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# The Conservation and Population Ecology of the Imperiled Crawfish Frog (Lithobates areolatus) in Human-altered Landscapes

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Conservation and Population Ecology of the Imperiled Crawfish Frog (*Lithobates areolatus*) in
Human-altered Landscapes


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by


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**Abstract**

The Crawfish frog (*Lithobates areolatus*) is an imperiled amphibian currently experiencing severe declines across its range. As a species with unique habitat requirements that is threatened by habitat loss, understanding their status across the landscape and how they respond to environmental stressors is key to developing effective conservation strategies that maintain and expand viable populations. We used a combination of observational, experimental, and theoretical approaches to understand the status of Crawfish Frog populations in Northwest Arkansas (NWA), their individual and population-level response to human-induced changes in vegetation surrounding breeding wetlands, and the effects of fire management on larval development within breeding wetlands. Specifically, in Chapter 1, we performed a landscape-level assessment of Crawfish Frog status at 81 sites across NWA. Crawfish Frog occupancy was relatively low (~26%), but occupancy probability increased strongly with density of prairie mounds, a proxy for habitat quality, and modestly with prevalence of clay/gravelly silt loam soils. In Chapters 2 and 3, we performed a series of mesocosm experiments to investigate how vegetation (Native Prairie or Non-Native Tall-Fescue Grass) surrounding wetlands interacted with timing of oviposition and density-dependence to affect larval amphibian development. We found that Fescue-dominated wetlands might act as ecological traps resulting in complete reproductive failure of breeding Crawfish Frogs within a season, due to higher microbial respiration. Demographic population revealed that populations breeding in Fescue-dominated wetlands had a more variable population size and had a 100-500% higher probability of quasi-extinction within 200 years, compared to populations breeding in prairie-dominated wetlands. Finally, we performed an experiment to measure the effect of ash-deposition and reduced vegetation due to fire on larval Crawfish Frog development. Our results suggested that the

quantity of ash used in our experiment had negligible effects on the development of aquatic amphibian larvae, but reduction of vegetation biomass by fire results in lower survival and biomass production if burning occurs during the dormant season, prior to wetlands filling. Collectively, our results indicate that Crawfish Frogs need high quality natural prairie habitat for population persistence and although relict populations exist in agricultural landscapes, these are poor replacements for natural grasslands.

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Chapter 2

Kross, C.S, A.K. Dodd, P.L. Mariage, J.D. Willson. Accepted. Timing of oviposition influences the effects of non-native grass on amphibian development. Oecologia.

# Introduction

The Crawfish Frog (*Lithobates areolatus*) is an open-canopy-associated amphibian that is currently experiencing precipitous declines throughout its range (Parris and Redmer 2005). The IUCN (Hammerson and Parris 2004) has listed the Crawfish Frog as near threatened; the species is listed as state endangered in Iowa, Indiana, and Illinois, and is a species of greatest conservation need in Arkansas, Oklahoma, Missouri, Kansas, Kentucky, Mississippi, and Louisiana. Due to the species' unique habitat requirements, habitat loss is thought to be the primary cause of decline. Open-canopy habitats used by the Crawfish Frog, such as grasslands and their associated ephemeral wetlands, have been largely replaced with agricultural crops or permanent ponds (Samson and Knopf 1994). The continued destruction of their natural habitat puts this species at an increased risk of extinction and requires knowledge about how the species responds to environmental stressors (e.g., non-native species, climate change, etc) and management techniques (e.g. prescribed fire).

Crawfish frogs are unique among North American amphibian species because adults are obligate crayfish burrow users and have been documented to migrate over a kilometer to and from their breeding wetland (Heemeyer et al. 2012; Heemeyer and Lannoo 2012). Adults are highly terrestrial; spending all but the brief (1-4 week) breeding season in crayfish burrows in upland habitat. Telemetry studies have shown that Crawfish Frogs tend to prefer open-canopy grassland habitat with multiple crayfish burrows (Williams et al. 2012a, Heemeyer et al. 2012). Crayfish burrows serve as important refugia from predation and high daytime temperatures (Engbrecht et al. 2012, Heemeyer and Lannoo 2012, Kwiatkowski et al. 2017). Additionally, Crawfish Frogs were often found in areas with clays soils at a refuge in Indiana (Williams et al. 2012a, 2012b). Outside of their breeding season, Crawfish Frogs are rarely detectable (Parris and

Redmer 2005, Heemeyer et al. 2012). However, while males are within wetlands, their calls are distinctive and easily detected; as such, call surveys have been a recommended method for monitoring populations and identifying potential new populations (Williams et al. 2012b; 2013). Although several studies have examined Crawfish Frog habitat-use, occupancy, and detection in localized protected areas, little is known about patterns of Crawfish Frog occupancy and detection at the landscape-level.

