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Effects of prescribed fire on Cope’s Gray Treefrog (Hyla chrysoscelis) across habitat scales and life stages

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Effects of prescribed fire on Cope’s Gray Treefrog (*Hyla chrysoscelis*) across habitat scales and life stages

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Abstract

EFFECTS OF PRESCRIBED FIRE ON COPE’D GRAY TREEFROG (HYLA
CHRYSOSCELIS) ACROSS HABITAT SCALES AND LIFE STAGES

By Logan McDonald, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2017.

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Fire may alter both aquatic and terrestrial habitat used by all amphibian life stages, yet, our knowledge of its effects on amphibians is primarily limited to adult responses. I present an integrated approach to test the response of Cope’s Gray Treefrog (Hyla chrysoscelis) to fire by examining responses in tadpole performance and survivorship, adult abundance, and oviposition. Tadpoles raised with burned leaf litter had similar survival, but total mass and total length were 440% and 170% greater, respectively, for tadpoles raised in unburned litter. I assessed terrestrial and aquatic oviposition cues by embedding burned and unburned litter treatments within burned and unburned terrestrial plots. Oviposition was an order of magnitude higher in unburned plots,
regardless of the litter treatment. This difference was not statistically significant or driven by adult abundance. My results indicate the need to explore the dynamic effects forest management practices can have on amphibians across life stages.
Introduction

The effects of forest management techniques on wildlife are variable depending upon the technique applied, focal taxa, and region (deMaynadier and Hunter 1995, Greene et al. 2016, Russell et al. 1999, Russell et al. 2004). These management practices may alter a suite of habitat parameters critical to amphibians, such as soil moisture and temperature, coarse woody debris, hydro-period, canopy cover, understory cover, water temperature, and nutrient cycling (Bixby et al. 2015, Blomquist and Hunter 2009, Rothermel and Luhring 2005, Semlitsch et al. 2009). Alterations to these habitat characteristics, even at the microhabitat and microclimate scale, may have implications for amphibian population dynamics (Earl and Semlitsch 2015). Prescribed fire management is widely applied in North American ecosystems to return to natural fire intervals, reduce fuel loads, improve wildlife habitat, control nuisance species, and achieve other management objectives (Ryan et al. 2013). Fire is a particularly complex landscape level disturbance that may create variation in both the terrestrial and embedded aquatic habitats. Yet, research examining the effects of forest management practices on amphibians has primarily focused on timber thinning and removal, with less emphasis on the effects of prescribed burning. This is surprising given that fire may alter some of the same habitat parameters that amphibians are particularly sensitive to, such as temperature and moisture. Prescribed fire is often applied in areas of high amphibian diversity, such as the southeast, making it increasingly important to understand its impact on this taxa. Research that has explored the amphibian response to fire has largely focused on a single scale and one life-stage. For organisms with complex life cycles
across the terrestrial-aquatic interface, a multi-life stage (Biek et al. 2002) and multi-habitat approach is necessary to fully understand the dynamic role that prescribed burning may have on amphibian populations.

Our knowledge of amphibian response to fire is primarily restricted to short time scale responses of adult populations in the terrestrial environment. In terrestrial systems, burning can reduce duff and understory and canopy cover, increase solar radiation, decrease humidity, and increase temperature (Debano et al. 1998). The impacts of fire on amphibians are highly variable by taxa and region. Fire has been found to increase adult movement or displacement due to microhabitat changes that impact thermoregulatory requirements and predation risks (O’Donnell et al. 2016). A meta-analysis revealed that fire in southeastern pine habitats generally reduces amphibian biodiversity indices over short time scales (Grant et al. 1994, Greene et al. 2016, Schurbon and Fauth 2003). Even within pine habitats, the impacts of fire on amphibian abundance was variable, with some studies showing no impact (Perry et al. 2012) or even a positive impact on amphibians (Langford et al. 2007). In the western United States, *Bufo boreas* may benefit from burns in habitat where fuel loads exceed historical maximums because burned forest was associated with preferred thermal requirements (Hossack et al. 2009). In Virginia shelterwood-harvested Oak-dominated forests, adult amphibian captures did not differ in a study comparing burned and unburned areas (Keyser et al. 2004). Clearly the responses in terrestrial adult populations can heavily depend on the species, habitat, and context of the fire.

