TERRESTRIAL HABITAT SELECTION BY THE DUSKY GOPHER FROG (*Rana sevosa)*

A thesis presented to the faculty of the Graduate School at Western Carolina University in partial fulfillment of the requirements for a degree of Master of Science in Biology.

By

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In dedication to my courageous grandmother Carmella Mildred Shollenberger.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

ABSTRACT

TERRESTRIAL HABITAT SELECTION BY THE DUSKY GOPHER FROG (*Rana sevosa)*

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The endangered dusky gopher frog (*Rana* sevosa) inhabits underground refuges created by fire and burrowing animals in longleaf pine forests. Prescribed fire can result in a mosaic of habitat patches having different characteristics. Fire suppression may lead to fewer underground refuges due to decreased disturbance and a reduction in vegetation required by burrow-making animals. I examined terrestrial refuge and prey availability as well as habitat choice of dusky gopher frogs (*Rana sevosa*) in a longleaf pine (*Pinus palustris*) forest (Harrison County, MS) managed with winter and early spring fires. In the first study I hypothesized that gopher frogs selected terrestrial home sites characteristic of well burned habitat. I tracked 13 adult and 4 juvenile gopher frogs from a breeding/metamorphosing site to terrestrial burrows using radio telemetry. I then characterized habitat at home sites and at randomly-chosen sites.

In the second study I hypothesized that newly- metamorphosed gopher frogs would not be able to find appropriate underground refuges as quickly in fire-suppressed habitat as in well-burned habitat. I tested this by releasing captive-reared newly-metamorphosed gopher frogs into 15 x 15m terrestrial field enclosures in fire-suppressed or well-burned longleaf pine habitat. I used fluorescent powder to track the distance traveled by individual frogs before passing within 1 cm of an appropriate underground refuge, defined as any ground depression at least 2 cm deep and 1 cm wide.

In the third study I hypothesized that fewer prey are available to gopher frogs in fire-suppressed habitats. I tested this by allowing newly- metamorphosed gopher frogs to forage in small cages in fire-suppressed and well-burned habitats, and then collecting their feces. I then sampled arthropods using fly paper at the same locations. Vegetation characteristics and soil moisture levels were measured at each release site.

In the first study, average distance traveled, measured from the center of the pond, was 158.22m (std. dev. = 52.02, min = 94.3m, max = 239.6m). Nonmetric multidimensional scaling of habitat variables revealed that the gopher frog home sites grouped together when plotted. Compared to random sites, gopher frog home sites had a higher percent cover of grass and bare ground and a lower percent cover of shrubs and leaf litter, more underground refuges (mostly stump holes and small mammal burrows), and a more open canopy .

In the second study, mean distance to a refuge was significantly lower in well-burned habitat (well-burned mean = 4.34 m ± 0.18 SE, fire-suppressed mean = 7.6 m ± 1.08 SE). The proportion of frogs tracked at least 6.05 m that found a refuge was significantly higher in well-burned habitat (well-burned = 0.88 ± 0.065 SE, fire-suppressed = 0.32 ± 0.091 SE). Foraging success as measured by mean feces weight did not vary significantly between habitat types in the third study (Exp. 1: $p = 0.53$, Exp. 2: $p = 0.83$). The mean number of arthropods captured was significantly higher in well-burned habitat than fire-suppressed habitat for experiment 1 ($p < 0.0001$), but not for experiment 2 ($p = 0.39$). Total vegetation cover, herbaceous cover and soil moisture were significantly higher in well-burned habitat. Shrub cover was significantly higher in fire-suppressed habitat.

I conclude that animals that have a small home range may select patches with particular characteristics within a fire-maintained area because the animals require a specific microhabitat The habitat characteristics found at gopher frog home sites are associated with the effects of fire. Reduced availability of refuges in fire-suppressed habitat may decrease the survival of newly-metamorphosed gopher frogs emigrating from ponds. I found little evidence of differences in prey availability between well-burned and fire-suppressed sites that would influence habitat selection, but sampling was limited. The selection of characteristics by and benefits to gopher frogs influenced by fire suggests that more frequent or intense fires, or applying fire during the growing season, may increase the availability of preferred microhabitats.

CHARACTERISTICS OF TERRESTRIAL HOMESITES SELECTED BY DUSKY GOPHER FROGS

Introduction

The study of habitat selection by mobile organisms allows us insight into the requirements individuals have in a particular landscape (Brown 1988). The physical and biotic conditions of a selected habitat patch can be compared to adjacent areas in order to define differences (Rosenweig 1981). Landscapes differ from each other in the amount of habitat diversity they contain and as a consequence fluctuations can influence the survival of particular populations of species (Danielson 1992). Therefore, it is important to observe habitat selection in order to better inform conservation decisions (Johnson 1980), especially where rare species occur.

In longleaf pine forests along the coastal plain of the south Atlantic and north Gulf regions of North America, the vast amount of productivity occurs at ground level due to largely open mid- and over-story canopies. A diverse ground layer composed of grass and herbaceous plants provides food for herbivores, some of which in turn excavate burrows. Burrows provide these animals and other using their burrows an escape from constant exposure that's allows them thermal or moisture regulation, predator refuge and protection from fire.

Gopher frogs (*Rana sevosa* and *R. capito*), are rare and secretive anuran found within these coastal longleaf pine forests (Richter et. al 2001). Adults have spatially separated breeding and foraging habitats (Godley 1992). They inhabit underground refugia, created by other animals or fire, and feed on insects and arthropods they ambush in or near their refuge (Franz 1986, Franz et. al 1988, Means 2005). When breeding, gopher frogs migrate, up to several hundred meters, to ephemeral ponds which fill seasonally during large rain events. Breeding events typically last < 1 month, after which frogs quickly return to their terrestrial home site, displaying high site fidelity (Bailey 1990, Greenberg 2001, Blihovde 2006).

Natural low intensity fires frequently occur within longleaf pine (*Pinus palustris*) - grass (*Andropogon* sp., *Aristada* sp.) forests of the southeastern coastal plain, USA (Jose et. al 2006). Typically ignited by lightning strikes in the growing season (Komarek 1974), fire is most effective when fuel loads, composed of leaf litter and woody debris, are at their driest and highest points. Fire creates gaps in the mid and over story canopies (Glitzenstein et al. 1995, Harrington and Edwards 1999) thus maintaining shrubs and trees at a level where herbaceous vegetation and grass are able to compete. Because of the random nature of ignition, sections of savanna burn at different times creating a mosaic of microhabitat patches of disturbed ground and highly diverse communities of plant species (Noss 1988, Abrahamson and Abrahamson 1996, Gilliam et al. 2006). By killing trees and shrubs and burning out cavities in stumps and roots, fire can also create subterranean refuges for many small animal species (Meshaka Jr. and Babbitt 2005, Means 2005, 2006). Due to extensive fire suppression throughout the last century, over 95% of longleaf pine forests within the southeastern US have disappeared or become degraded (Jose et. al 2006). As a consequence endemic species have declined or become extirpated.