The biology of larval crawfish frogs has been understudied compared to adults. Most work has focused on head-starting (Stiles et al. 2016) or the effect of density and competition on development (Parris and Semlitsch 1998, Williams et al. 2012). Larval Crawfish Frogs require ephemeral wetlands free of fish and competitors for optimal survival (Parris and Semlitsch 1998, but see McKnight and Ligon 2016). Additionally, larval survival in natural wetlands can be near 0% in some years (Stiles et al. 2016), which can increase extinction risk (Hels and Nachman 2002, Vonesh and De la Cruz 2002). However, no work has been done to determine how larval Crawfish Frogs respond to human-induced changes in habitat, which is especially needed to understand patterns of extinction risk across human-altered landscapes.

As a result of habitat loss, Crawfish Frogs are frequently found breeding within agricultural wetlands (Baecher et al. 2018, Polis 2018), which are often surrounded by a mono-culture of non-native vegetation. Vegetation entering wetlands (i.e. subsidies) provide an essential energy base in aquatic systems (Earl and  Semlitsch 2013), and changes to the species of litter entering a wetland can affect community composition and alter food web dynamics (Rubbo and Kiesecker 2004). In temporary wetlands, amphibians are often the top consumers (Rubbo and Kiesecker 2004), and litter has been shown to exert important bottom-up effects on amphibian production (Maerz et al. 2005, Earl and Semlitsch 2013). For example, tannins

produced from Eurasian Purple Loosestrife (*Lythrum salicaria*) and Chinese Tallow (*Triadica sebifera*) resulted in reductions of tadpole survival (Maerz et. al. 2005; Adams & Saenz 2012). Additionally, Rubbo et al. (2008) found that three different species of amphibian raised in tanks with oak leaves had higher survival to metamorphosis compared to those raised in tanks with maple leaves. However, no studies have expressly determined how non-native agricultural grasses, such as Tall-grass Fescue (*Lolium arundinaceum*), affect the development of amphibians that breed within them.

Crawfish Frogs have been shown to be poor intra- and inter-specific competitors (Parris and Semlitsch 1998, Williams 2012). Density-dependent competition for resources is a key aspect of population regulation for many species (Turchin 1999), especially for species with complex life cycles that lead to high densities within particular life stages (Wilbur 1980). For this reason, amphibians have served as model organisms for addressing questions related to the role density-dependence plays in population regulation (Wilbur 1976, 1977, Skelly and Kiesecker 2001, Vonesh and De la Cruz 2002). High larval densities can result in reduced growth rates, small juvenile body sizes, and increased time required to attain reproductive maturity (Van Buskirk and Smith 1991, Altwegg 2003). In extreme cases, high density can result in complete reproductive failure due to increased development times and failure to metamorphose prior to drying of an ephemeral breeding wetland (Taylor et al. 2005). Although amphibians are among the most threatened vertebrate groups (IUCN 2019), few studies have examined interactions between density-dependence and the effects of anthropogenic stressors – Those studies that have (e.g., Vonesh and De la Cruz 2002, Willson et al. 2012) have found that experimental studies can be misleading, if not considered within the context of density-dependence. Furthermore, little work has evaluated how rearing habitat and density-dependence interact to affect recruitment and

subsequent population dynamics. Understanding how vegetation changes affect *L. areolatus* and their population dynamics could provide important implications for conservation and management