A meta-analysis exploring the effects of fire on aquatic ecosystems demonstrated that fire can dynamically alter aquatic habitats embedded in burned landscapes by altering nutrient cycling and inputs, microclimate, and water chemistry (Benda et al. 2003, Bixby et al. 2015, Coombs and Melack 2013, Gresswell 1999). Riverine systems experienced loading of nitrogen...
and phosphorous and reductions in dissolved oxygen and pH following fire (Dahm et al. 2015, Sherson et al. 2015). In wetland systems, the reduction of vegetation can allow for increased allochthonous nutrient inputs and solar radiation (Beakes et al. 2014, Cooper et al. 2015, Gresswell 1999, Minshall et al. 1989, Verkaik et al. 2013) which has implications for primary production and food web dynamics. However, data from fire impacted lentic systems is limited (Bixby et al. 2015) and few studies have examined the responses of amphibians in aquatic life stages.

Aquatic amphibian larvae are sensitive to fine scale habitat changes to leaf litter quantity and quality, water temperature and chemistry, and canopy cover (Alvarez and Nicieza 2002, Lillie and Knowlton 1897, Schiesari 2006, Stoler and Relyea 2011, Werner and Glennemeier 1999, Williams et al. 2008). All of these parameters are susceptible to alteration via fire disturbance. Given the potential for fire to alter aquatic habitats, more consideration should be given to the effects of fire on the aquatic life stages of amphibians. The few studies that have explored the effects of fire on aquatic amphibian life stages have focused solely on the aquatic habitat, without examining the corresponding influence of the terrestrial habitat. Aquatic post-fire surveys found that fire can have negative impacts on egg mass abundance, and tadpole survivorship and performance in mountainous, western regions where fire frequency is low (Gamradt and Kats 1997, Hossack et al. 2006). In the fire adapted Florida scrub, Oak Toad (Anaxyrus quercicus) larvae had higher survivorship and performance in recently burned pools (Noss and Rothermel 2015). Special consideration should be given to vernal pool breeding amphibians due to the unique hydrology of their breeding habitat. Fires that occur during the late spring or summer may alter water chemistry from runoff, but are unlikely to directly burn leaf litter within vernal pool depressions that are saturated with water. The same vernal pools
may experience a direct reduction in leaf litter and pool substrate during fall burns when these
depressions are dry. Leaf litter type and quality has well-known effects on tadpole growth and
survival (Rubbo et al. 2008, Rubbo and Kiesécker 2004), but no studies to our knowledge have
examined the impacts of burned leaf litter on tadpole growth and development.

Variation in the quality of potential aquatic breeding habitats can also have important
implications for the distribution of eggs on a landscape. Oviposition habitat selection (OHS),
when females selectively oviposit in response to habitat cues, has been demonstrated in multiple
taxa with complex life cycles (Aray and Blaustein 2006, Ries and Fagan 2003). Abiotic and
biotic habitat characteristics, such as predator presence, conspecific density, nutrient inputs, and
pesticides are known to influence OHS behavior and tadpole performance (Binckley and
Resetarits 2008, Marsh and Borrell 2001, Relyea and Werner 1999, Semlitsch and Gibbons
1988, Vonesh and Buck 2007, Wilbur 1980, Xue et al. 2006). Females can confer a fitness
advantage to offspring when OHS aligns with habitat conditions that increase larval performance
(Pinar and Resetarits 2017). For organisms with complex life cycles, both the terrestrial
landscape and embedded aquatic patches may be highly variable and influence OHS. However,
work examining this behavior has largely focused on OHS in mesocosms embedded into a single
landscape type. Field surveys have examined oviposition in multiple aquatic sites across the
landscape, but have not determined whether oviposition differences across sites are attributed to
OHS behavior or differences in adult abundances (Gamradt and Kats 1997). These field surveys
are also limited in their ability to separate aquatic and terrestrial factors that may influence
oviposition. A predation cue in a single pool, nested within a patch of pools, can reduce
oviposition in neighboring pools without predators (Resetarits 2005); this suggests that OHS can
operate at multiple scales, but previous studies have only considered the aquatic habitat. To our
knowledge, no studies have attempted to simultaneously assess whether OHS decisions are made in response to terrestrial or aquatic conditions. This raises the question of whether responses to variation in habitat quality at larger scales override or magnify habitat selection behavior at other nested scales and whether there is an interaction between the heterogeneity of terrestrial and aquatic systems. Prescribed fire inherently provides multiple levels of variation (Debano et al. 1998, Pilliod et al. 2003) and it is of interest to consider if OHS in response to fire is based on terrestrial, aquatic, or a combination of the terrestrial-aquatic cues.

