned somewhat less frequently than the surrounding uplands, but probably at least every 4-10 years (Frost 1995, Kirkman 1995). These natural fires (i.e., from lightning strikes) were essential to maintaining the high-quality habitat conditions (open canopy cover and high herbaceous

groundcover) within this ecosystem upon which most amphibians and other wildlife species were dependent. However, exclusion of fire from wetlands, primarily from a shift to dormant season fire, has led to low-quality habitat conditions (dense canopy cover and low herbaceous groundcover and understory) that do not support as high a diversity of wildlife. Because high-quality wetlands and their ecotones within the longleaf pine uplands harbor much higher wildlife diversity (Kirkman *et al*. 1998, Hinman and Brewer 2007, Jones *et al.* 2010), it is important to understand how to retain or restore wetlands towards these historic conditions.

Fire (natural or prescribed) is necessary for maintaining and restoring the habitat conditions on which breeding amphibians in these ephemeral depression wetlands depend (Russell *et al.* 1999, Means *et al*. 2004, Bishop and Haas 2005). Reticulated flatwoods salamanders breed in ephemeral wetlands with a well-developed herbaceous groundcover and relatively open canopy that are embedded within the longleaf pine system (Sekerak *et al*. 1996, Gorman *et al*. 2009). Adult salamanders migrate to the wetlands in late fall, and lay their eggs in the dry wetland basin. The eggs hatch into aquatic larvae once winter rains inundate the wetland. The larvae feed predominantly on aquatic invertebrates (Whiles *et al.* 2004), taking approximately 3-4.5 months to grow and develop (Palis 1995), and then metamorphose into a terrestrial form in the spring. Complete reproductive failure occurs when ponds dry before the larvae can reach metamorphosis within this time frame.

Frequently-burned wetlands may be more suitable for reproduction of flatwoods salamander and other amphibians because frequent fire results in increased hydroperiod by reducing woody vegetation, increased herbaceous groundcover, increased water temperatures, increased dissolved oxygen, and increased invertebrate prey (important for larval salamanders) or algal growth (important for anuran tadpoles) (deSzalay and Resh 1997, Skelly *et al*. 2002, Gorman *et al*. 2009, Sacerdote and King 2009, Shulse *et al.* 2012). Shortened hydroperiod has negative consequences for amphibian reproduction in ephemeral wetlands, and increased woody vegetation (because of increasing evapotranspiration) can result in shortened hydroperiods (Huxman *et al*. 2005). Several studies have demonstrated a positive relationship between increased amounts of herbaceous groundcover and the presence or abundance of larval amphibians (Gorman *et al*. 2009, Shulse *et al.* 2012). Long periods without fire result in an accumulation of plant litter that can reduce dissolved oxygen below levels required by amphibians (Sacerdote and King 2009). Removal of a woody midstory reduces canopy cover allowing increased insolation and higher water temperatures, and increased in situ photosynthesis, which in turn allows more rapid growth and development of larval amphibians (Skelly *et al.* 2002, Sacerdote and King 2009).

Prescribed fire has been a widely used technique for restoring habitat conditions to the longleaf pine ecosystem (Brockway *et al.* 2005, Van Lear *et al.* 2005). As in the uplands, fire-suppression in wetlands within this system fosters the growth of invasive hardwoods that results in the development of a dense woody midstory. In addition, there is a corresponding loss of the herbaceous component due to inadequate sunlight reaching the ground to foster the growth of herbaceous groundcover (Martin and Kirkman 2009). Although there is some debate about the importance of burn season for managing longleaf pine uplands (e.g., Palik *et al.* 2002), season of fire clearly has implications for management of seasonally ephemeral wetlands. In the Florida Panhandle, these wetlands are typically inundated during the winter months, but are dry during the normal lightning season in the late spring and summer when the wetlands are most susceptible to burning (Bishop and Haas 2005, Gorman and Haas, Virginia Tech, Blacksburg, VA, unpublished data).

Management prescriptions to restore wet lowlands and the surrounding flatwoods call for growing-season fire, but recognize that dormant-season fires may be necessary to reduce fuel loads before growing-season burning. Moreover, because the dense woody midstories in these wetlands may be fire-resistant, as seen with other hardwood species (Kane *et al.* 2008), long-term frequent burning may be necessary to restore herbaceous understory vegetation (Waldrop *et al.* 1987, Brockway and Lewis 1997, Brockway *et al.* 2005).

A better understanding of the role of fire in maintaining wildlife populations was identified as one of seven key information needs for longleaf pine restoration efforts (Brockway *et al*. 2005). Here, we describe the short-term results from an on-going adaptive management study. The goal of our current study is to develop techniques to restore ephemeral wetlands to enhance reproductive success of flatwoods salamanders and other winter-breeding amphibians in a longleaf pine ecosystem. Because of the challenges of implementing growing-season fire (see Knapp *et al*. 2009), we are particularly interested in evaluating whether and how quickly alternatives to growing-season fire can produce suitable conditions for amphibian breeding.

OBJECTIVES

The overall objectives of this ongoing study were designed to allow managers to develop appropriate management strategies to maintain and restore breeding habitat for the reticulated flatwoods salamander and a diverse suite of other amphibians and plants that occur in the same habitats. Here we present a final report for the first year of funding from the Department of Defense, Legacy Resource Management Program and focus on the following two objectives:

1. Understand how a range of potential vegetation management treatments influences herbaceous vegetation, hydroperiod, and use by the amphibian community (with emphasis on reticulated flatwoods salamanders).
2. Monitor drift fences to capture reticulated flatwoods salamanders and other amphibians.

METHODS

Study area

Our study area was located on Eglin Air Force Base (Eglin) in the counties of Okaloosa, Santa Rosa, and Walton in northwestern Florida. Eglin is a large military installation that spans 187,774 ha. The topography of the study area is level to rolling with the highest elevation at ~ 75 m and slopes that generally range from 0-30 % (Eglin Air Force Base 2002). Most of the upland habitat on Eglin is a longleaf pine (*Pinus palustris* Mill.) and turkey oak (*Quercus laevis* Walter) sandhill community. However, our sites were 25 ephemeral wetlands (ranging in size from 0.3 – 5.9 ha) located in the western portion of Eglin, usually surrounded by wet or mesic flatwoods. These wetlands had overstories of longleaf pine, slash pine (*Pinus elliottii* Engelm.), pond cypress (*Taxodium ascendens* Brongn.), and black gum (*Nyssa sylvatica* Marshall) and had open to dense midstories dominated by myrtle-leaved holly (*Ilex myrtifolia* Walter), buckwheat tree (*Cliftonia monophylla* [Lam.] Britton ex sarg.), and Apalachicola St. Johnswort (*Hypericum chapmanii* P. Adams).