Crawfish frogs primarily occur in disturbance-adapted systems (e.g. grasslands). Prescribed fire is an important management tool for many disturbance-adapted terrestrial systems and their associated wetlands (Gilliam and Platt 1999, Kirkman 1995, Lugo 1995, Gray et al. 2013, Swan et al. 2015). Although some studies have addressed the effect of fire on terrestrial life stages of amphibians (Russell et al. 1999, Chelgren et al. 2011), there is a paucity of studies examining how fire might indirectly affect wetland communities and amphibian larvae within fire-maintained ecosystems (Pilliod et al. 2003). Prescribed fire is often implemented during the dormant (Late Fall - Early Spring) season (Knapp et al. 2009), when many amphibian species breed. Thus, fire could affect larval development via two pathways: (1) reduction of vegetative biomass, which serves as an important nutrient base in aquatic systems, and (2) deposition of ash from surrounding terrestrial habitat. Understanding the effects of fire on larval Crawfish Frog development is an important step in optimizing management strategies for this species.

This dissertation focuses on understanding the population and conservation biology of Crawfish Frogs. I use a combination of observational, experimental, and theoretical approaches to understand how the species responds to different environmental stressors. I had four primary questions, each its own chapter and prepared as a publication in peer-reviewed journals:

1) What is the status of Crawfish Frogs across Northwest Arkansas and what landscape and habitat variables are most important in predicting their occupancy at the landscape level?

2) Does replacement of native prairie vegetation with non-native Tall-grass Fescue affect larval development and recruitment of Crawfish Frogs?

3) How does larval density-dependence interact with vegetation type to affect extinction risk of Crawfish Frog populations within prairie and agricultural wetlands?

4) Does ash addition and reduced vegetation biomass as a result of fire affect the development of larval Crawfish Frogs?

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**Chapter 1**

Population decline and landscape-scale occupancy of the Crawfish Frog (*Lithobates areolatus*)

in Northwest Arkansas

Chelsea S. Kross

and

John D. Willson

**Abstract**

The Crawfish Frog (*Lithobates areolatus*) is an imperiled amphibian, unique among ranid frogs due to its obligate use of crayfish burrows, highly terrestrial behavior, and reliance on open-canopy habitats within the central USA. Currently listed as threatened by the IUCN, and as state endangered, threatened, or of greatest conservation need in most states where it occurs, the Crawfish Frog serves as an indicator of high-quality prairie and wetland habitat, and potentially as an umbrella species for biodiversity conservation in the region. However, few studies have sought to identify site characteristics that most strongly associated with *L. areolatus* occupancy or rigorously assessed the status of populations in core areas of the species' range in Arkansas, Oklahoma, and Kansas. Within Northwest Arkansas, we used an occupancy modeling framework to 1) determine landscape characteristics that are most important predictors of *L. areolatus* populations, 2) assess the status of current and historical populations, and 3) identify potential areas for habitat conservation to support population persistence. We completed 405 time-constrained auditory surveys across 81 potential and historical *L. areolatus* breeding sites over two breeding seasons (March – April 2016 and 2017). Overall the naïve occupancy and detection were 26% and 32.5%, respectively. We did not detect *L. areolatus* at 37.5% (6/16) of historic locations during our study, indicating these populations have likely gone extinct. Occupancy probability was strongly related to density of prairie mounds within 1.2 km of breeding wetlands and was weakly related to clay and chert/gravel loam soil. Our results suggest that: 1) *L. areolatus* is widespread throughout Northwest Arkansas but is threatened by the expanding human population, 2) detection probability is high under optimal conditions (cool temperature and recent rain), and 3) prairie mound density is useful proxy for habitat quality for

this species. Thus, our study provides a framework for rigorous assessment of the status of *L. areolatus* populations across their range.

## Introduction

*Lithobates areolatus* (Crawfish Frog) is currently experiencing precipitous declines throughout its range in the eastern Great Plains of the central United States (Parris and Redmer 2005). *Lithobates areolatu*s is unique among North American amphibian species because adults are obligate crayfish burrow users and have been documented to migrate over a kilometer to and from their breeding wetlands (Heemeyer et al. 2012; Heemeyer and Lannoo 2012). Adult *L. areolatus* are highly terrestrial, spending all but the brief (1-4 week) breeding season in crayfish burrows in open canopy upland habitat. Larval *L. areolatus* require fish-free ephemeral wetlands with limited competitors for optimal survival (Parris and Semlitsch 1998, but see McKnight and Ligon 2016). Due to the species' unique habitat requirements, habitat loss is thought to be the primary cause of decline. The IUCN (Hammerson and Parris 2004) has listed *L. areolatus* as near threatened; the species is listed as state endangered in Iowa and Indiana, and is considered a species of greatest conservation need in Arkansas, Illinois, Kansas, Kentucky, Louisiana, Missouri, Mississippi, and Oklahoma. As such, *L. areolatus* serves as an indicator of high-quality wetland and upland habitat, and potentially as an umbrella species for biodiversity conservation throughout its range.