Adult gopher frogs spend the majority of their lives (~3-4 yrs., although some individuals have been recorded to live up to 12 years [Pechmann and Tupy, *unpublished data*)]) in an underground refuge within terrestrial habitat surrounding ephemeral ponds. When not underground they can be found in an area cleared of debris directly outside of the refuge (Goin and Netting 1940, Blihovde 2006). Gopher frogs have been documented in upland habitat as near as 49 m (Richter et al. 2001) to as much as 3.5 km (Humphries and Sisson 2012) away from the nearest breeding pond. Refugia have been reported as mainly gopher tortoise burrows (Franz 1986, Blihovde 2006, Roznik and Johnson 2009a) in Florida where gopher frogs (*R. capito*) are found. Where tortoises are absent the dusky gopher frog (*R. sevosa*) in Mississippi has been documented using decaying or burned-out cavities of stump holes, root mounds and associated holes (Richter et al. 2001). Gopher frogs found in North Carolina (*Rana capito*), out of the range of gopher tortoises, and have also been reported to occupy stump holes (Humphries and Sisson 2012).

Fire suppression has greatly reduced both sources of refugia (Noss 1988, Means 2006, Roznik and Johnson 2009a, Tupy *Thesis Ch. 2*) Prescribed burns have recently been implemented as a solution to loss of natural fire, but the time of year and reoccurrence interval differs from natural cycles and may only be producing minimal effects on shrub and tree densities (Smith et. al 2000). Because of habitat loss and degradation; gopher frogs in Mississippi were declared endangered by US Fish and Wildlife Service in 2001, but defined as an isolated population (Glen's Pond, Harrison County, MS), of *Rana capito* at the time. An electrophoretic study that compared the allozymes in DNA of gopher frogs in Mississippi to populations found elsewhere, determined that the population in Mississippi had one single, fixed genetic locus difference (Young and Crother 2001). The dusky gopher frog or *Rana sevosa* (Goin and Netting 1940) is now generally accepted as a separate species from other gopher frogs (United States Fish and Wildlife Service [USFWS] 2012). The historical range of *R. sevosa* is thought to have been west of Mobile Bay (AL), throughout southern MS and westward as far as the southeastern parishes of Louisiana (USFWS 2001).

The purpose of this study was to test whether gopher frogs select habitat that is more characteristic of fire-maintained longleaf pine forest than that of fire-suppressed forest, I studied one of the last known dusky gopher frog populations, at Glen's Pond. I radio tracked 17 individuals from the breeding pond to upland terrestrial home sites. I compared habitat characteristics around the home site to those of nearby randomly selected sites. If frogs were found to select habitat more characteristic of well burned longleaf pine forests then key variables could be used to help identify desired fire outcomes or aid in searches for new areas for gopher frogs.

Methods

Rana sevosa were captured emigrating after breeding (adults) or metamorphosis (juveniles) in 2010 using a drift fence encircling Glen's Pond. A radio transmitter (juveniles: A1015 [0.63 g, 45 d life] or adults: A1040 [2.2 g, 101 d life], antenna 15° angle, Advanced Telemetry Systems, Isanti, MN) was strung through elastic string along with glass beads to make a belt and was fitted around the waist of a frog so that it trailed behind (Muths 2003, Roznik and Johnson 2009b) . Transmitters were no more than 10% of the body weight of an animal (Richards et al. 1994). Individuals were released on the opposite side of the drift fence within 15 minutes of capture and fitting. Frogs were tracked every 24 hours after release until the transmitter was lost or removed at the end of its battery life. Frogs that stayed at a single underground refuge > 20 days were considered to be at a home site (h).

I compared the characteristics of home sites to those of twenty random sites (r) between the drift fence and the maximum distance a gopher frog was tracked to. I centered random vegetation sampling plots on r points and home site vegetation plots on the frog's burrow. Both types of sites were visually assessed for percent cover of grass, herbs, vines, shrubs, woody debris, pine seedlings, leaf litter, bare ground and standing dead vegetation within a 1 x 1 m quadrat. The number of trees and the circumference (at breast height) of each were recorded within a 15 x 15 m quadrat. The length of intersecting coarse woody debris (≥ 5cm diameter) was recorded along a 15 m straight line centered on each sampling point and extending in a randomly chosen direction. I also counted available underground refuges, defined as a hole in the ground, ≥ 1 cm wide and ≥ 2 cm deep, within 1m of this line, as this was the minimum size hole I observed a frog fit in. I took a digital photo of the canopy at each sampling point using a fish-eye lens held at 1m height and measured percent canopy openness using Gap Light Analyzer (GLA v.2, Institute of Ecosystem Studies, Millbrook, NY)

Percent cover data from the 9 variables in the 1 x 1m quadrats plus percent openness from canopy photos was explored for relatedness between home sites and random sites through the visual orientation technique of nonmetric multi-dimensional scaling (NMDS). I also examined differences between home and random sites for single variables using T-tests.

Results

Thirteen adults (7 female, 6 male) and four newly metamorphosed (mets) *R. sevosa* were captured and released with transmitters 23 January – 29 June 2010. Adult frogs were tracked for an average of 98 days (range [R] 62 – 132 days, standard deviation [SD] 62 days. Juvenile frogs were tracked for an average of 46.25 d (R 29-61 d, SD = 17.2 d). Average total distance to final location, measured from the center of the pond was: all individuals 158.2 m (R 84.3-239.6 m, SD = 46.6 m), female adults = 153.3m (R 84.3-239.6m, SD = 55.0m), male adults = 156.0 m (R 87.6-216.7 m, SD = 53.5 m), juveniles = 170.2 m (R 147.2-194.4 m, SD = 53.5 m; Fig. 1). Average distance from center of the pond to the high water mark was 61.1 m. Average distance from home site to nearest neighbor was 47.1 m (R 23.7 – 119.6 m, SD = 29.9 m). All gopher frogs were tracked for more than 20 days. I used each final location as a home site sampling point (h; Fig. 1). All final home site locations were classified as stump holes because they showed at least some signs of woody structure. There was also evidence of excavation by other animals (non gopher frog) at some of the home sites. It took adults an average of 1.2 days (R 1 – 3, SD = 0.6 d) and juveniles average of 5.8 days (R 4 – 9, SD = 2.2 d) to reach final refuge.

Graphing NMDS ordination results of the nine 1 x 1m quadrat variables and percent openness from canopy photos showed clumping of home site and not of random site values (Fig. 2) and providing evidence that home site characteristics were more similar to one another than to random sites.   T-tests showed that home sites had significantly ($p \leq 0.03$) greater percent cover of grass and bare ground but less percent cover of shrubs and leaf litter (Fig 3).   Home sites also had significantly ($p \leq 0.03$) more holes, fewer trees and more canopy openness (Fig. 3)   There was no significant difference between home and random sites in percent cover of herbs ($h\dot{X}$= 10.47%, $r\dot{X}$ = 7.10%), vines ($h\dot{X}$= 7.88%, $r\dot{X}$ = 5.5%), woody debris ($h\dot{X}$= 8.88%, $r\dot{X}$ = 5.85%), pine seedlings ($h\dot{X}$= 0.35%, $r\dot{X}$ = 1.5%) and standing dead vegetation ($h\dot{X}$= 13.29%, $r\dot{X}$ = 18.25%),   or length of intersections of coarse woody debris ($h\dot{X}$= 102.24cm, $r\dot{X}$ = 89.26cm) all $p \geq 0.09$.