Cope’s Gray Treefrog (*Hyla chrysoscelis*) is a well-studied species that is known to show differential OHS and tadpole performance in response to aspects of aquatic habitat quality (Hocking et al. 2007, Resetarits and Wilbur 1989, Rubbo and Kiesécker 2004, Rubbo et al. 2008, Vonesh et al. 2009). We present a novel approach testing stage specific effects of prescribed fire on amphibians. Our three part study examined tadpole performance, adult abundance, and oviposition. We tested tadpole performance in mesocosms with burned or unburned leaf litter to investigate if tadpoles experience fitness consequences in response to a late season fire common in the southeastern US. We measured adult abundance across burned and unburned plots to determine if differences in oviposition were representative of OHS behavior, differences in adult abundance in plots with different fire histories, or both. In order to disentangle terrestrial and aquatic effects, we monitored oviposition in experimental mesocosms with burned and unburned leaf litter that were embedded within replicate burned and unburned terrestrial plots. We compared oviposition by adults to tadpole performance to determine if females selectively oviposited in pools that conferred a fitness advantage to their offspring.

Given our knowledge of tadpole sensitivity to aquatic conditions and the potential impacts of fire on aquatic habitats, we expected that tadpoles would have reduced performance
in burned leaf litter conditions. If fire negatively impacts tadpoles, we would then expect to see greater oviposition by adult frogs in pools with unburned leaf litter. Although the reported effects of fire on adult amphibians are variable, we predicted two possible responses for adult abundance and oviposition. First, adult abundance could be lower in burned environments due to direct and indirect mortality, and then differences in oviposition between burned and unburned plots would be partially accounted for by differences in the proportion of adults in each plot. Alternatively, adult abundance could be similar in burned and unburned plots, and then oviposition differences would be solely due to OHS behavior. The latter suggests that adults may choose not to oviposit in a burned plot, or that they are moving from burned to unburned plots to oviposit. We expect the greatest oviposition in unburned plots and in pools with unburned leaf litter. Adult frogs would likely experience cues from the terrestrial plot first, however, Cope’s Gray Treefrog has also demonstrated an ability to discern habitat quality differences across aquatic pools (Resetarits and Wilbur 1989). We predict that oviposition decisions will be determined by both the terrestrial plot and aquatic cues, but that terrestrial cues will be stronger than the aquatic cues: we expect greater oviposition in burned leaf litter pools embedded in an unburned plot than in an unburned leaf litter pools embedded in a burned plot.
Methods: Study Site

We conducted our study on tadpole performance, oviposition, and adult abundance at U.S. Army Garrison Fort A.P. Hill, VA (Figure 1). Fort A.P. Hill’s 30,673 ha of habitat is located primarily in the Coastal Plain of northwestern Caroline County. The Natural Resources Division at Fort A.P. Hill maintains an annual prescribed burn regime, often using wetlands as a burn line, making it ideal for studying the effects of fire on amphibians. We nested our design into prescribed burns that Fort A.P. Hill conducted during the spring of 2017 (February- March). By utilizing prescribed burns at an army installation, we were able to embed our mesocosms into prescribed burns applied by natural resource managers. We screened all plots that were successfully burned, but as a result of weather conditions during the spring burn season, only four burned plots that were separated from an unburned plot by a wetland met the criteria for our study.
Tadpole growth and development (measured here as length, mass, and developmental stage), was examined through a common garden experiment, in a shaded forest plot, that manipulated leaf litter treatments in small mesocosms (n = 24, depth = 0.15m, max diameter = 0.28m, volume = 26.50L). We sought to simulate a fall burn where leaf litter may be directly burned in a dry vernal pool depression and then incorporated into a pool as it fills in the spring. Mixed leaf litter was collected from one mixed hardwood forest and half of this litter was burned on an asphalt pad in order to prevent loss of the ash into the soil. The mass of the burned leaf litter treatments was based on the mass of the leaf litter prior to burning. Thirty-four days after burning the leaf litter, we stocked mesocosms with 0.30g/L of leaf litter, a ratio that has been used in previous mesocosms experiments (Kiesecker and Skelly 2001, Rubbo et al. 2008, Werner and Anholt 1996). The leaf litter treatments consisted of 100% unburned (mass = 7.95g, n = 8), 100% burned (mass prior to burning = 7.95g, n = 8), or mixed (50% burned and 50% unburned, n = 8) leaf litter. Leaf litter treatments were randomly assigned to pools filled with well water and inoculated with 15mL of concentrated zoo- and phytoplankton collected from a nearby wetland. Ceramic tiles (15.24cm x 15.24 cm) were deployed in each pool as a substrate for periphyton growth.