Due to the declining status of *L. areolatus*, an understanding of both range-wide patterns of habitat-use and probability of detection is important for developing effective conservation and management strategies. Telemetry studies have shown that *L. areolatus* select open-canopy grassland habitat with a high-density of crayfish burrows (Williams et al. 2012a; Heemeyer et al. 2012). Crayfish burrows serve as an important refuge from predation, desiccation, and high

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daytime temperatures (Engbrecht et al. 2012; Heemeyer and Lannoo 2012; Kwiatkowski et al. 2017). Additionally, some studies have used auditory recording systems and call-surveys to determine patterns of occupancy and detection (Williams et al. 2012b, 2013). However, these studies were small in scale and only monitored areas with known *L. areolatus* populations. While the previously mentioned studies have provided a baseline for understanding the habitat-use of *L. areolatus*, these studies have primarily focused on protected areas and been limited to a few populations at their northern and southern range limits. Landscape-scale studies are needed to thoroughly assess the status of *L. areolatus* across its range and identify important factors associated with populations outside of protected areas.

Occupancy analysis has emerged as a powerful tool for understanding distributions and habitat-use of many amphibian species across landscapes (Weir et al. 2005; Gould et al. 2019; Guzy et al. 2019), and Northwest Arkansas (NWA) offers a unique opportunity for completing a landscape scale assessment of *L. areolatus* occupancy. *Lithobates areolatus* have been documented throughout Arkansas, with most records within the Northwestern regions and Arkansas River Valley (AGFC 2015). Historically, Northwest Arkansas had extensive tallgrass prairie habitat (Transeau 1935), but prairies were among the first habitats to be converted for agriculture and little if any intact prairie habitat currently exists in the region. Currently, low-intensity agriculture (i.e., hayfields and cattle pastures) and rural habitats are being destroyed and replaced by urban development to accommodate growing city centers. The NWA region is one of the fastest growing areas within the state and the human population was expected to double in size between 2014 and 2020 (UScensus 2014). As the population continues to grow, natural and rural lands will be replaced by suburban and urban development leading to an increasingly patchy landscape. Northwest Arkansas is centrally located within the *L. areolatus* range, where

little work has been done to rigorously assess their status. Although, *L. areolatus* populations have been documented throughout the Northwest Arkansas region for over 20 years (e.g. Trauth et al. 2004), monitoring has not been consistent, standardized, or comprehensive across potential *L. areolatus* habitat.

We used an occupancy modeling framework to 1) determine landscape characteristics that are most important predictors of *L. areolatus* populations, 2) assess the status of current and historical populations, and 3) identify potential areas for habitat conservation to support population persistence. We had two primary hypothesizes: 1) *L. areolatus* occupancy would be negatively associated with urban land cover and positively associated with historic prairie and prairie mound density, and 2) historic records near urban centers would be locally extirpated. Documenting patterns of *L. areolatus* occupancy across Northwest Arkansas, new localities, and the loss of previously reported breeding populations provides baseline data for long-term monitoring, potential landscape factors that might influence occupancy, and helps identify potential conservation priorities for this species.

**Methods**

Occupancy Sampling

We completed five time-constrained auditory surveys at each of 81open-canopy ephemeral wetland sites spanning Benton and Washington counties in Northwest Arkansas (Figure 1). In 2016, we surveyed 60 sites three times and those same sites twice in 2017. In 2017, we added an additional 21 sites, which we surveyed 5 times. We completed surveys between 9 March and 18 April in 2016 and between 9 March and 6 May 2017. We began surveys immediately following the first heavy rain event in March and site visits were completed within six days of a rain event. Sites were centered on potentially suitable breeding wetlands and were

selected based on historical records (N=15), proximity to historical prairie habitat, and known *L. areolatus* habitat preferences (i.e., open canopy) using aerial imagery and remote-sensing data. After identifying potential breeding sites, we ground-truthed sites in February 2016 and 2017 to ensure remote-sensing data were accurate. All sites were located along or near roads, and the majority of sites were on private property. Sites included remnant and restored prairies, low- and high-intensity agriculture fields (hayfields, cattle pastures, and row crops), as well as urban areas (e.g., urban preserves, urbanized historic prairie).