Treatments

We selected 25 wetlands, including 21 wetlands with overgrown midstories, which were part of previous monitoring efforts. Occupied and “potential” breeding wetlands for flatwoods salamanders had been identified by Florida Natural Areas Inventory based on soil surveys and some ground-based surveys in the early 1990s. This list had been supplemented over the years

by our previous work and by other biologists. We selected a subset of these occupied and potential wetlands for study based on the condition of woody vegetation. We assigned the 21 overgrown-midstory wetlands to four different treatment categories using a stratified random approach based on wetland size. The remaining four sites were not treated and were considered high-quality sites and represented the desired condition. Because fire treatments were not under our control, some sites originally assigned as untreated low-quality sites were burned in a wildfire, while other sites assigned to a prescribed-burn treatment or a combined mechanical and prescribed-burn treatment were not burned as scheduled. This resulted in the following treatment categories:

1) *high-quality* (n = 4) – sites were occupied by reticulated flatwoods salamanders between 2006-2008 and had characteristics typical of reproductive habitat for this species (i.e., high amounts of herbaceous groundcover and moderate canopy cover; Gorman *et al*. 2009).
2) *mechanical/herbicide* (n = 8) – sites with an overgrown midstory that received a mechanical treatment and herbicide application.
3) *mechanical/herbicide* + *burn* (n = 2) – sites with an overgrown midstory that received a mechanical treatment and herbicide application and received a burn after the mechanical/herbicide work was completed.
4) *burn only* (n = 4) – sites with an overgrown midstory and where only fire was applied.
5) *low-quality* (n = 7) – sites with an overgrown midstory that received no treatment and were not recently occupied by flatwoods salamanders.

Mechanical treatments were conducted on the midstory vegetation (i.e., woody vegetation with a diameter at breast height (dbh) < 12.7 cm, excluding only pines and cedars) of the wetlands using hand-held saws (i.e., chainsaws and brush saws). Additionally, we treated the ecotone (i.e., the transition zone between the surrounding uplands and the wetlands) and in some cases cut smaller vegetation with machetes. We followed cutting with a cut-stump application of herbicide. We used only herbicides that were approved for use within aquatic environments (i.e., Triclopyr). We treated the mechanical/herbicide wetlands (n = 10) in August – September 2010 and any stems that were resprouting were retreated with a basal application of herbicide (i.e., Triclopyr) or in a few cases a foliar application (using a mixture of Triclopyr and Imazapyr) in September 2011. Fire crews were able to prescribe-burn only one wetland (in December 2010), but five wetlands were burned during a wildfire (in June 2011). Our sample sizes and inability to control the timing of fire treatments precluded us from analyzing the effects of fire seasonality.

Vegetation Sampling

To evaluate if habitat management practices improve conditions for flatwoods salamanders and other wetland-breeding amphibians, we collected pre- (Fall 2009) and post-treatment (Fall 2011) vegetation data at all sites (n = 25). We established multiple vegetation plots per wetland to acquire wetland-scale habitat characteristics. Plots were oriented along a transect that started in the ecotone surrounding the wetland and followed the long axis of the wetland. At both ends of the transect (i.e., within the ecotone), we also established two additional vegetation plots perpendicular to the transect to increase the number of plots within the ecotone, which is thought to be important for amphibian use. In total wetlands received from 7-21 vegetation plots depending on the size of the wetland.

Within each vegetation plot, we examined percent canopy cover (including woody midstory and overstory in a single measure), herbaceous groundcover, woody debris, and basal area (m^2

ha^{-1}), as potential descriptors of amphibian habitat. We measured percent canopy cover using a spherical densiometer, and basal area of woody vegetation using a Jim-Gem Cruz-All® (Forestry Suppliers Inc., Jackson, MS). We used the Daubenmire (1959) cover class scale to estimate the percent herbaceous groundcover and woody debris by visually estimating the percentage of each variable in a 0.5 m x 0.2 m plot.

Amphibian Sampling

Pre- and post-treatment amphibian sampling included surveying for larval amphibians, including flatwoods salamanders and ornate chorus frog (*Pseudacris ornata*), a Florida Species of Greatest Conservation Need that breeds in similar wetland types across the southeastern United States. We could only sample for amphibians at wetlands that contained water (at least approximately 3 cm deep in substantial portions of the pond for dipnetting; any standing water for call surveys), so the number of wetlands sampled varied somewhat across sampling periods. We conducted timed, systematic, dip-net surveys for larval amphibians twice per month from December–April 2009-2010 and 2011-2012 in each of the 25 wetlands (Bishop *et al*. 2006, Gorman *et al*. 2009), when water levels were sufficient to allow sampling. Also, we conducted nighttime call-surveys to assess the use of these wetlands by calling anurans (i.e., frogs and toads). We listened for amphibians at two calling stations per wetland, one at each end of the vegetation transect. Call surveys started 30 minutes after sunset and ended before 0200 hours. Each survey was five minutes long, and surveys were conducted at each calling station twice per month (when water was present) from December–April 2009-2010 and 2011-2012. Sample sizes for both dipnetting and call surveys were reduced in 2011-2012 when many ponds were full less than 1 or 2 of these months.

Additionally, we completely encircled the three ephemeral wetlands with drift fences and placed funnel traps on the inside and outside, following Palis (1997). On rainy nights, we checked drift fences on a continuous basis, alternating between ponds, with each trap checked approximately every two to three hours. On dry nights, when soil temperatures were at or below 15° C (reducing fire ant activity; Porter and Tschinkel 1987), we checked traps near dusk and just after dawn. On warmer dry nights or on humid nights, we attempted to attend traps every 5-7 hours through the night, but when there was little to no amphibian activity and staff availability was limited, we occasionally left traps unattended for 8-9 hours.

Upon capture of adult flatwoods salamanders, we uniquely marked individuals using visual implant elastomer (VIE) or passive integrated transponder (PIT) tags. Yearlings or small adults and gravid females were not PIT tagged. All marked animals received a batch toe-clip indicating the pond of capture. We ran the drift fences during three breeding seasons for flatwoods salamanders. Unfortunately, drought conditions prevented any successful reproduction during this season.

Table 1. Dates of operation for three drift fences in Oglesby Flatwoods, Eglin Air Force Base, Okaloosa County, Florida. Ponds were completely encircled and fences were operated continuously during dates shown below.

Season	Pond 4	Pond 5	Pond 51
2011-2012	10/31/2011-3/23/2012	10/31/2011-3/23/2012	10/31/2011-12/18/2011

Statistical Analysis

To understand pre- and post-treatment differences among sites, we used a Repeated Measures Analysis of Variance (ANOVA) using each habitat variable (percent canopy cover, percent herbaceous groundcover, basal area, and percent woody debris cover) and amphibian species richness as the response for a total of five separate analyses. We removed the two sites that were mechanical/herbicide treated + burn from this analysis, because only two replicates were available and it created an unbalanced statistical design. (We retain mention of this treatment in the paper as results from this treatment will be available in future analyses.) We considered treatment as a fixed effect and year as a repeated effect and our model included both main effects and an interaction of the main effects (year × treatment). Additionally, we used five planned contrasts that examined the differences among (1) the high-quality sites versus mechanical/herbicide treated sites, (2) low-quality sites versus mechanical/herbicide treated sites, (3) mechanical/herbicide treated sites versus burn only sites, (4) high-quality sites versus burn only, and (5) low-quality sites versus burn only sites. We performed contrasts with data pooled across years if there was no significant interaction between treatment and year in the overall ANOVA model. Contrasts were interpreted separately for each year if there was a significant interaction between treatment and year. We used a Modified Levene's test to evaluate the homogeneity of variances and normal probability plots to ensure the data sets met assumptions of normality and homoscedasticity. All analyses were performed in SAS 9.2 (SAS Institute, Cary, North Carolina, USA), and we used an alpha level of 0.05.