Eggs were collected from experimental pools described below in an unburned plot, mixed, and allowed to hatch. Ten tadpoles were randomly assigned to each pool (n = 240) on 26 June 2016. Tadpoles within each pool were photographed at the time of stocking to determine
pre-experiment length using ImageJ (Image J software, version 1.49; National Institutes of Health, Bethesda, MD, U.S.A., Schneider et al. 2012). Six additional representative samples of ten tadpoles (n = 60) were randomly selected prior to the experiment and euthanized (IACUC #AD10000450). These tadpoles served as a proxy to determine if tadpole dry mass differed across groups at the time of stocking. After tadpoles were stocked, mesocosms were covered with fiberglass mesh to exclude predators and left undisturbed for three weeks. After three weeks, on 18 July, tadpoles within each pool were captured and photographed. Each tadpole photo was measured in ImageJ to determine total length. Tadpoles were also observed for hind and front limb development. Tadpoles were then euthanized in a buffered MS-222 solution and immediately frozen. Tadpole dry mass for each pool was determined by placing tadpoles in the drying oven for 72 hours at 60°C. Pool water parameters (pH, temperature, and dissolved oxygen) were recorded in each pool using a Hydrolab M15 Sonde. Algal biofilms, which have served as a proxy for periphyton biomass (Relyea 2005), were scraped from tiles deployed in each pool and dried at 60°C for 72 hours in order to assess if tadpole food availability differed across treatments. These algal samples were then combusted in a muffle furnace for 3 hours at 475°C to determine ash free dry mass, or organic content.
Methods: Adult Abundance

To assess adult abundance across the terrestrial plots, we established four paired research plots that were bisected by a wetland during the summer of 2016 (Figure 1). Each plot contained a burned and unburned treatment, separated by a wetland. Treatments contained a 40 x 50m grid of

20 hylid refugia (n=160) attached to trees, beginning 30m from a wetland (Figure 2). Hylid refugia were made of 1.27cm diameter PVC cut in 1m lengths that were painted blue to increase occupancy (Cohen et al. 2016). The use of hylid refugia (Boughton 2000) is minimally invasive, as tree frogs are able to freely enter and exit the refugia. Hylid refugia allows for treefrogs to be sampled without dependence on breeding calls to locate individuals. We measured snout to vent length (SVL) and mass, and marked adults occupying refugia. Visible alphanumeric tags (Visible Implant Tags Alphanumeric Format, Northwest Marine Technology, Shaw Island, WA; Buckmeier and Irwin 2000, Pittman 2008) were injected subcutaneously within the tibial region of the hind leg to identify individuals.
Methods: Oviposition

Six experimental mesocosms (depth = 0.19m, diameter = 1.14m, volume = 200L) were deployed 30m from the wetland within each terrestrial treatment. Mesocosms (n = 48) were filled with well water and covered with fiberglass mesh on 2 May to prevent invertebrate and predator colonization. The leaf litter mass to water volume ratio (0.30g/L) in oviposition mesocosms matched that of the tadpole performance mesocosms. Unburned leaf litter (mass = 60g) was randomly assigned to three mesocosms in each subplot. The burned leaf litter treatment was comprised of half unburned litter (mass = 30g) and half burned litter (mass prior to burning = 30g) that was randomly assigned to three mesocosms in each subplot.