We completed surveys following the North American Amphibian Monitoring Program (NAAMP; Weir and Mossman 2005) protocol. In short, we began surveys 30-minutes after sunset and listened at each site for 5-minutes. Longer listening periods for *L. areolatus* have been shown to increase detection (Williams et al. 2012b; 2013), however the large number of sites and geographic area monitored in our study combined with the brief breeding season made longer survey periods untenable. We recorded all species present and scored calling intensity on a 1 to 3 scale (1=individuals calling with little to no overlap, 2= calling overlaps, but individuals are still identifiable, 3= full chorus); intensity scores were collapsed to 0 (Non-detection) or 1 (Detection) for analysis. During each survey, we recorded temperature and determined the number of days since the last rain event. Both temperature and rain have been shown to be important for *L. areolatus* detection (Williams et al. 2012b).

Landscape Data Collection and Analysis

We measured a variety of variables at each of our sites using a GIS (ArcMap v.10.7.1 ESRI, Redlands, CA) with the Aerial Imagery base map, as well as a soil raster file (Arkansas GIS 2013) and historic prairie extent layer acquired from the Arkansas Natural Heritage Commission, and 2016 landcover data from the National Land Cover Database (NLCD 2016).

Within ArcGIS, we created a 1.2 km buffer surrounding each breeding wetland, representing the recommended buffer proposed by Heemeyer et al. (2012) for the conservation of *L. areolatus* populations, and counted the number of prairie mounds and wetlands within the buffer, based on aerial imagery. Prairie mounds, also known as pimple mounds or mima mounds, are small dome-shaped hillocks that can be found throughout native prairies of the southern mid-continent of North America (Melton 1954, Seifert et al. 2009). Due to an inability to gain access at many of our private property sites (N=73), we were unable to measure potentially important habitat variables, such as vegetation and crayfish burrow density. Instead, we used presence of prairie mounds, which are easily identified from aerial imagery, as an indication of both historic prairie habitat presence and lack of plowing or other intensive habitat alteration. We also tabulated the % urban land cover within the buffer at each site using the tabulate area tool within the Spatial Analyst ArcGIS extension. We did not include agricultural land cover due to an inability to differentiate intact or restored prairie habitats from pasture and the presence of high-intensity agriculture only at a few sites and at low proportions. Using the historic prairie extent layer, we determined the percentage of each buffer within historic prairie extent using the tabulate area tool. Finally, we tabulated % soil type within each site buffer. The soil layer contained over 70 soil types, which we lumped into 14 broader categories (e.g. silt loam, clay, silty clay, etc) based on Harper et al. (1969). To reduce the number of soil covariates, we ran a Principle Components Analysis (PCA) to identify patterns in soil composition across sites. We included soil PC1, soil PC2, and soil PC5 as covariates for model selection. We included soil PC1 and PC2, which explained 17.5% and 12.8% of the variation, respectively. Soil PC1 was characterized by a strong positive association with sandy and stony loam soils. Soil PC2 was characterized by a strong negative association with silt loam and strong positive association with chert loam and

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gravelly silt loam. We also included soil PC5 because it had strong positive associations with Silty Clay and Silty Clay Loam soils, which did not weigh heavily on other PC's, but we suspected clay soils might be important in mediating occupancy (Williams et al. 2013). The three soil PC's cumulatively explained 40% of the variation in soil types among sites.