We used a two-sample t-test to compare the means of vegetation characteristics that were present at sites occupied by reticulated flatwoods salamanders and ornate chorus frogs as compared to unoccupied sites (i.e., sites where these two species were not detected). We restricted this analysis to the pretreatment year (2009-2010), because water was present in all wetlands during this winter breeding season.

RESULTS

We detected an interaction effect between year and treatment ($F_{3, 19} = 4.99$, $P = 0.010$) on canopy cover. Further, we detected a difference between high-quality sites and mechanical/herbicide treated sites pre-treatment (contrast 1, $F_{1, 19} = 4.58$, $P = 0.046$), but not post-treatment (contrast 1, $F_{1, 19} = 0.20$, $P = 0.6662$), with canopy cover being 19 % higher in mechanically treated sites before treatment, but similar following treatment (Figure 1). No other contrasts were significant for canopy cover in either year (contrasts 2, 3, 4, 5, $F_{1, 19} \leq 4.00$, $P \geq 0.060$).

We did not observe a significant interaction effect between year and treatment ($F_{3, 19} = 2.33$, $P = 0.106$) or treatment alone on percent herbaceous groundcover ($F_{3, 19} = 1.90$, $P = 0.164$), but we did detect a significant difference for year ($F_{1, 19} = 23.79$, $P < 0.001$) with the post-treatment year being lower than pre-treatment (Figure 2). Additionally, we detected a difference between high-quality sites and mechanical/herbicide treated sites (contrast 1, $F_{1, 19} = 5.53$, $P = 0.030$), with herbaceous groundcover being 25 % higher in high-quality sites pre-treatment and 20 % higher post-treatment (Figure 2). No other contrasts were significant for herbaceous groundcover (contrasts 2, 3, 4, 5, $F_{1, 19} \leq 2.24$, $P \geq 0.151$).

For basal area, we did not observe a significant effect of the year and treatment interaction ($F_{3, 19} = 0.60$, $P = 0.620$), or year alone ($F_{1, 19} = 0.87$, $P = 0.636$), but we did detect a difference among treatments ($F_{3, 19} = 3.46$, $P = 0.037$). We detected a difference between high-quality sites

and mechanical/herbicide treated sites (contrast 1, $F_{1, 19} = 4.80$, $P = 0.041$), with basal area being 4.4 m^2 ha^{-1} higher in mechanically treated sites before treatment (Figure 3). No other contrasts were significant for basal area (contrasts 2, 3, 4, 5, $F_{1, 19} \leq 3.08$, $P \geq 0.096$).

For woody debris, we did not observe a significant effect of the year and treatment interaction ($F_{3, 19} = 2.06$, $P = 0.140$), treatment alone ($F_{3, 19} = 2.82$, $P = 0.067$), or year alone ($F_{1, 19} = 0.015$, $P = 0.914$). We detected a difference between mechanical/herbicide treated sites and burn only sites (contrast 3, $F_{1, 19} = 4.01$, $P = 0.015$), with woody debris being 10 % higher in mechanically treated sites post-treatment, but only 4 % higher pre-treatment (Figure 4). No other contrasts were significant for woody debris (contrasts 1, 2, 4, 5, $F_{1, 19} \leq 4.02$, $P \geq 0.059$).

We detected a total of 18 amphibian species (Table 1), with the greatest number of species (8) occurring pre-treatment at the high-quality sites. For amphibian species richness we did not observe a significant effect of the year and treatment interaction ($F_{3, 19} = 0.42$, $P = 0.738$) or treatment alone ($F_{3, 19} = 2.39$, $P = 0.101$), but we did detect a difference between years ($F_{1, 19} = 59.51$, $P \leq 0.001$). This difference is likely only related to substantial differences in water levels between years. In the pre-treatment year, all 25 wetlands had sufficient water to dipnet and conduct call surveys, whereas post-treatment only 18 wetlands had sufficient water for dipnetting (~3 or more cm of water) and 22 wetlands had sufficient water for call surveys post-treatment. Species richness was higher pre-treatment than post-treatment (Figure 5). Also, we detected a difference between high-quality sites and mechanical/herbicide treated sites (contrast 1, $F_{1, 19} = 6.21$, $P = 0.022$), with amphibian species richness being higher in high-quality sites (i.e., ~3 > pre-treatment and~2 species > post-treatment; Figure 5). No other contrasts were significant for species richness (contrasts 2, 3, 4, 5, $F_{1, 19} \leq 4.00$, $P \geq 0.060$).

During the pretreatment year, when all wetlands had sufficient water levels, there was a significant difference in percent canopy cover and percent herbaceous groundcover between sites occupied by reticulated flatwoods salamanders (canopy: df = 23, $t = -3.59$, $P = 0.002$; herbaceous: df = 23, $t = 3.25$, $P = 0.004$) and ornate chorus frogs (canopy: df = 23, $t = -2.55$, $P = 0.018$; herbaceous: df = 23, $t = 2.64$, $P = 0.015$) and unoccupied sites (Figure 6). For both species, occupied sites had lower percent canopy cover and higher percent herbaceous groundcover (Figure 6). However, we did not detect a significant difference in basal area or percent cover of woody debris at sites occupied by reticulated flatwoods salamanders (basal area: df = 23, $t = -1.62$, $P = 0.120$; woody debris: df = 23, $t = 0.47$, $P = 0.642$) and ornate chorus frogs (basal area: df = 23, $t = -1.68$, $P = 0.107$; woody debris: df = 23, $t = 0.38$, $P = 0.709$) compared to unoccupied sites.

Drift fence captures of A. bishopi

At pond 4 we captured the first *A. bishopi* on 3 November 2011 and the last capture was made on 19 March 2012. In total we made 145 captures of 106 individuals (39 within-season recaptures) over 144 nights of running the drift fences (Table 2). The greatest number of captures occurred on 27-28 November 2011 with 28 captures. Further, 74% of captures occurred in November and December 2011.

At pond 5 we captured the first *A. bishopi* on 3 November 2011 and the last capture was made on 20 March 2012. In total we made 86 captures of 54 individuals (32 within-season recaptures) over 144 nights of running the drift fences (Table 2). The greatest number of captures occurred on 9-10 November 2011 with 12 captures. Further, 70% of captures occurred in November and December 2011.

At pond 51 no captures of *A. bishopi* were made. This drift fence was operated continuously for 48 days during a time of peak movement at ponds 4 and 5.