From 17 May to 29 July, a weekly rotation was established to survey two sites per week. During a site’s survey week, mesh nets were pushed down into the water to allow oviposition. Adult treefrogs were able to freely enter and exit the mesocosms through their own behavior. Within 24 hours, eggs deposited in the mesocosms were counted and then immediately relocated to an adjacent suitable aquatic habitat. Water chemistry parameters (pH, conductivity, temperature, and dissolved oxygen) were recorded in each pool throughout the duration of the study using a Hydrolab M15 Sonde. Air temperature and humidity were recorded (iButton Hygrochron Temperature/Humidity Logger model DS1923, Maxim Integrated) at randomly generated locations and attached to wooden stakes within each 40 x 50m hylid refugia grid. Water temperature was continuously monitored by deploying an iButton coated in PlastiDip into a randomly selected pool in each subplot.
Methods: Analysis

In order to assess the effects of leaf litter on tadpole performance, one-way ANOVAs and Tukey HSD comparison of means were conducted to evaluate tadpole mass and length, and environmental pool parameters. Effect sizes ($\eta^2$) were calculated for significant results. Initial tadpole dry mass was determined via the representative sample of 60 tadpoles. Tadpole development was evaluated by comparing hind and front limb development, and completed metamorphosis. Kruskal-Wallis tests and Dunn’s pairwise comparisons were used to evaluate tadpole development and survivorship. Adult captures were compared using a Wilcoxon Ranked Sum Test due to small sample size. A two sample t-test was applied to determine if average adult SVL and mass differed across plot treatments. To assess oviposition, we used a randomized complete block design with sites as blocks. The treatment structure had a split plot design: terrestrial treatments as whole plots and leaf litter treatment as the split plots. Replication within the split plot allowed for the testing of multiple interaction terms in our model. We tested the effect of plot treatment, leaf litter treatment, and the interaction of plot and litter treatments on oviposition using a generalized linear mixed model, with site as an effect. Site was included as an effect due to variation across our research plots. The oviposition count data was logged transformed due to failure in model convergence when the data was fit to Poisson, quasi-Poisson, negative binomial, or zero-inflated distributions (Ives 2015).
Results: Tadpole Performance

Tadpole survivorship was high and did not differ across treatments ($H_{(2)}=1.89$, $p=0.39$, Figure 3a), but litter treatments altered development and size. Achieving hind limb development (Gosner stage26) by the end of the experiment differed across treatments ($H_{(2)}= 19.23$, $p < 0.0001$, Figure 3b), and was most common in the unburned treatment ($\mu = 3.25 \pm 0.32$; mean $\pm$ SE for all reported descriptive statistics), occasionally encountered in the mixed treatment ($\mu = 0.5 \pm 0.32$), and never encountered in the burned treatment ($\mu = 0.00 \pm 0.32$). The number of tadpoles that had front limb development ($H_{(2)}= 2.00$, $p = .37$) and completed metamorphosis ($H_{(2)}= 4.17$, $p=0.12$) did not differ across treatments. However, front limb development ($n=1$) and completed metamorphosis ($n=3$) were only observed in the unburned leaf litter treatments.

There was no difference in initial length and mass of tadpoles across treatments when pools were stocked ($F_{(5, 57)}=0.62$, $p=0.68$). After three weeks, tadpole mass differed across leaf litter treatments and these treatments accounted for 73.4% of the variation in mass ($F_{(2, 21)} = 28.91$, $p < 0.0001$, $\eta^2 = .734$, Figure 3c). Post-hoc comparison of means revealed that total mass was 4.4x greater in the unburned treatments ($\mu = 0.023g \pm 0.016$, $p<0.0001$) than in the burned treatments ($\mu = 0.0052g \pm 0.016$, $p<0.0001$). Average tadpole total length (cm) also differed and leaf litter treatments accounted for 65.5% of the variation ($F_{(2, 209)} = 197.59$, $p < 0.0001$, $\eta^2 = .655$, Figure 3d). A post-hoc comparison of means revealed that average total length was 1.7x longer in unburned leaf litter ($\mu = 3.39cm \pm 0.055$, $p <0.0001$) than in burned leaf litter treatments ($\mu = 1.95cm \pm 0.050$, $p<0.0001$).
Two of our four environmental mesocosm parameters varied across treatments. Dissolved oxygen ($F_{(2, 21)} = 230.36, p<0.0001, \eta^2 = .956$, Figure 4b) and pH ($F_{(2, 21)} = 88.43, p<0.0001, \eta^2 = .894$, Figure 4c) were higher in the burned leaf litter treatments. Water temperature ($F_{(2, 21)} = 0.15, p=0.86$, Figure 4a) and periphyton biomass ($F_{(2, 21)} = 3.20, p=0.062$, Figure 4d) were not significantly different across treatments.
Results: Adult Abundance