Discussion

The selection of home sites with more open canopy, grass and bare ground by gopher frogs provides insight into preferred habitat.   This habitat may have been the historically dominant patch type in places like Glen's Pond before fire suppression was so wide spread.   It has been shown in numerous studies within different regions, that amphibian species are drawn to characteristics of their native ecosystem.   Wood frogs (*Rana sylvatica*) are a classic example of an amphibian that has been observed migrating toward native closed canopy forests when faced with a choice between this and open fields or clear-cut areas (DeMaynadier and Hunter 1999, Rothermel and Semlitsch 2002).   Not only did these studies demonstrate native habitat selection, but Rothermel and Semlitsch (2002) went on to find survival was higher for wood frogs which migrated to closed canopy forests.

Although habitat patchiness is a natural component in longleaf pine ecosystems due to fire (Komarek 1974, Glitzenstein et al. 1995, Gilliam et al. 2006), the gopher frogs in this study tended to select home sites with characteristics that displayed the most profound effects from fire. Random sites, on the other hand, were more characteristic of fire suppressed habitat, which tends to be a very common patch type currently around Glen's Pond. Forest heterogeneity comes about when fire creates disturbance (Platt et. al 1988). Lack of fire efficacy may be a result of prescribed burns being applied during winter months (Abrahamson and Abrahamson 1996). Where some patches may be more completely burned and therefore more suitable for gopher frogs, the majority of the landscape may still be rarely burned and less suitable for the frogs. With the help of Hurricane Katrina in 2005 the majority of trees around Glen's Pond were knocked down (Michael Sisson, *personal communication*); consequently more stump holes and root mounds were created, aiding in refuge creation. However, managers' inability to burn after the storm left a large amount of land unburned for years (4 yrs), allowing the shrubby mid-story in particular to thrive. Further, fire suppression may indirectly affect gopher frogs, as declines in populations of herbivorous, burrow excavating species, such as the gopher tortoise and small mammals, have also been noted in many fire-suppressed longleaf pine forests (Noss 1988, Means 2006).

I suggest that the most important component of well-burned terrestrial habitat for gopher frogs is the availability of underground refuges. A more open canopy, grass and bare ground as well as less leaf litter and shrubs may simply be indicators of a habitat having more holes. Roznik and Johnson (2009b) found that the greater number of holes available to juvenile gopher frogs, the higher the chances for survival. Risk of mortality was reduced to 4% for frog in holes compared to those frogs left exposed. Newly metamorphosed gopher frogs encountered an underground refuge more quickly (in a shorter distance) when released into an enclosure in well-burned habitat compared to an enclosure in fire-suppressed habitat (Tupy, *Thesis Ch. 2*).

Enclosures in well-burned habitat also contained a higher number of holes compared to enclosures in fire-suppressed habitat during visual surveys (Tupy, *Thesis Ch.* 2). During surveys of terrestrial areas around ponds, Thurgate (2006) counted a greater number of burrows/holes at sites where gopher frogs were present, suggesting that a site that has burned more recently, more often, and/or at a more optimal time of year will contain more appropriate underground refugia for gopher frogs.

More available underground refugia may be the most important factor for gopher frog survival but other characteristics at home sites may also be beneficial. A more open canopy might allow for better thermoregulation, in conjunction with a cool burrow. Gopher frogs are often observed sitting outside of their refuge at all times of day (Tupy, *personal observation*). It is thought that this is in part ambush predation behavior, but the frog may also be basking. Metamorphosed gopher frogs prey on arthropods, but arthropod density comparisons between well burned and fire suppressed habitats were inconclusive (Tupy, *Thesis Ch. 2*). Furthermore, after weighing feces collected from juvenile gopher frogs recaptured in either habitat type, we found no significant difference in their ability to forage (Tupy, *Thesis Ch. 2*). Habitat with a more open canopy may allow greater mobility for actively foraging animals (Denton and Beebee 1994) but may not matter to gopher frogs as much because of their sedentary nature. Once at a terrestrial home site they almost never stray from the immediate vicinity (Tupy, *personnel observation*).

The gopher frogs tracked in this study traveled relatively short distances compared to other populations of gopher frogs studied using similar methodology. For example, Roznik and Johnson (2009a) tracked juvenile gopher frogs (*R.* capito) in Florida up to 691 m. This contrast may be due to the abundance of underground refugia available around Glen's Pond. Gopher frogs tracked during this study may have had little difficulty finding a suitable home site despite a predominantly fire suppressed landscape. This is supported by one other study where Richter et al. (2001) tracked 12 adult *R sevosa* at Glen's Pond using radio transmitters and found similar results: mean travel distance 173 m, maximum distance 299 m). Another radio telemetry study observed *R. capito* in NC were observed traveling several kilometers away from breeding sites (mean 1.3 km, max 3.5 km), with a lack of suitable stump holes or burrows noted as a limiting factor (Humphries and Sisson 2012).

I observed no mortality of juveniles when tracked using radio telemetry (N=4). Juveniles moved an average of 4.3 times and transmitter life averaged 32.3 days. In contrast, Roznik and Johnson (2009a) observed greater mortality (87.5%) while tracking juvenile gopher frogs in Florida. Sample size was larger compared to our study, but only 4 frogs (12.5%) survived past the first month. Greater susceptibility to predation from transmitter weight was ruled out by Roznik and Johnson (2009a), but their release time (during the day), may have made individuals more vulnerable. In this study, our juveniles were captured at night and released ≤ 15 min after fitting with a transmitter. All individuals found an underground refuge and three out of four remained at it until transmitter expiration (30 d). The fourth frog quickly found another refuge not far from their previous occupancy. Two juveniles were fitted with new transmitters after expiration of their original transmitter and tracked for an additional 30 days. Both remained in the same underground refuge.

The habitat characteristics found at gopher frog home sites are associated with the effects of fire. Reduced availability of refuges in fire-suppressed habitat may decrease the survival of newly-metamorphosed gopher frogs emigrating from ponds. Benefits to and the selection of characteristics by gopher frogs influenced by fire suggest that more frequent or intense fires, or applying fire during the growing season, may increase the availability of preferred microhabitats.