Overall, we made 231 captures of 160 individuals (71 within-season recaptures). Of the 160 adults, 72 were female, 70 were male, and 18 were unknown. Also, 23% of the individuals were first captured in year 1 and the remaining individuals were all new captures. We captured the first gravid females shortly after midnight on 10 November 2011. Previous to this, we had captured 9 males on 3-4 November, and 17 males and 2 females with unknown reproductive status earlier in the night of 9 November 2011.

Table 2. Frequency of amphibian species encounters (number of sites occupied/number of sites sampled) during dipnet and call surveys at 25 wetlands on Eglin Air Force Base, Florida from December - April 2009-2010 (Pre) and 2011-2012 (Post). All sites were sampled in pre-treatment year, but 3 sites were not surveyed for anurans and 7 sites were not dipnetted in the post-treatment year.

	Proportion of sites occupied	
Species	Pre	Post
Barking treefrog (*Hyla gratiosa*)	0.36	0.00
Bronze frog (*Lithobates clamitans*)	0.36	0.00
Central newt (*Notophthalmus viridescens*)	0.16	0.06
Dwarf salamander (*Eurycea quadridigitata*)	0.40	0.06
Eastern narrowmouth toad (*Gastrophryne carolinensis*)	0.00	0.14
Gray treefrog (*Hyla chrysoscelis*)	0.00	0.09
Green treefrog (*Hyla cinerea*)	0.04	0.00
Greenhouse frog (*Eleutherodactylus planirostris*)	0.00	0.05
Oak toad (*Anaxyrus quercicus*)	0.00	0.18
Ornate chorus frog (*Pseudacris ornata*)	0.52	0.32
Pig frog (*Lithobates grylio*)	0.36	0.00
Pine woods treefrog (*Hyla femoralis*)	0.08	0.23
Reticulated flatwoods salamander (*Ambystoma bishopi*)	0.32	0.06
Southern chorus frog (*Pseudacris nigrita*)	0.64	0.09
Southern cricket frog (*Acris gryllus*)	1.00	0.59
Southern leopard frog (*Lithobates sphenocephalus*)	1.00	0.55
Southern toad (*Anaxyrus terrestris*)	0.60	0.14
Squirrel treefrog (*Hyla squirella*)	0.00	0.05

DISCUSSION

Longleaf pine savannas and the embedded ephemeral wetlands support a diverse amphibian community, including 17 species that are found only in this ecosystem (Means 2006). However, natural resource managers have not always recognized the importance of fire to maintaining habitat quality for these species (Russell *et al*. 1999, Schurbon and Fauth 2003, Means *et al*. 2004, Schurbon and Fauth 2004). Our data from 25 ephemeral ponds in longleaf pine flatwoods clearly documented that two amphibians of conservation concern, the reticulated flatwoods

salamander and the ornate chorus frog, occupy sites with an open canopy and well-developed herbaceous understory, characteristics associated with frequent fire.

The preliminary results from the management experiment were more equivocal. Our mechanical treatments of ephemeral wetlands successfully reduced percent canopy cover to levels that were similar to those of high-quality sites (Figure 1). However, herbaceous groundcover, a critical component of larval flatwoods salamander habitat (Gorman *et al*. 2009, Sekerak *et al*. 1996), did not respond in the short-term to these treatments. The failure of herbaceous plants to respond positively to the treatments may be a result of several factors. In upland longleaf pine stands, herbaceous groundcover responded positively both to increased light levels and increased soil moisture levels associated with removal of hardwoods and shrubs (Harrington 2006). Perhaps in our study, herbaceous groundcover grew less in the drought year (post-treatment) than in the year with high precipitation (pre-treatment). This is supported by an overall decline of herbaceous groundcover in the drought year across treatments, including the high-quality sites (Figure 2). Further, the lack of response may be an artifact of insufficient time. Herbaceous understory of planted longleaf stands on the Savannah River Site in South Carolina took over three years to respond to removal of understory hardwoods and shrubs (Harrington 2006). Because restoration of wetland vegetation can take several years to progress, researchers and managers should expect >3 years to observe successful outcomes (Martin and Kirkman 2009, De Steven *et al*. 2010). Additionally, while we did not have sufficient sample sizes to include in our formal analyses, we did observe an increase in herbaceous groundcover in the mechanical/herbicide + fire treatment. Several studies have documented the importance of fire to growth and reproduction of herbaceous groundcover in the longleaf pine ecosystem (Walker and Silletti 2006, Fill *et al*. 2012), but we did not see an immediate response to fire alone. The large shrubs and trees in these overgrown stands were not immediately killed by the fires, suggesting that mechanical treatment may be required to reset conditions in sites that have experienced long-term fire suppression. We were unable to determine the length of time that a given wetland had gone without fire before our study, because generally these wetlands are not explicitly evaluated for burn success (Bishop and Haas 2005). The apparent increase in herbaceous groundcover in the two sites subjected to mechanical/herbicide + fire treatment provides tentative but tantalizing support that this combined treatment may effectively facilitate herbaceous plant growth.

Amphibian species richness varied across years, but the primary influence on amphibian use of wetland sites post-treatment was insufficient water levels because of drought in 2011-2012. Because other studies have found that amphibians may take four years or more to colonize newly created or restored wetlands (e.g., Pechmann *et al*. 2001, Sacerdote 2009), we anticipated amphibians would take longer to respond than one or two years post-treatment. We are continuing to collect data on amphibian community and the influence of these treatments.

We observed a 12.5 % reduction in basal area from pre- to post-treatment for mechanical/herbicide treated sites (n = 8). This reduction was not significant and did not reduce basal area for mechanical/herbicide treated sites to levels similar to high-quality sites (Figure 3). The minimal reduction in basal area was likely related to our treatment methods that focused on removal of small to medium sized woody stems (< 12.7 cm dbh) from the midstory, while retaining the larger trees. The reduction that we saw was consistent with reductions in basal area for other studies conducting midstory removals (Parrott *et al*. 2011, Bailey *et al*. 2011). Similarly, burn only treatments did not reduce basal area to levels similar to high-quality sites. Larger stems may not be readily removed during a low-intensity fire.

Because the response of vegetation and amphibians can take several years, and because amphibian populations show large annual fluctuations, we are continuing to monitor the response of vegetation and amphibians at these sites. We also installed hydrological monitoring wells post-treatment so that we can document whether evapotranspiration is lower, and hydroperiod longer, in sites from which the woody midstory is removed. In January and February 2012, prescribed fire was applied to an additional four study ponds, making treatments more balanced. Being able to compare the full suite of treatments will allow us to assess whether mechanical/herbicide treatments alone can serve as a surrogate for fire, whether fire alone can restore densely overgrown stands, or whether the combination of mechanical/herbicide + burn treatments is necessary to restore habitat components important to rare and declining amphibians. We hope the recent drought will end and we will be able to collect additional data, so we can better compare herbaceous groundcover and amphibians to pre-treatment (non-drought) conditions. If after 4-5 years, flatwoods salamanders and ornate chorus frogs have not colonized sites and herbaceous groundcover has not recovered to levels comparable to that of the high-quality ponds, even in the mechanical/herbicide + burn treatment, we will have to consider other restoration actions such as seeding native grasses.