A total of 21 adults were captured during our study and the number of detected individuals in burned (n = 10) and unburned treatments (n = 11) did not differ (Z=0.09, p=0.77, Figure 5). Adults in both treatments had similar morphometric measurements. Average SVL in burned (39.07mm ± 1.43) and unburned (39.64mm ± 1.15) treatments (t (17) = 0.31, p = 0.76), as well as mass, in burned (6.18g ± 0.56) and unburned (5.90 g ± 0.57) terrestrial treatments, did not differ (t (17) = 0.36, p = 0.72).
Results: Oviposition

One of our four research plots experienced zero net oviposition and was excluded from our analysis. Only one adult treefrog was captured at this site and we suspect the lack of oviposition was related to an absence of adults. Across the other three plots, oviposition occurred from 16 June – 19 July and we counted 24,313 total eggs that were laid over ten occasions. When site effect was accounted for, oviposition was not affected by plot treatment ($F_{(1)}=1.35$, $p=0.33$), leaf litter treatment ($F_{(1)}=0.01$, $p=0.91$) or the interaction between plot and litter treatments ($F_{(1)}=0.59$, $p=0.50$, Figure 6). Though not statistically significant, the unburned terrestrial and burned leaf litter treatment received the highest oviposition ($\mu = 1197\text{eggs} \pm 607$), followed by unburned terrestrial and unburned leaf litter ($\mu = 1087\text{eggs} \pm 556$), burned terrestrial and burned leaf litter ($\mu = 136\text{eggs} \pm 104$), and burned terrestrial and unburned leaf litter ($\mu = 133\text{eggs} \pm 93$).

Average water temperature ($F_{(1)}=2.02$, $p=0.25$; $F_{(1)}=0.0$, $p=0.97$; $F_{(1)}=0.44$, $p=0.55$, Figure 7a), dissolved oxygen ($F_{(1)}=0.030$, $p=0.86$; $F_{(1)}=0.030$, $p=0.88$; $F_{(1)}=0.15$, $p=0.73$, Figure 7b), and pH ($F_{(1)}=4.42$, $p=0.13$; $F_{(1)}=1.54$, $p=0.300$; $F_{(1)}=0.00$, $p=0.95$, Figure 7c) did not differ across terrestrial treatments, leaf litter treatments, or an interaction of treatments, respectively.
Discussion

Our study on the effects of fire on Cope’s Gray Treefrog suggests that treefrogs may be differentially impacted by fire at different life stages and that environmental cues for oviposition may be driven by terrestrial habitat quality. While we saw no difference in survivorship, we found clear differences in tadpole performance across aquatic leaf litter treatments, and smaller size at metamorphosis may translate into reduced adult fitness. However, our finding of similar adult abundances and morphometric measurements across burned and unburned plots suggests that adults were not negatively impacted by spring burns at this site. Oviposition was highly variable across our sites, with more eggs counted in the unburned plots, but this difference was not significantly different given our statistical power with three plots. Qualitatively, the mean number of eggs oviposited in the unburned plots was nearly an order of magnitude higher than the pools in the burned plots. We recognize that our inferences from adult captures and oviposition are limited due to sample size. However, the trend of higher oviposition in unburned plots, paired with equivalent adult abundances, indicates support for the hypothesis that oviposition responses to fire are driven by behavior as opposed to presence of adults. Our capture results indicate that adults were equivalent in both habitats and potential differences in numbers of eggs suggests treefrogs were choosing not to oviposit in burned plots and moving to unburned plots to oviposit.

In our mesocosm experiment, our results show that *H. chrysoscelis* tadpoles exhibit higher performance with unburned leaf litter than in treatments with burned leaf litter. These
results are consistent with previous studies that found increased performance in response to leaf litter quality. Wood frog tadpole survivorship, mass at metamorphosis, and morphology is affected by litter species and nutrient content (Stephen et al. 2013, Stoler and Relyea 2013). Leaf litter species effects can vary across amphibian species and influence community structure (Stoler and Relyea 2016).