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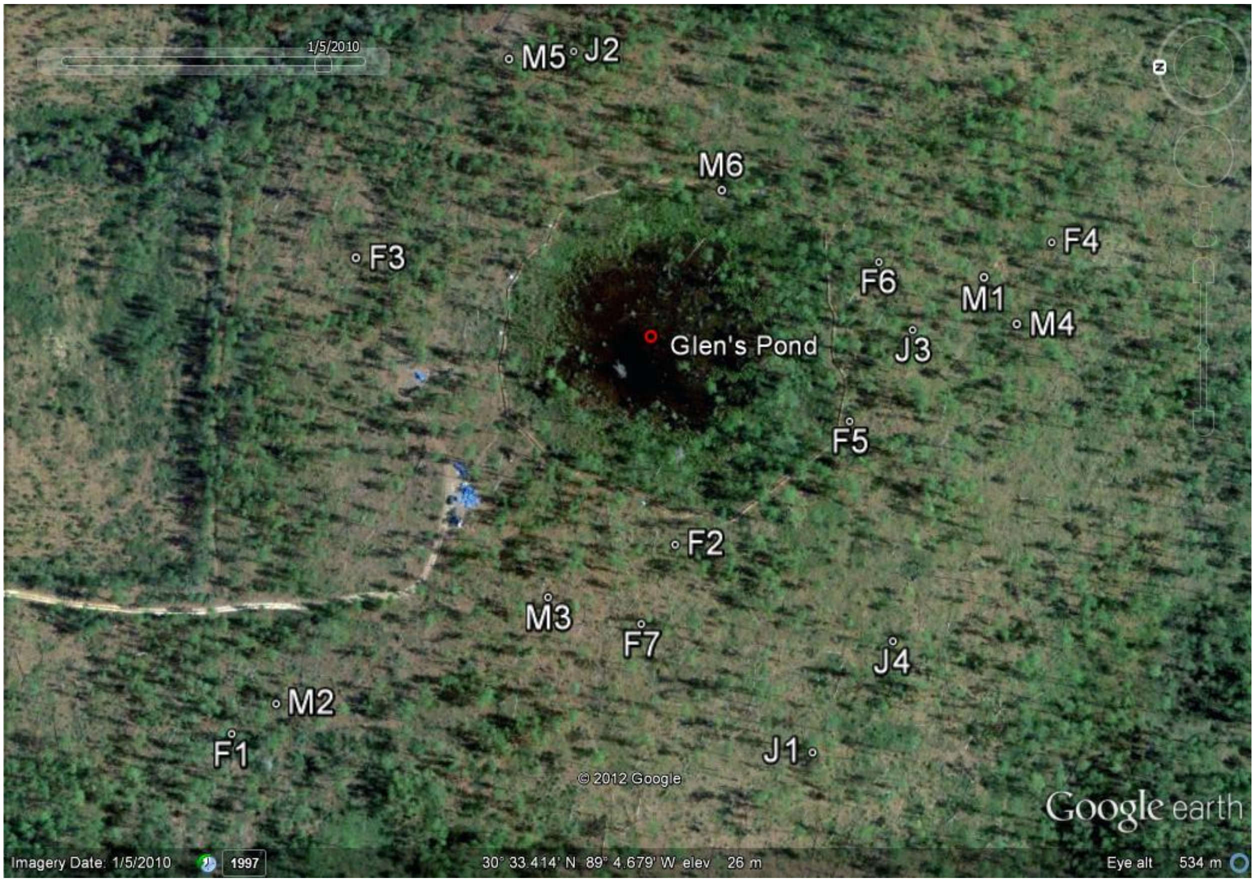

FIGURE 1: GoogleEarth™ aerial photo (2010) of the Glen's Pond Area with plotted final locations of gopher frogs tracked using radio telemetry. The red circle marks the middle of Glen's Pond, used as the point from which to measure each frog's distance from the pond. M = adult male, F = adult female, J = juvenile, unknown sex.

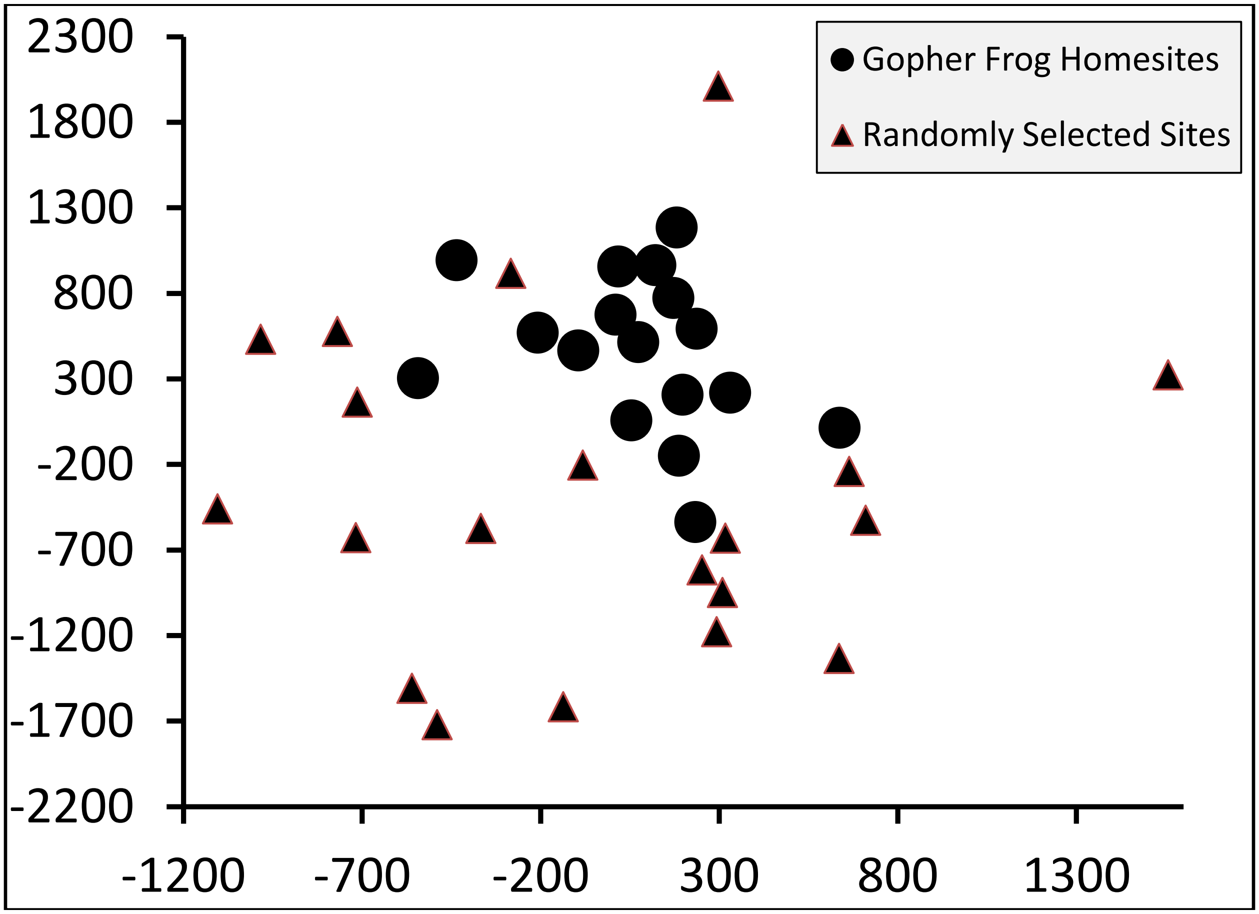

FIGURE 2: Nonmetric multidimensional scaling ordination (X 1,000 for scale) of values from the nine 1 x1 m vegetation quadrat variables and canopy openness (stress = 14.141).