Drift Fencing

The number of adult reticulated flatwoods salamanders using the two sites that we have drift-fenced is comparable to that of the single site drift-fenced by Palis (1997) approximately 18-20 years ago. The site (pond 14) fenced by Palis (1997) was 0.8 ha whereas the two sites that we sampled were each smaller (pond 4 = 0.45 ha and pond 5 = 0.36 ha), but collectively equal the same area. Interestingly, no movement between ponds 4 and 5 has been detected thus far. These sites are < 375 m apart and therefore some movement in the form of dispersing metamorphs is highly likely. Additionally, four other sites (three that have been discovered since 2010) are within the vicinity.

Although 257 salamanders were captured in 1993-1995 at pond 14 (Palis 1997) we have not documented larval occupancy at that site despite sampling it for larvae since 2003. In a study of *A. cingulatum,* Palis et al. (2006) noted that droughts that last >4 years may cause a sharp decline in the adult breeding population, possibly explaining the local extirpation at pond 14 and raising a concern for these current sites. Models of marbled salamander (*Ambystoma opacum*) populations demonstrate that short adult life spans in combination with a series of catastrophic reproductive failures can lead to high probability of extinction (Taylor et al. 2006). With additional years of study we could calculate a survival estimate for adult *A. bishopi*.

Estimates of adult survival are required in order to estimate extinction probabilities or population viability of reticulated flatwoods salamanders (Taylor et al. 2006). However, calculating survival estimates from animals that are detectable only when they enter breeding ponds requires a long sampling period, because of the probability in a given year of a certain percentage of the adult population not breeding and not entering the ponds (Kinkead and Otis 2007). Estimates of the probability of females breeding in consecutive years are also influenced by this issue. If non-breeding females do not migrate to breeding ponds where they can be captured, then by default most of the females that are captured in consecutive years will be breeding in consecutive years. We hope to be able to collect several additional seasons of data, so we can employ capture-mark-recapture techniques to estimate recapture probabilities. A longer duration and larger sample size may be required in order to be able to estimate population size and minimum survival rates.

CONCLUSIONS

The results of our study suggest that mechanical treatments of ephemeral wetlands can successfully reduce canopy cover to levels that are similar to those of high-quality. However, herbaceous groundcover, a critical component of larval flatwoods salamander habitat, may not always respond in the short-term. The delay of herbaceous plants to respond positively to the treatments may be a result of several factors and we are continuing to investigate and monitor the results of these treatments. In some instances these treatments may need to be applied in conjunction with prescribed fire and the results of this approach will be forthcoming.

We documented a larger than expected breeding population of reticulated flatwoods salamanders at two nearby breeding wetlands. These two adjacent wetlands are used by apparently separate groups of breeding adults. However, because we had relatively low recapture rates, we are unlikely to have detected movements between ponds if they occur at low frequency. Given the complete reproductive failure over the past three years, the likelihood that females do not enter the breeding population until at least age 2 (Palis 1997), and the apparent short life-span of flatwoods salamanders (Palis et al. 2006), we expect that the population could decline more precipitously in subsequent years, especially if dry conditions persist.

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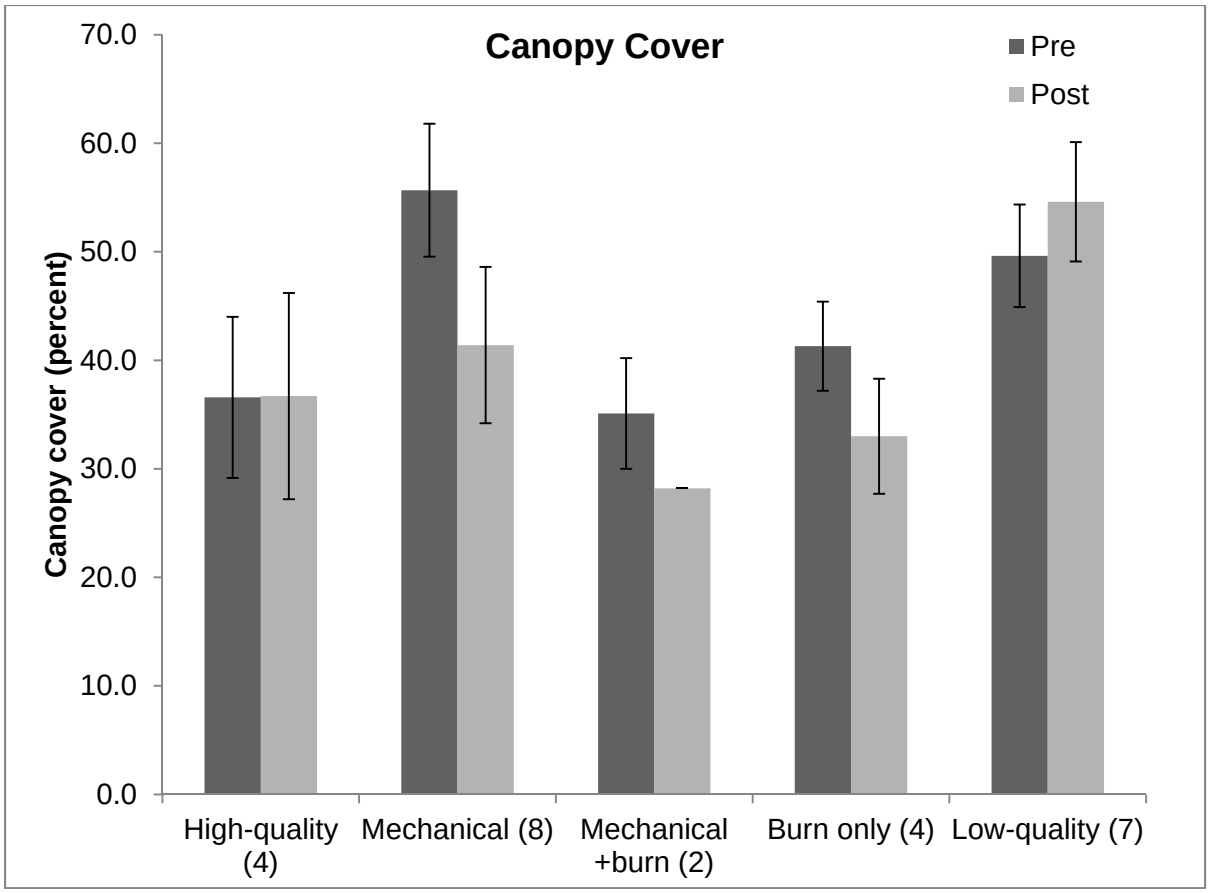

Figure 1. Comparison of percent canopy cover across five treatments at 25 wetlands on Eglin Air Force Base, Florida (sample size in parentheses, error bars represent standard error) from 2009-2010 (Pre) and 2011-2012 (Post). High-quality sites represent sites recently occupied by reticulated flatwoods salamanders (*Ambystoma bishopi*) and have habitat characteristics reported for the species. Mechanical sites had the midstory removed and were treated with herbicide, Mechanical + burn sites had the midstory removed and were treated with herbicide and burned (these sites were not included in statistical analyses), and Burn only sites received only fire as a treatment. Low-quality sites were not treated, had an overgrown midstory, and were not recently occupied by flatwoods salamanders.