The variation in performance in this study is not clearly explained by four typical environmental drivers of tadpole performance: water temperature, dissolved oxygen, pH, and periphyton. Our common garden design controlled for water temperature, which did not differ across our treatments. Decomposition of leaf litter may explain the reduced oxygen availability in unburned leaf litter treatments. However, as tadpoles from unburned treatments out performed tadpoles in burned treatments, oxygen was not a limiting factor. Studies investigating the effects of pH on amphibians have focused on acidic conditions, which are known to negatively impact tadpole survivorship and performance (Freda 1986, Pierce 1985), but few have explored the effects of basic aquatic conditions. Average pH was higher across all leaf litter treatments than in vernal pools and road ruts surveyed at Fort A.P. Hill in 2015 (average pH =5.46 ± 0.084 SE, n= 62, personal observation). In our study, the burned leaf litter treatments were more basic than the unburned treatments, but all pools fell within a narrow pH range of 7.0-7.7. Given this range, differential tadpole performance in our experiment may be largely driven by other water chemistry parameters. Leaf litter carbon, nitrogen and phosphorus content (Rowland et al. 2016, Stoler and Relyea 2013, Stoler and Relyea 2016, Williams et al. 2008), associated microbial communities (Earl and Semlitsch 2012), and leachates such as saponins and tannins (Earl et al. 2012, Martin and Blossey 2013) may be contributors to larval performance. High levels of trace elements, often associated with fire (Gresswell 1999), may also negatively impacted tadpole
performance. Survival may vary in natural systems, as water quality is expected to change with burn intensity, and landscape and aquatic characteristics. It is plausible that tadpole mortality could occur in higher intensity burns due to greater changes in water chemistry than seen in our experiment.

Periphyton biomass did not statistically differ across treatments, but we expect that nutrient availability may be a larger driver of performance than our measurements indicate. We used a small tile added to each tank to measure periphyton growth, but this method may not have provided a reliable index due to the differences in structure provided in treatments with leaf litter. In treatments with leaf litter, the leaves likely provided an important growing surface for periphyton and grazing area for tadpoles, which could have reduced growth on the tile. Tiles in treatments with leaf litter were also likely partially shaded by leaves while the tiles were completely exposed in the burned treatment. Our results do indicate that tadpoles were not overly food limited in the burned treatment. Additional studies are needed to separate the effects of burned leaf litter on water chemistry and habitat structure. A reduction in physical leaf litter structure and refugia could also increase predation rates, which can interact with reductions in size to have important consequences for development, size at metamorphosis, and survival.

*Hyla chrysoscelis* oviposition preference has been demonstrated to match larval performance (Pintar and Resetarits 2017) and by laying eggs in unburned leaf litter pools, females may confer a fitness advantage to their offspring. In accordance with preference-performance theory, we expected to see greater oviposition in pools with unburned leaf litter in unburned terrestrial plots. If adults prefer unburned pools (assuming there are no differences in adult abundance), but use terrestrial cues, there would be greater oviposition in all unburned terrestrial plots, with no difference in aquatic leaf litter treatments. If aquatic cues drive OHS,
then we would see equal oviposition across plots, but higher oviposition in unburned aquatic leaf litter treatments. If both terrestrial and aquatic cues are used, oviposition would differ across plots and leaf litter treatments. Although not significant, we saw a trend for increased oviposition in unburned terrestrial plots, but we saw no oviposition response to our aquatic leaf litter treatments. This would suggest that oviposition in response to fire is based on terrestrial cues. However, our measures of pool parameters revealed that differences in water chemistry in the tadpole mesocosms were not mirrored in the oviposition mesocosms. The lack of variation in pH and dissolved oxygen across the oviposition treatments may be due to the relatively longer duration of the oviposition experiment. Any initial differences in oviposition pool parameters from the leaf litter treatments may have been diluted by intense rainfall washing out our small pools throughout the experiment, and it is possible that oviposition did not differ across leaf litter treatments because there were no detectable differences from treatments established in May remaining in June when oviposition occurred. If fire induced shifts in water parameters are rapidly neutralized and adults do not oviposit based on aquatic conditions, then tadpoles may be differentially impacted by time of egg laying following a fire, with tadpoles from eggs that are laid immediately after a fire having reduced performance compared to eggs laid weeks to months after a fire.