Occupancy Analysis

We used a single-season occupancy model to estimate *L. areolatus* occupancy at wetland breeding sites, and to explore the influence of six site-specific covariates (prairie mound density, number of wetlands, % buffer within historic prairie, % urban land cover, soil PC1, soil PC2, soil PC5), while accounting for imperfect detection (MacKenzie et al. 2002). We included days since last rain, and temperature as sampling covariates, as well as year, due to some sites being sampled over two years. We constructed our models using the 'unmarked' package (Fiske and Chandler 2011) within R v. 4.0.0 (R core team 2020) and used an information-theoretic approach to model selection (Burnham and Anderson 2002). Prior to running all models, we checked that covariates were not strongly correlated (all $R^2 < 0.50$) and standardized each covariate using a *z* transformation. To determine *L. areolatus* occupancy at breeding wetlands, we used a two-step approach (similar to Peterman et al. 2013). First, we modeled combinations of our detection covariates (rain, temperature, and year), while holding site occupancy constant. All detection models that were used in selection included year as a covariate, except the null model (Table 1). We then modeled occupancy using the best supported parameterization for detection probability (Mackenzie et al. 2018). For occupancy probability estimation, we modeled all possible covariate combinations ($2^7 = 128$ possible models). We performed a goodness-of-fit test with parametric bootstrapping and calculated an overdispersion parameter (ĉ) based on our global model to adjust parameter standard errors and for model selection (Mackenzie and Bailey 2004).

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We used the MuMIn package (Bartoń 2020) to calculate Akaike's Information Criterion (AIC) and quasi-Akaike's Information Criterion (QAIC) values for model selection. QAIC adjusts for overdispersion by incorporating ĉ, and QAIC weights ($\leq$ 2) for model averaging (Hamer and Mahoney 2010). We also calculated relative covariate importance (w+), which is the sum of QAIC weights that included a given covariate (Burnham and Anderson 2002; Fuller et al. 2016). For our occupancy model selection table, we removed parameters with 85% confidence intervals that included zero (Arnold 2010). However, all covariates were included for model averaging and relative importance estimates.

## Results

We completed 405 auditory surveys between March 2016 and May 2017 and detected *L. areolatus* at 18 (22%) out of the 81 sites. All but two *L. areolatus* sites were located on private property. We failed to detect *L. areolatus* at 6 (37.5%) of 16 historic localities (Figure 1). These sites of apparent extirpation were primarily located near urban centers in Washington County (cities of Fayetteville, Siloam Springs, and Gentry) and in several cases urban and suburban development had unequivocally destroyed wetland and upland habitats needed by *L. areolatus* (Figure 1). We documented *L. areolatus* at 8 new localities. Extant *L. areolatus* populations were predominately located in three large sections of historic prairie in western Benton county, with only three sites in Washington county (Figure 1). We did not record any breeding *L. areolatus* at sites located in a large, but isolated, historic prairie region of central Benton county or in historic prairie regions of central and western Washington County (Figure 1).

In our occupancy analysis, the global model fit the *L. areolatus* data well, but the goodness-of-fit test suggested there was some overdispersion (ĉ=1.84), so we used QAIC weights for model selection and averaging. Our top detection model included days since rain,

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temperature, and year (Table 1). Relative importance values (w+) for days since rain were both one; temperature had a relative importance of 0.44. Naïve detection probability was 32.5% (0.06 SE). However, when days since rain, year, and temperature were included to inform detection our estimated detection probability was 97% (0.05 SE) under optimal conditions after 5 surveys. Detection probability was highest (>60%) during cooler temperatures (<12°C) and within 24 hrs of rainfall (Figure 2).

Occupancy model selection and relative importance results indicated that prairie mound density was the most important factor in predicting *L. areolatus* occupancy. Mound density, soil PC2, and soil PC5 were the only covariates that had 85% confidence intervals that did not overlap zero. The best supported model for site occupancy included only Mound Density (Table 2), however models that included soil covariates were also highly ranked (ΔQAIC < 2). Mound density had a relative importance close to 1 (0.98); all other covariates had a lower relative importance ranging between 0.28 and 0.37 (Table 2). Naïve occupancy of breeding wetlands included in our study was 26% (0.06 SE). Model averaged estimates of occupancy probability ranged from 0.02 (95% CI 0.00-0.08) when mound density was lowest to 0.99 (95% CI 0.99-1.00) at the highest mound density (Figure 3). The mean model averaged occupancy probability was 0.73 (95% CI 0.63-0.83). Once mound density reached 50 mounds per $km^2$, occupancy probability increased dramatically and plateaued near 100% probability when mound density was near 150 mounds per $km^2$ (Figure 3). Soil PC2 and soil PC5 were both positively associated with occupancy but had only minor effects on occupancy probability. Specifically, mean model-averaged occupancy probability increased from 0.35 (95% CI 0.07-0.63) to 0.40 (95% CI 0.00 – 0.89) with increasing soil PC2 scores and increased from 0.33 (95 % CI 0.02-0.65) to 0.42 (95% CI 0.00-0.88) with increasing soil PC5 scores. Thus, *L. areolatus* occupancy increased slightly