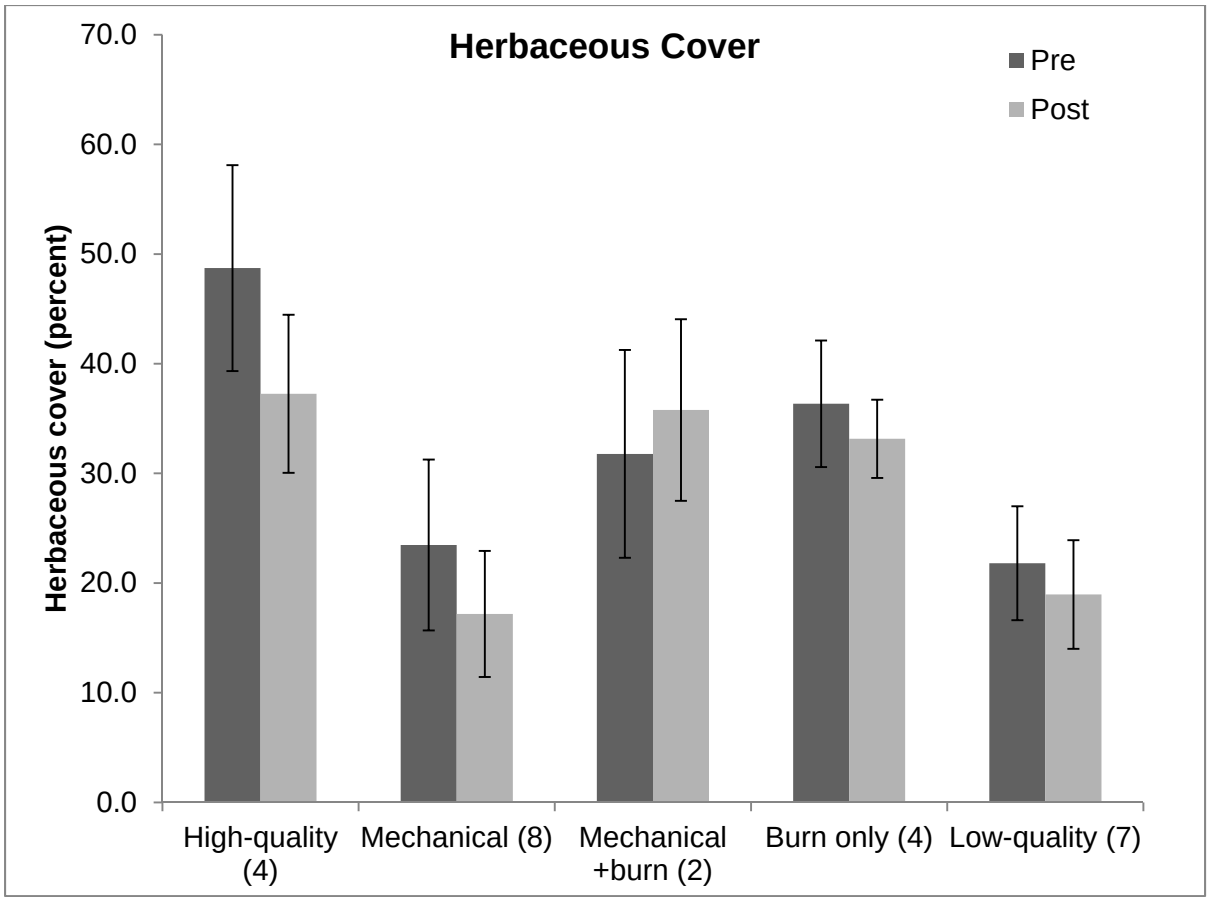

Figure 2. Comparison of percent herbaceous groundcover across five treatments at 25 wetlands on Eglin Air Force Base, Florida (sample size in parentheses, error bars represent standard error) from 2009-2010 (Pre) and 2011-2012 (Post). High-quality sites represent sites recently occupied by reticulated flatwoods salamanders (*Ambystoma bishopi*) and have habitat characteristics reported for the species. Mechanical sites had the midstory removed and were treated with herbicide, Mechanical + burn sites had the midstory removed and were treated with herbicide and burned (these sites were not included in statistical analyses), and Burn only sites received only fire as a treatment. Low-quality sites were not treated, had an overgrown midstory, and were not recently occupied by flatwoods salamanders.