Pre-fire adult abundance and oviposition records would have improved our ability to understand the effects of fire on *H. chrysoscelis*, but sampling the prior year at Fort AP Hill is not feasible due to the large number of potential plots to be burned. Anticipated burn plans are highly dependent upon spring conditions and anticipated burns are not always executed. If our trend toward adult OHS based on terrestrial cues after fire is representative of adult behavior, then managers should consider terrestrial buffers around vernal pools. For fall burns in
particular, such buffers would reduce the negative effects of fire on tadpole performance by preventing dry vernal pool depressions from being directly burned. Implementation of this management option would require extensive surveys to locate and map these pools.

Despite the broad application of prescribed fire in North American systems, our understanding of fire effects on specific amphibian life stage and populations is limited. Larval amphibian response to fire is likely species dependent (Pilliod et al. 2003) and driven by breeding season phenology relative to the natural fire season, region, and evolutionary association with fire. Forest managers should consider how departures from burn seasons may impact species differentially (Knapp 2009), as there is a potential for novelty or ecological traps associated with altered fire phenology. We see an increasing demand for these studies as climate change is expected to increase the frequency and intensity of fires (Aponte et al. 2016, Moritz et al. 2012, Ruthrof et al. 2016, Westerling et al. 2011). Exploring species specific responses to fire, across life stages and habitats, will be instrumental in establishing effective wildlife management plans in the context of prescribed burns and shifting fire regimes.
Literature Cited
Literature Cited


Appendix 1

Figures

Figure 1. Locations of research plots (n=4) established at Fort A.P. Hill, VA where a burned and unburned subplot were separated by a wetland (black dot). The extent of the spring burns in these locations is indicated in orange.
Figure 2. Research plot design with a terrestrial burned and unburned subplot on either side of a wetland. Each terrestrial subplot contains six mesocosms with burned (n = 3, green and black leaves) and unburned (n = 3, all green leaves) leaf litter and a 40 x 50m grid of hylid refugia (black circles outlined in blue).
Figure 3. Mean and standard error of (a) tadpole survivorship, (b) number of tadpoles with hind limb development, (c) tadpole dry mass (g), and (d) tadpole total length (cm) in burned, mixed, and unburned tadpole leaf litter treatments from 26 June to 18 July. Letters on bars denote differences of $p < 0.05$. 
Figure 4. Mean and standard error of (a) water temperature (°C), (b) dissolved oxygen (mg/L), (c) pH, and (d) periphyton dry mass (µg/cm²) in burned, mixed, and unburned tadpole leaf litter treatments from 26 June to 18 July. Letters on bars denote differences of p < 0.05.

Figure 5. Mean and standard error of adult captures from refugia in burned and unburned plots from 17 May to 29 July.
Figure 6. Mean and standard error of eggs oviposited in experimental mesocosms across terrestrial and leaf litter treatments from 17 May to 29 July. UTUL = unburned terrestrial * unburned leaf litter. UTBL = unburned terrestrial * burned leaf litter. BTUL = burned terrestrial * unburned leaf litter. BTBL = burned terrestrial * burned leaf litter.
Figure 7. Mean and standard error of (a) water temperature (°C), (b) dissolved oxygen (mg/L), and (c) pH in oviposition mesocosms across terrestrial and leaf treatments from 17 May to 29 July. UTUL = unburned terrestrial * unburned leaf litter. UTBL = unburned terrestrial * burned leaf litter. BTUL = burned terrestrial * unburned leaf litter. BTBL = burned terrestrial * burned leaf litter.
Logan Alexandra McDonald was born on January 6, 1992, in Escambia County, Florida. She graduated from Niceville Senior High School, Niceville, FL in 2010. Logan received her Bachelor of Science in Biology and her Bachelor of Arts in Environmental Studies from Florida Gulf Coast University, Fort Myers, FL in 2014. After earning her bachelor degrees, Logan conducted research as a salamander field technician at Mountain Lake Biological Station, Pembroke, VA. In 2015, she completed a wildlife internship with the National Park Service in the Santa Monica Mountains National Recreation Area, Calabasas, CA. Logan began her transition into the graduate program at Virginia Commonwealth University, Richmond, VA by leading an amphibian research crew at Fort A.P. Hill, VA under an Army Corps of Engineers cooperative agreement. She received research grants from The Animal Behavior Society, The Virginia Herpetological Society, and the VCU Rice Rivers Center. In March 2017, Logan accepted a position with the Florida Fish and Wildlife Conservation Commission as a biological scientist to coordinate citizen science efforts.