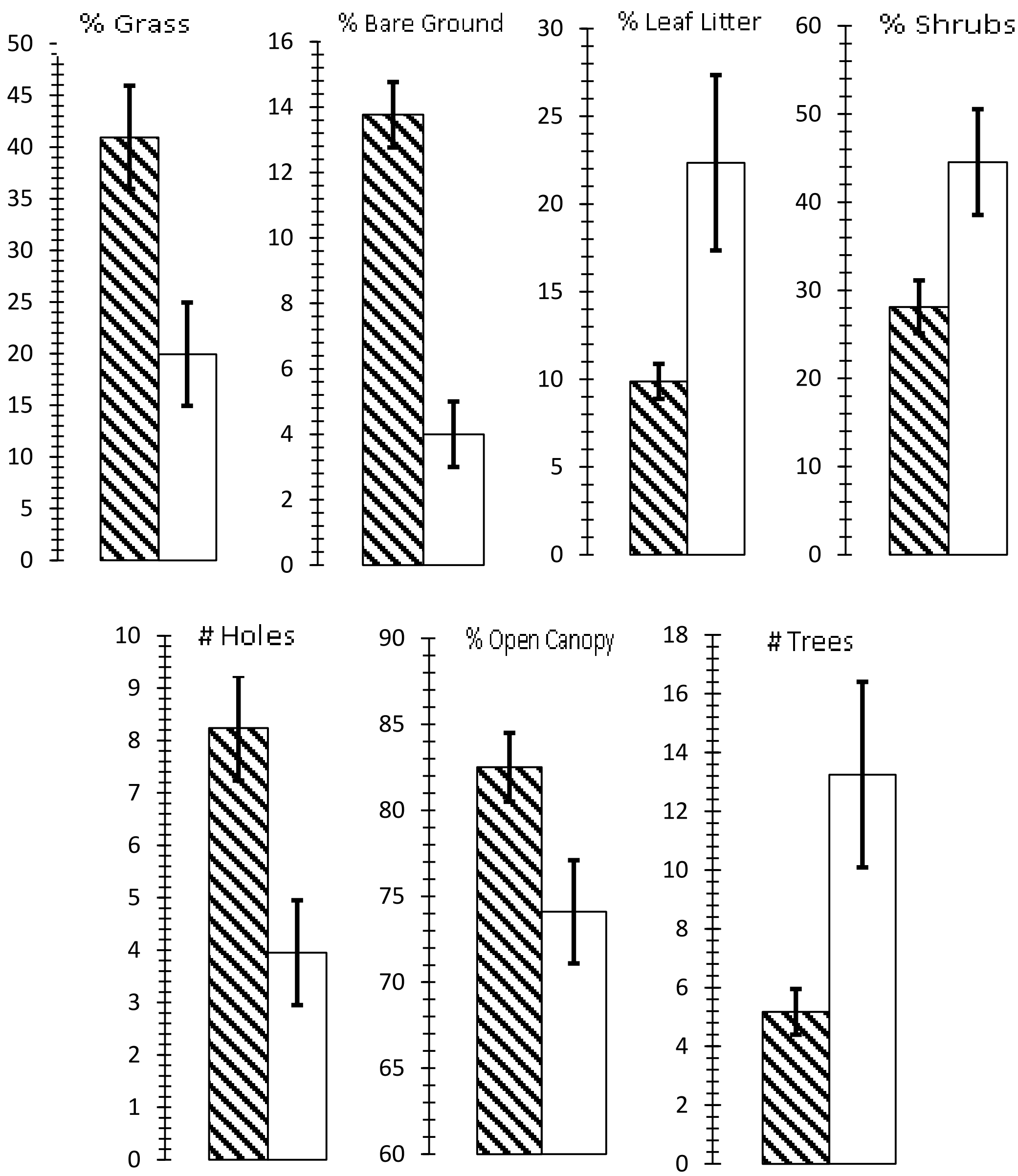

FIGURE 3: Variables that differed significantly ($p \leq 0.03$) between gopher frog home sites and random sites (mean ± 1 SE). Bars with downward horizontal lines = gopher frog home sites, open bars = random sites.

TERRESTRIAL REFUGE AND PREY AVAILABILITY FOR THE DUSKY GOPHER FROG IN FIRE-SUPPRESSED AND FIRE-MANAGED HABITATS

Introduction

The availability of refugia is an important component of habitat selection by small mobile organisms. This is especially true for organisms that are unable to create refugia yet depend on them. Gopher frogs (*Rana sevosa* and *R. capito*) are incapable of excavating and therefore rely on burrows or underground refugia created by other means (Wright and Wright 1949, Franz 1986). They use refugia to forage, avoid predators, and regulate body temperature or moisture levels.

Fire is a natural component of the longleaf pine (*Pinus palustris*) ecosystem in the southeastern U.S where gopher frogs are found. By limiting growth of the mid- and over story canopies, fire allows a diverse herbaceous ground layer to flourish (Glitzenstein et. al 1995, Harrington and Edwards 1999). This in turn provides food for burrowing herbivores; whose burrows are important to numerous commensals (Lee 1968, Franz 1986, Brown 1997, Means 2006), including the gopher frog. Fire also burns through dead stumps and roots, creating underground cavities which animals use as additional refuge (Means 2005).

Adult gopher frogs spend the majority of their lives in and around an underground refuge, only leaving to migrate to nearby ephemeral rain-fed wetlands to breed (Allen 1932, Palis 1997). Newly metamorphosed gopher frogs (metamorphs) typically exit larval sites in spring or summer, often when these wetlands begin to dry (Semlitsch et. al 1995, Palis 1998, Richter 1998). Metamorphs must navigate the unfamiliar terrestrial landscape and quickly seek out underground refuges (Braid et. al 2001). Underground refuges not only provide a permanent home site, but also allow migrating gopher frogs to hide and rest during movements. The chances for survival of a metamorph greatly increase if more underground refugia are available at any given time when they are exposed in the terrestrial landscape during migrations (Roznik and Johnson 2009b)

Gopher frogs are prey generalists and have been observed feeding on arthropods (Test 1893, Goin and Netting 1940). A variety of herbivorous arthropods are found in longleaf pine forests due to its highly diverse vegetative ground layer (Provencher et. al 2003), yet fire suppression can lead to a reduction in ground herbs (Walker and Peet 1983, Noss 1988). A lack of fire allows woody vegetation to persist at the expense of herbaceous vegetation, which is likely to affect the arthropod community.

The suppression of fire has been a rampant problem for the remaining longleaf pine forests in the southeast throughout the last century (Noss 1988). I hypothesize that fire suppression can degrade terrestrial habitat for gopher frogs in two ways: by increasing the time needed to find a refuge, and by decreasing availability of arthropod prey. The objectives of this study were 1) to test how long gopher frogs take to find underground refuges in fire-suppressed habitat compared to fire- maintained habitat; 2) investigate whether prey abundance was lower in fire-suppressed habitat compared to fire-maintained habitat.

Methods

The main study site was composed of sixteen 15 x15 m terrestrial enclosures located 527 – 979 m west of Glen's Pond (DeSoto National Forest, Harrison County, MS), an isolated breeding site for *R. sevosa*. Eight enclosures were located in fire-maintained or "well-burned" habitat and the other eight enclosures were located in fire-suppressed habitat. Enclosures were located in four spatial blocks; located an average of 366, apart, with the northernmost enclosure located 1,400m from the southernmost enclosure. Each block contained two pairs of enclosures: one pair well- burned, one pair fire-suppressed, located an average of 198 m apart from each other. Well-burned habitat experienced prescribed fire at two year intervals for ten years prior to this study. Fire-suppressed habitat had not been burned 5 years prior to the study, and was burned only twice in the last ten years.