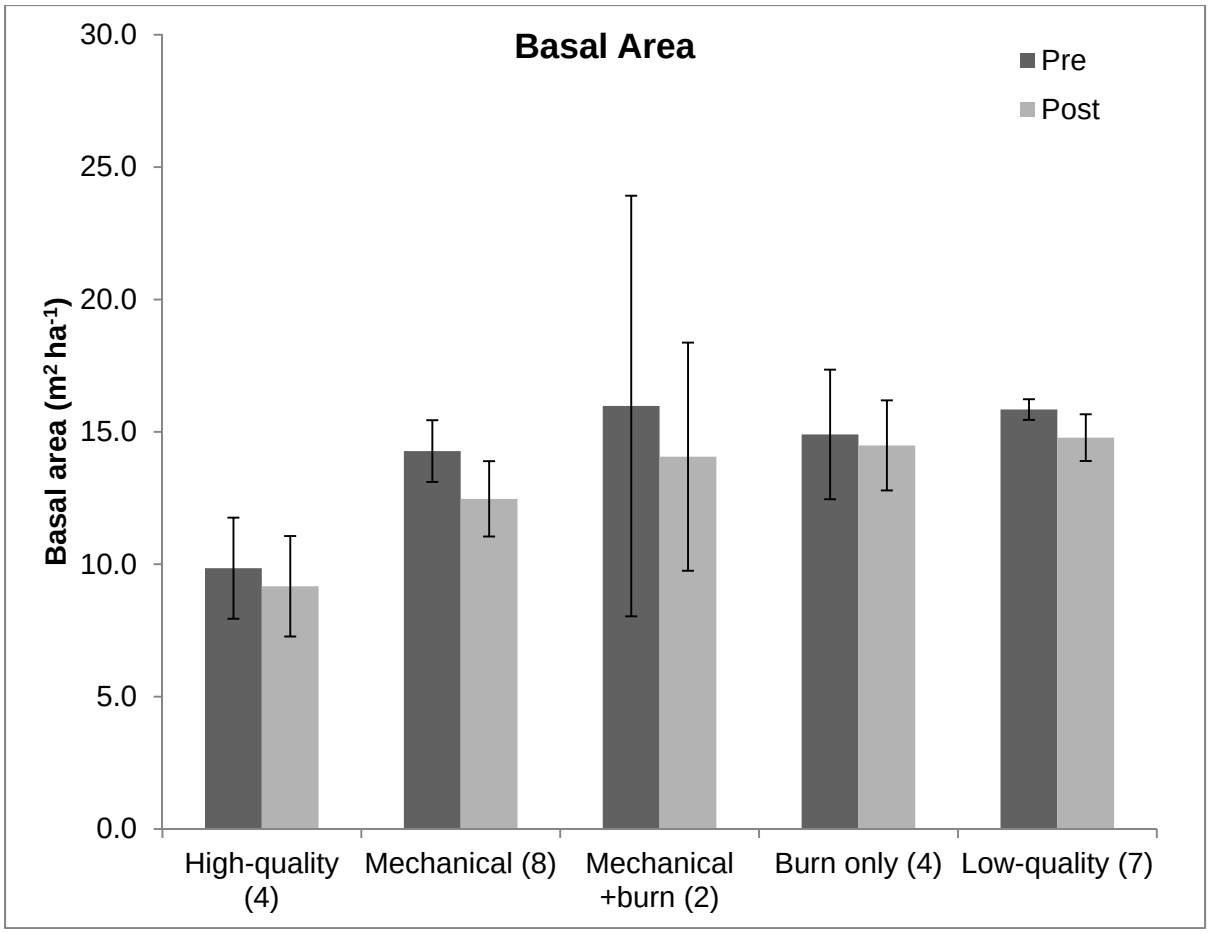

Figure 3. Comparison of basal area (m^2/ha) across five treatments at 25 sites (sample size in parentheses, error bars represent standard error) from 2009-2010 (Pre) and 2011-2012 (Post). High-quality sites represent sites recently occupied by reticulated flatwoods salamanders (*Ambystoma bishopi*) and have habitat characteristics reported for the species. Mechanical sites had the midstory removed and were treated with herbicide, Mechanical + burn sites had the midstory removed and were treated with herbicide and burned (these sites were not included in statistical analyses), and Burn only sites received only fire as a treatment. Low-quality sites were not treated, had an overgrown midstory, and were not recently occupied by flatwoods salamanders.

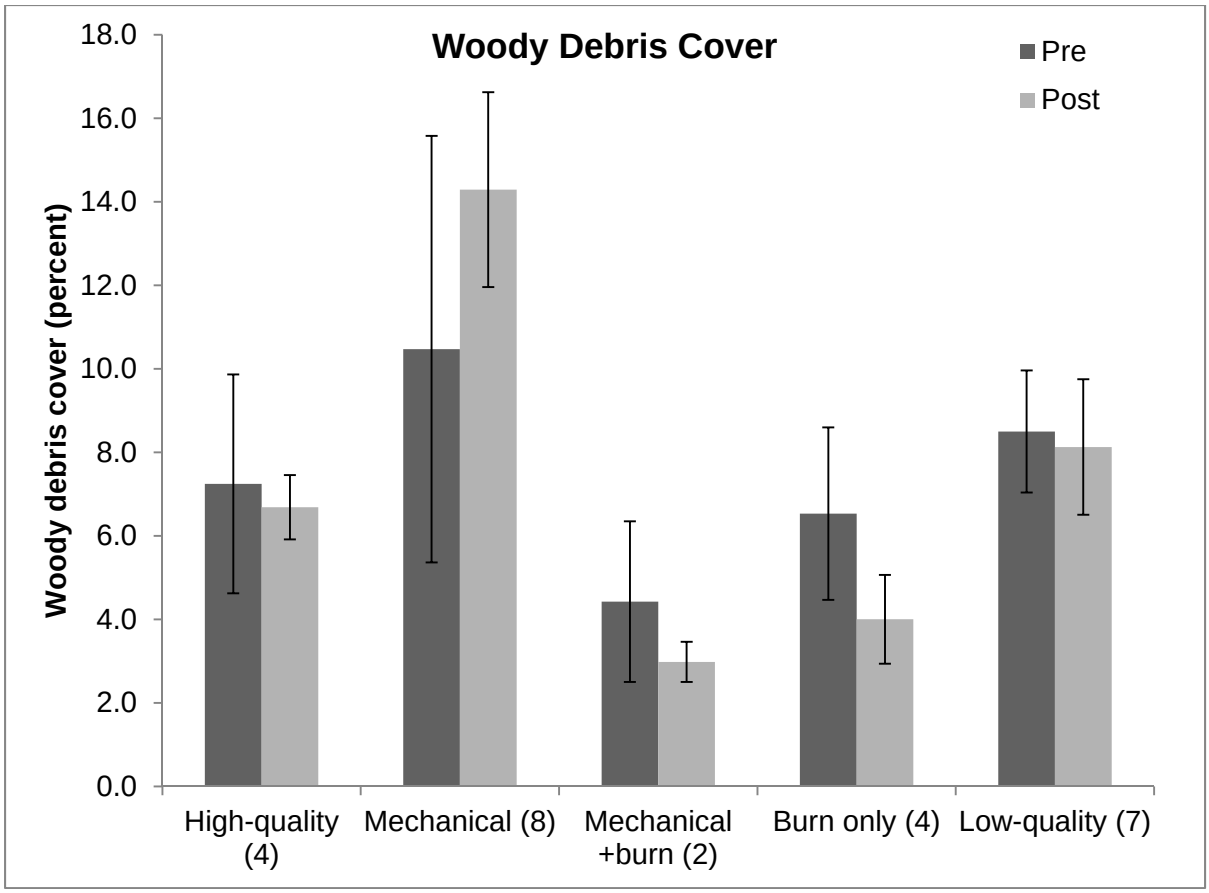

Figure 4. Comparison of percent woody debris across five treatments at 25 sites (sample size in parentheses, error bars represent standard error) from 2009-2010 (Pre) and 2011-2012 (Post). High-quality sites represent sites recently occupied by reticulated flatwoods salamanders (*Ambystoma bishopi*) and have habitat characteristics reported for the species. Mechanical sites had the midstory removed and were treated with herbicide, Mechanical + burn sites had the midstory removed and were treated with herbicide and burned (these sites were not included in statistical analyses), and Burn only sites received only fire as a treatment. Low-quality sites were not treated, had an overgrown midstory, and were not recently occupied by flatwoods salamanders.

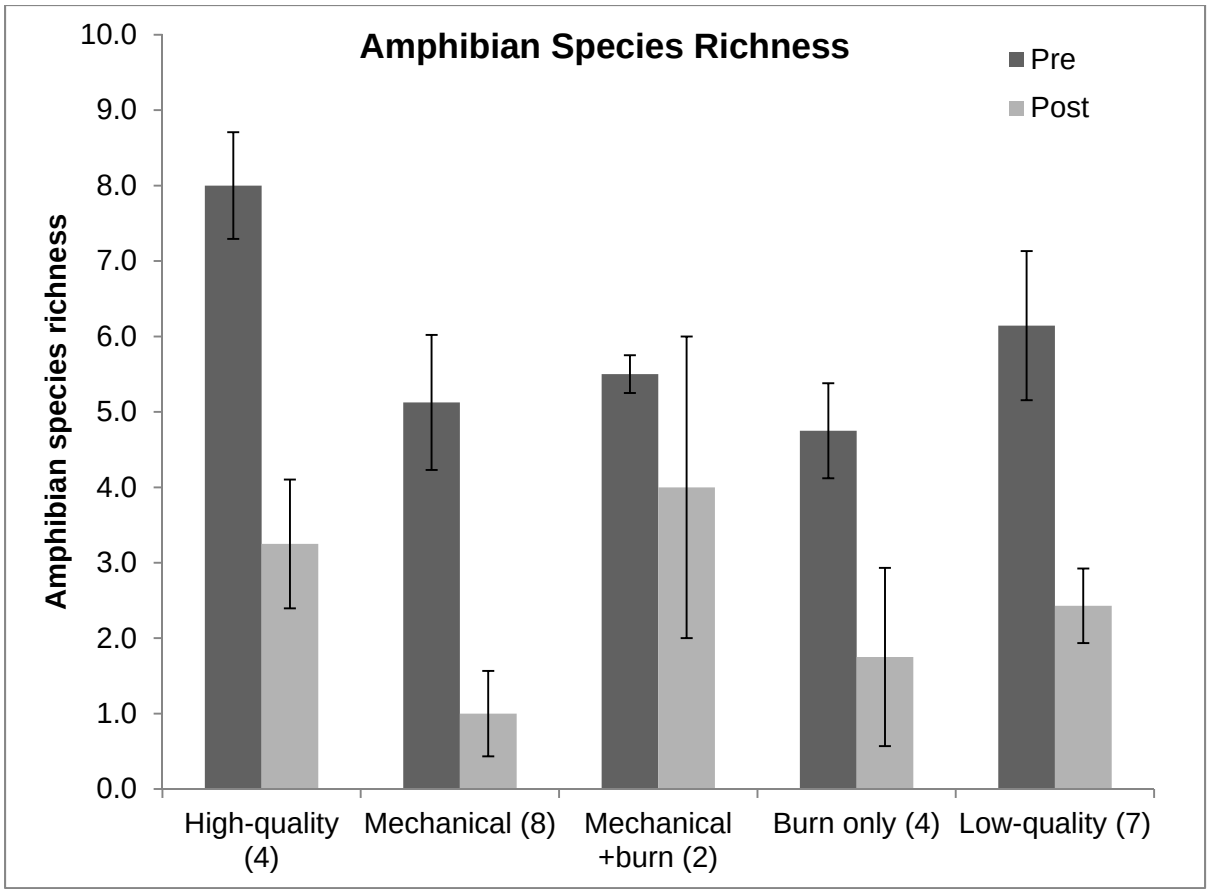

Figure 5. Comparison of amphibian species richness across five treatments at 25 sites (sample size in parentheses, error bars represent standard error) from 2009-2010 (Pre) and 2011-2012 (Post). High-quality sites represent sites recently occupied by reticulated flatwoods salamanders (*Ambystoma bishopi*) and have habitat characteristics reported for the species. Mechanical sites had the midstory removed and were treated with herbicide, Mechanical + burn sites had the midstory removed and were treated with herbicide and burned (these sites were not included in statistical analyses), and Burn only sites received only fire as a treatment. Low-quality sites were not treated, had an overgrown midstory, and were not recently occupied by flatwoods salamanders.

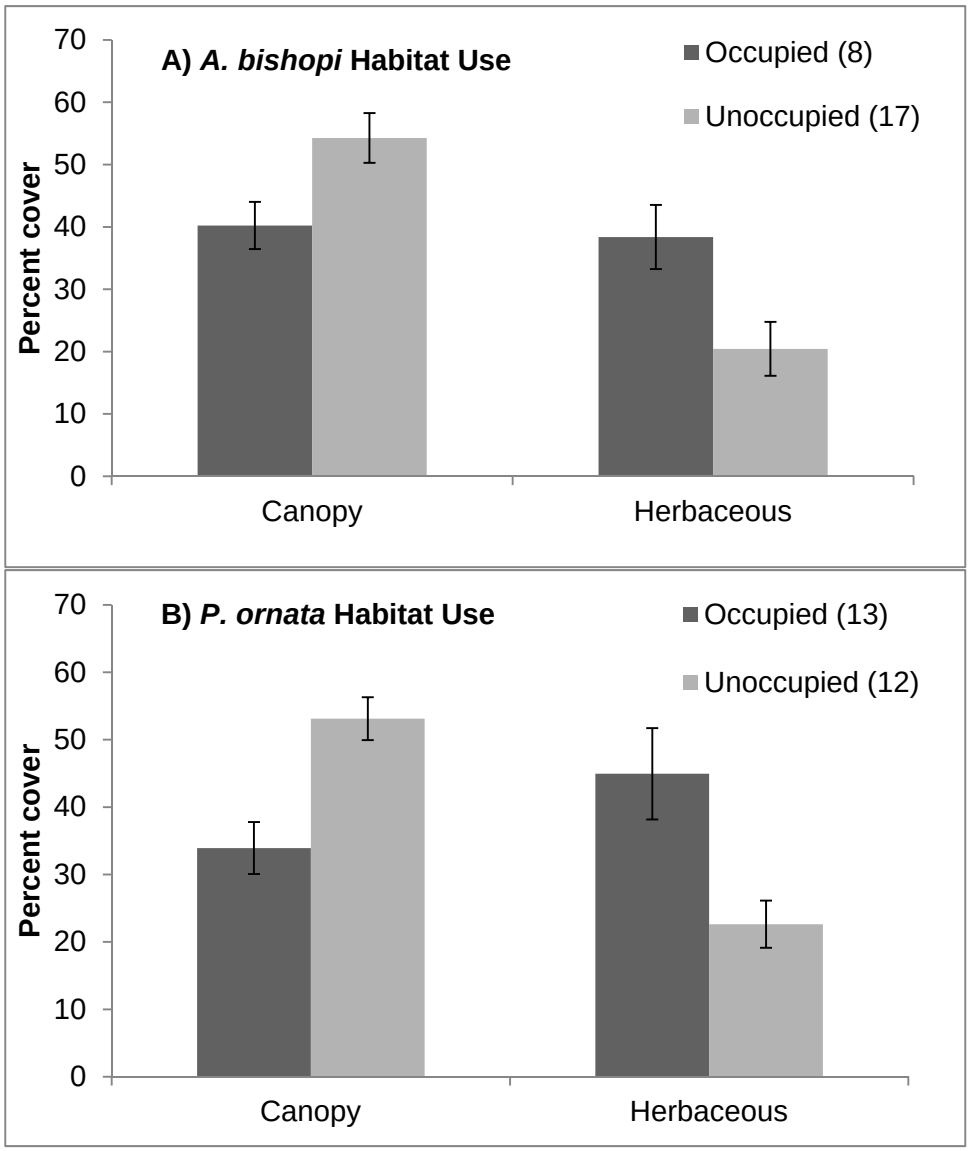

Figure 6. Percent canopy cover and herbaceous groundcover in wetlands occupied and unoccupied by A) reticulated flatwoods salamanders (*Ambystoma bishopi*) and B) ornate chorus frog (*Pseudacris ornata*), 2009-2010 (i.e., pre-treatment only) on Eglin Air Force Base, Florida (sample size in parentheses, error bars represent standard error).