Refuge availability was tested by tracking newly metamorphosed gopher frogs in enclosures using non toxic fluorescent powder (DayGlo Corp, Cleveland, OH) May – August 2008. Frogs used were obtained as eggs, deposited by breeding adults at Glen's Pond, and raised as tadpoles in cattle-watering tanks (350 gal) to metamorphosis. Metamorphs were used ≤5 days after tail absorption. Average weight of metamorphs was 2.09 g, snout vent length (SVL): 27.9 mm. Approximately ¾ of the frogs' bodies (excluding eyes, nostrils and mouth) were covered in fluorescent powder upon release into the enclosures. Frogs were released in the center of each enclosure after dusk, within 48 hours after rain, at ≥ 2 frog per night per enclosure. Twenty-four hours following release, the trail of fluorescent powder pigment was tracked using a portable, rechargeable UV black-light (Fisher Scientific, Hampton, NH; Graeter and Rothermel 2007). A suitable refuge was defined as an underground depression at least 1 cm wide by 2 cm deep, based on the size of a hole a metamorph could fit into. Total traceable trail of fluorescent powder was measured (m) until the frog was found or no more pigment could be illuminated. Distance was measured (m) to the first available refuge a frog used or "found" by passing within 5 cm.

Visual surveys for suitable refugia were conducted in the 16 enclosures and also at nearby sites (≤ 5 km from enclosures, DeSoto National Forest, Harrison County, MS) containing well-burned (n = 8) or fire-suppressed (n = 8) habitat. Nearby sites had similar burn histories to enclosure sites, were separated from each other by at least 50 m and spatially separated into 4 blocks composed of 4 sites (2 – well burned, 2 – fire suppressed). Refuges were counted within 1 m of two 15 m parallel transect lines, spaced 5 m apart, within each site or enclosure. I drew transect lines parallel to enclosure walls and chose a random cardinal direction for nearby sites.

I tested foraging success by releasing unfed metamorphs into small cages in well-burned or fire-suppressed habitat for 8 (experiment 1) or 3 days (experiment 2), June-August 2007. Frogs used were obtained as eggs, deposited by breeding adults at Glen's Pond, and raised as tadpoles in cattle tanks (350 gal) to metamorphosis. Metamorphs were used ≤ 5 days after tail absorption. Average weight of individuals used was 2.47 g, SVL = 30.2 mm. Cages were located within 20 m of a 15 x15 m terrestrial enclosure (see above). Cages were constructed of 0.6cm wire mesh, were 48 cm diameter and 61 cm tall, and contained an artificial underground burrow (PVC pipe: diameter = 0.635, length = 5 cm). Experiment 1 was composed of 5 cages per enclosure site (sites = 4 well-burned, 4 fire-suppressed, total = 40 cages). I placed the cages where the vegetation was no taller than the cages. Cages were located within 0.5 m of a small shrub to provide shade, and the tops of the cages were covered with screen. Experiment 2 was composed of 4 or 5 cages per enclosure site (sites = 3 well-burned, 3 fire-suppressed, total = 26 cages). Cages in well burned habitat were located as far from scattered shrubs as possible. Large shrubs were enclosed with cages in fire-suppressed habitat. The tops of the cages were left uncovered in both habitats. Frogs were recaptured after experiments and held in a laboratory for 24 hours to collect defecations. Feces were dried in an oven at 70 C for 24 hrs and weighed (g).

To measure prey abundance, arthropods were sampled by using fly paper (25.5x3.7 cm) suspended vertically up from touching the ground in the middle of each cage. Sampling occurred immediately after foraging experiments for 24 hours in experiment 1 and 48 hours in experiment 2. I then counted individual arthropods per fly paper.

I characterized vegetation in cages by estimating percent cover (%) of total vegetation, herbaceous vegetation and shrubs. Soil volumetric water content (%) was measured at each site using a Field Scout TDR 300 soil moisture probe (Spectrum Technologies, Plainfield, IL). All measurements were made on the same day following each foraging experiment.

I performed ANOVA to test for differences between well burned and fire suppressed habitat in the response variables measured, separately for each experiment. Count data were square root transformed and mass data were log transformed to reduce heterogeneity of variance.

Results

One hundred thirty-eight metamorphs covered in fluorescent powder were released into enclosures. Seventy-eight were tracked to a refuge. The average distance a frog traveled before first encountering a refuge was 6.05m. The proportion of frogs tracked up to 6.05 m that found a refuge was significantly higher ($p = 0.005$) in well-burned habitat (0.88 ± 0.065 SE) than in fire-suppressed habitat (0.32 ± 0.091 SE, Fig. 4). Mean distance traveled to a refuge in enclosures was significantly shorter ($p = 0.02$) in well-burned (4.34 m $\pm$ 0.18 SE) compared to fire-suppressed (7.6 m $\pm$ 1.08 SE) habitat (Fig. 5) Surveys for suitable refugia revealed that the average number of underground refugia per enclosure or nearby site was higher in well-burned than in fire suppressed habitat (Nearby Sites: $p = 1.62$ E-5, Enclosures: $p = 0.016$, Fig. 6).

Foraging success as measured by mean feces weight did not vary significantly between habitat types (Exp. 1: $p = 0.53$, Exp. 2: $p = 0.83$, Fig. 7). The mean number of arthropods captured was significantly higher in well-burned habitat than fire-suppressed habitat for experiment 1 ($p < 0.0001$), but not experiment 2 ($p = 0.39$, Fig. 8).

Total vegetation cover, herbaceous cover and soil moisture were higher in well-burned habitat (all $P \leq 0.0$; table 1). Shrub cover was higher in fire-suppressed habitat ($P \leq 0.0$; table 1).

There were no significant block effects or block by treatment interactions in any analyses (all $F \leq 0.95$, all $P \leq 0.001$).

Discussion

The native ecosystem of dusky (and other) gopher frogs is fire-maintained longleaf pine forests. Gopher frogs choose well-burned over fire-suppressed habitat (Tupy, *Thesis Ch. 1*; Roznik and Johnson 2009a). My results suggest that one reason for this is the greater availability of underground refuges. Tracking experiments using fluorescent powder in enclosures suggest that there is a difference in refuge availability between well-burned and fire-suppressed habitats. Surveys in two separate areas as well as the enclosures revealed more underground refugia in well-burned compared to fire-suppressed habitat. I also found that metamorphs reached an underground refuge in a shorter distance in well-burned habitat. These results suggest that fire plays an important role in creating appropriate underground refugia for newly-metamorphosed gopher frogs. Similarly, Roznik and Johnson (2009a) found that burrow densities were 80% higher in fire-maintained habitats compared to fire-suppressed in terrestrial areas around gopher frog (*R. capito)* breeding sites in Florida. Increased refuge availability is likely to result in increased survival rates (Roznik and Johnson 2009b). Therefore, areas containing gopher frogs would benefit from management burns at appropriate intervals or time of year to bring about more underground refugia. Fire is most effective when fuel loads, composed of leaf litter and woody debris, are at their driest and highest points. Fire prescribed more frequently creates gaps in the mid and over story canopies (Glitzenstein et al. 1995, Harrington and Edwards 1999) thus maintaining shrubs and trees at a level where herbaceous vegetation and grass are able to compete. More herbaceous vegetation would attract burrowing herbivores.

In addition to increased refugia, I thought that the thick ground layer of herbaceous vegetation that fire fosters would support a greater density of arthropods to forage upon than dense woody shrubs (Swengel 2001). Arthropod sampling revealed significantly higher numbers of arthropods in well-burned habitat compared to fire-suppressed habitat in experiment 1, but not in experiment 2. Also, foraging success as measured by mean feces weights did not differ significantly between well-burned and fire-suppressed habitats. The cages in fire-suppressed habitat in Exp 1 enclosed mostly bare ground, whereas cages in the well-burned habitat enclosed mostly grass. Thus, it was not surprising to find more arthropods in the cages that provided more cover and food resources for arthropods. The cages in fire-suppressed habitat contained much more vegetation in experiment 1 than in experiment 2 (Table 1). Although most of this vegetation was wood, it supported numbers of arthropods similar to those supported by the grass. The findings in these experiments should be considered preliminary, however. Prey availability is an important component for gopher frog survival and should be further explored. Gopher frogs rely on a variety of prey drawn to areas that contain high amounts of herbaceous ground forage (Provencher et. al 2003). Fire suppression leads to a loss in herbaceous vegetation; potentially having a tremendous impact on prey items for gopher frogs. Gopher frogs may also benefit from higher soil moisture; this was significantly greater at foraging cage sites in well-burned habitat.

Habitat destruction and alteration are the biggest causes of declines and disappearances in amphibian populations (Lips 1998, Wake and Morowitz 1991, Alford and Richards 1999, Semlitsch 2000). Currently, Glen's Pond is the only known productive population of *R. sevosa* remaining. Understanding the mechanisms by which habitat affects survival, reproductive success, and population dynamics is key to reversing the decline of *R. sevosa* and other amphibians.

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TABLE 1: Vegetation characteristics and soil water volume inside the cages. All differences between habitats were significant at P ≤ 0.01 in both experiments.

Experiment	Habitat	Total Veg. Cover (% ± SE)	Herbaceous cover (% ± SE)	Shrub cover (%± SE)	Soil water (%± SE)
1	**Fire Suppressed**	**13.0 ± 4.0**	**7.6 ± 4.1**	**5.4 ± 0.9**	**13.2 ± 0.6**
	Well Burned	**86.8 ± 4.0**	**85.4 ± 4.1**	**1.3 ± 0.9**	**17.1 ± 0.6**
2	**Fire Suppressed**	**73.7 ± 6.5**	**5.9 ± 4.6**	**67.8 ± 5.3**	**17.6 ± 0.8**
	Well Burned	**98.2 ± 6.5**	**95.8 ± 4.6**	**2.4 ± 5.3**	**21.9 ± 0.8**

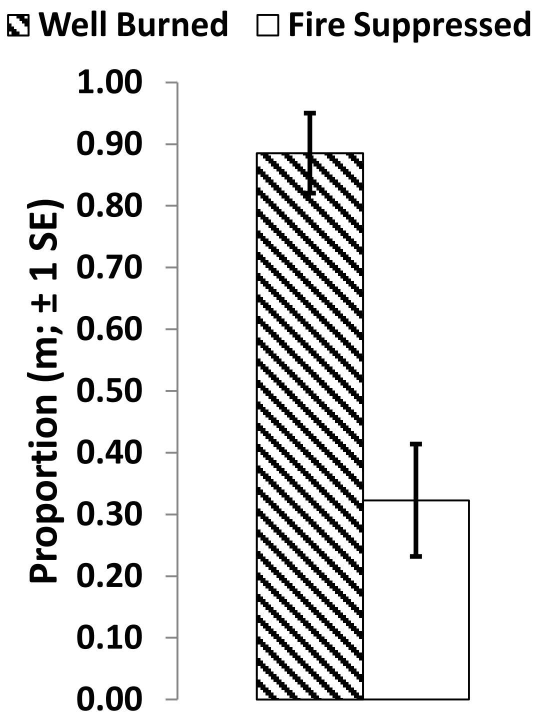

FIGURE 4: The proportion of frogs that found a refuge within 6.05 m when tracked using fluorescent powder in terrestrial enclosures.

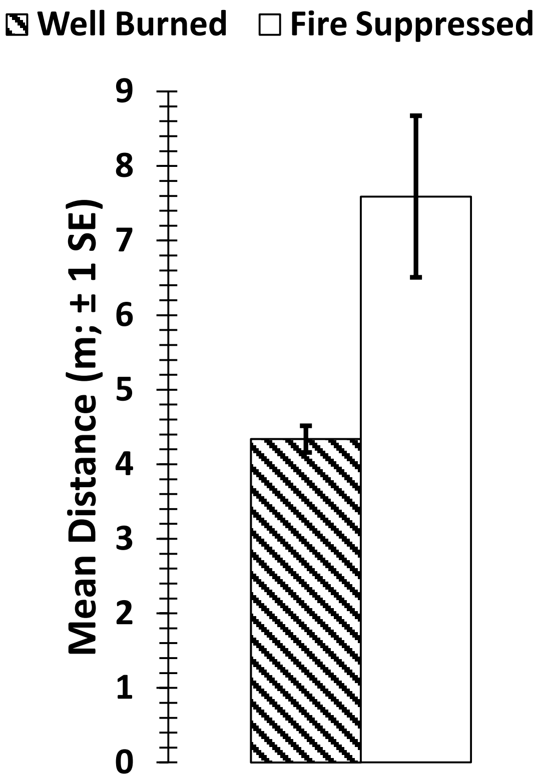

FIGURE 5: Mean distance traveled to a refuge by newly-metamorphosed gopher frogs in enclosures of well-burned habitat compared to fire-suppressed habitat.

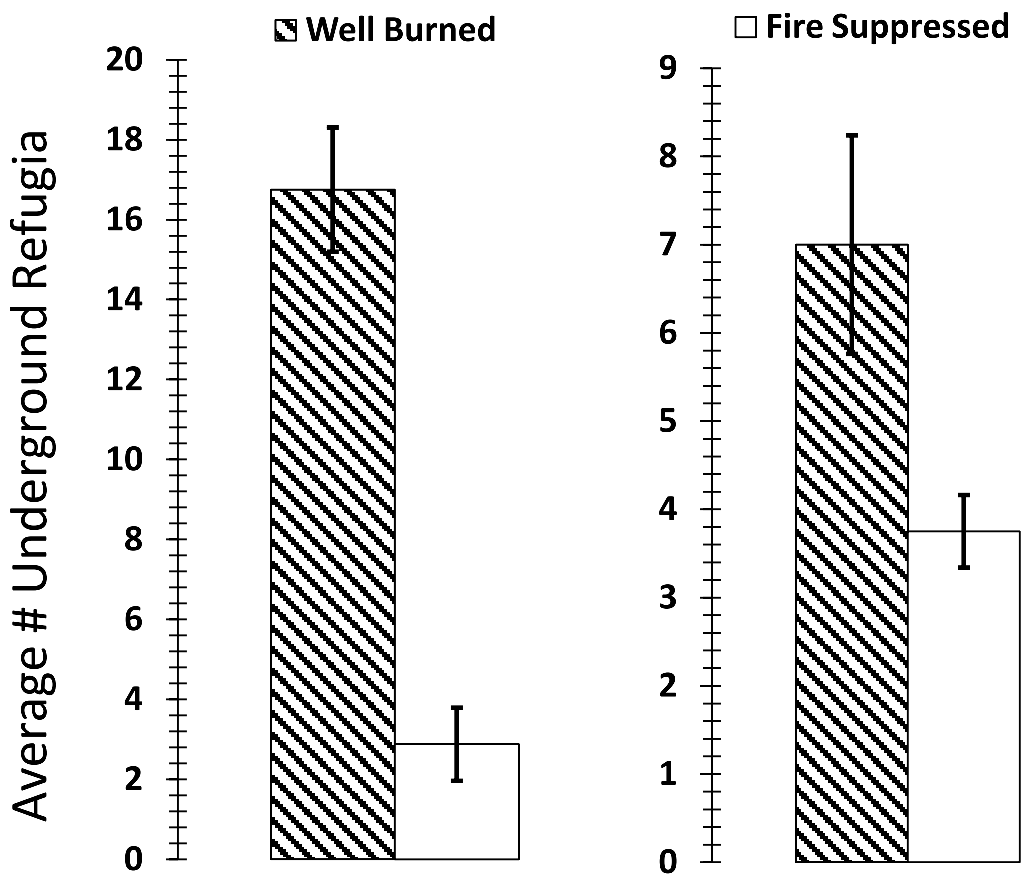

FIGURE 6: Average number of underground refugia per enclosure or nearby site in well-burned and fire-suppressed habitat. Left graph = nearby sites, right graph = enclosures.

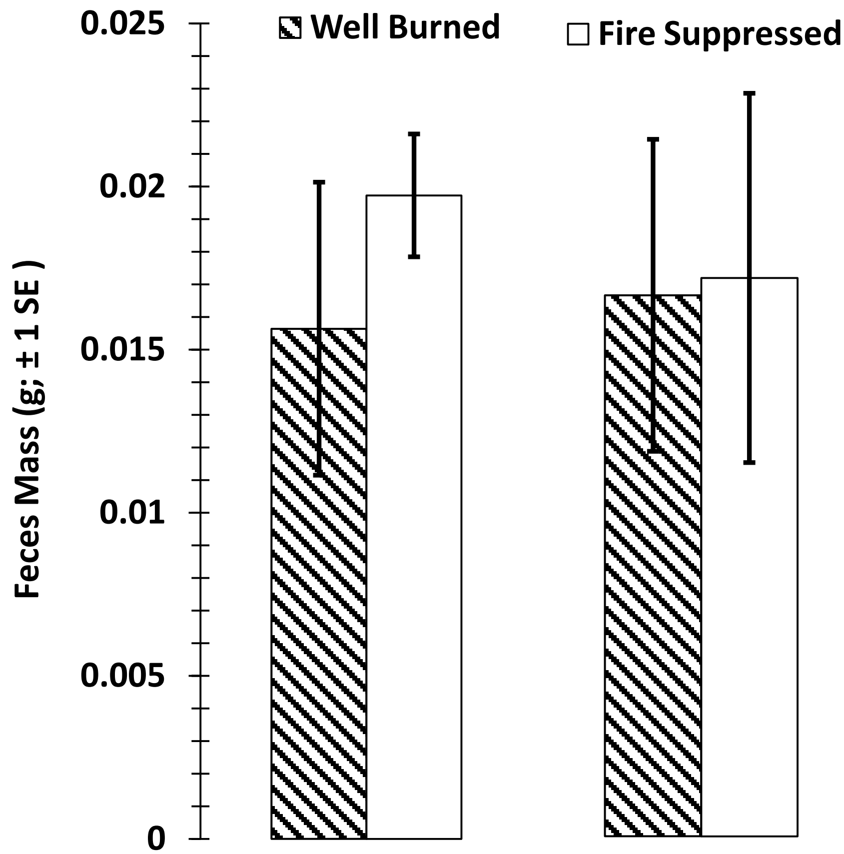

FIGURE 7: Mean feces mass of individual gopher frog metamorphs after foraging in well-burned or fire-suppressed habitat. Left graph = Exp. 1, right graph = Exp. 2.

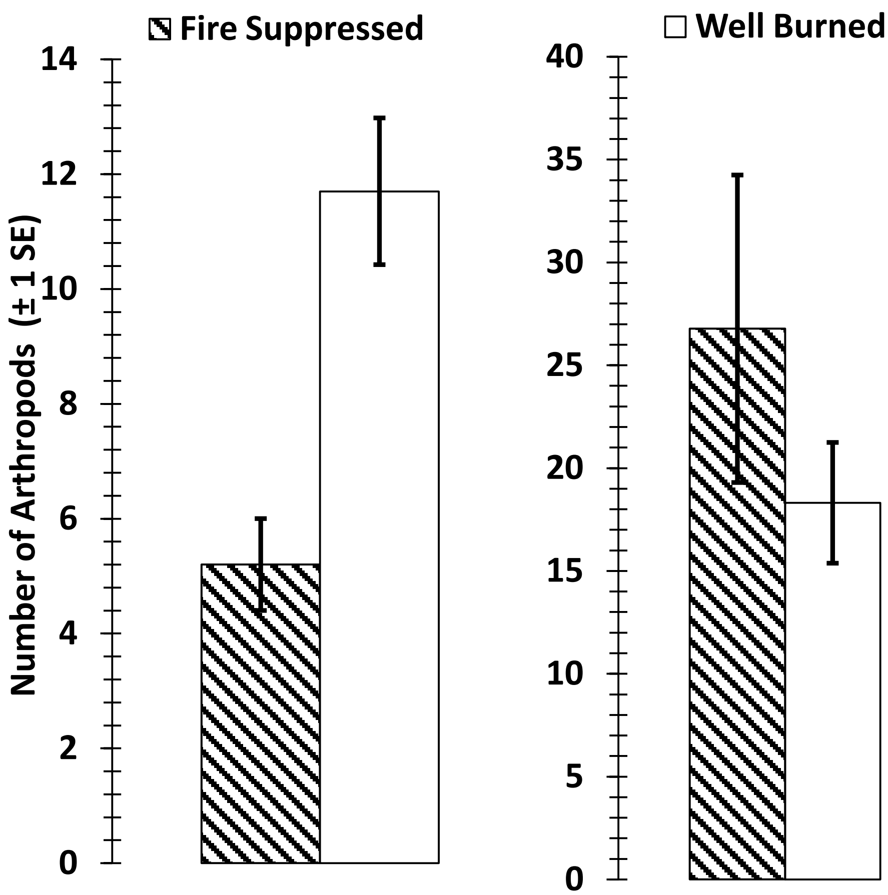

FIGURE 8: The mean number of arthropods (prey abundance) captured in cages immediately after foraging trials in fire-suppressed and well-burned habitats. Exp. 1 (left graph) and Exp. 2 (right graph). Sampling periods were 24 hours in Exp. 1, 48 hours in Exp. 2.