with the presence of chert and gravelly silt loam (soil PC2) and with increasing clay content (Soil PC5).

## Discussion

Our study represents the most comprehensive landscape-scale occupancy study of *L. areolatus* to date, focusing on an increasingly human-altered region at the core of its historic range: Northwest Arkansas.  Overall naïve occupancy was 26%, indicating that much of the apparently suitable habitat was unoccupied. While we confirmed the continued existence of 60% of historically-known breeding populations, we also documented 6 (38%) populations that had apparently been extirpated, primarily due to recent urban expansion in the region. *Lithobates areolatus* detection probability was high (>0.5) under ideal conditions (low temperature and recent rain), thus we expect that had *L. areolatus* been present at the historic sites, we would have detected them. Our results indicate that *L. areolatus* populations are more likely to be found in areas where habitat has remained relatively intact, as indicated by a high density of prairie mounds. The association with prairie mounds shows promise as an integrative proxy for habitat suitability that could be used to rapidly assess potentially suitable *L. areolatus* habitat from remote-sensing data. Weak, but positive associations with soil covariates hinted that soils may have been important in mediating occupancy historically, but these relationships may have been weakened by past and recent extirpations. *Lithoabates areolatus* is threatened throughout much of its range due to the loss and degradation of native prairie habitats; our results suggest that trend is no different in Northwest Arkansas.

The overall occupancy rate of *L. areolatus* was low across our study region, with fewer than 25% of sites having at least one detection. Low overall occupancy was driven in part by large patches of historic prairie in central Benton and western Washington counties (Figure 1).

These regions are not appreciably more degraded than other historic prairie areas in our study area. Thus, lack of historical or recent records suggests that *L. areolatus* may not have ever occurred in these areas. Reflecting the lack of intact prairie habitat in the region, *L. areolatus* was most commonly found in low-intensity agricultural areas, where there were large tracts of open canopy habitat with relatively low-intensity disturbance. In western Benton County, where most of our occupied sites occurred, land cover is primarily pasture and hayfield agriculture, with very little urban development. In contrast, five of the six extirpated populations were in areas with extensive recent urban development in both Benton and Washington counties, especially around the cities of Fayetteville and Siloam Springs. While some historical populations have likely been lost, our study documented several previously unrecorded breeding populations, suggesting that *L. areolatus* are still widespread throughout the region, but remain rare. Most of the remaining populations within the NWA region breed at wetlands on private property. Only two of the 18 sites with extant *L. areolatus* populations are protected, and even those are located within very small preserves that may not protect enough upland habitat for long-term population viability. At both protected sites, much of the calling activity occurred in privately-owned agricultural wetlands adjacent to the preserve (C. Kross, pers. obs.), suggesting that primary breeding wetlands may be located outside of preserves. The preservation of larger tracts of protected habitats is critical for the long-term persistence of *L. areolatus* within NWA.

Our top detection model was our global model, closely followed by a model only incorporating days since rain and year. Detection probability was highest (~70%) immediately following rain. Our results were similar to Williams et al. (2012b), the authors completed a two-year occupancy study on a wildlife refuge in southeastern Indiana and found *L. areolatus* detection probability increased when temperatures were >8°C and within 24 hours of a rain

event. In contrast, a follow-up to Williams et al (2012b) recorded calling activity over a two-month period at two known *L. areolatus* breeding wetlands and found that rain within 24 hours negatively affected *L. areolatus* detection (Williams et al. 2013). The reason for these contrasting effects of rain on detection might be because we performed short call surveys, which might be biased towards detecting calling individuals early during migration to their breeding wetlands (Williams et al. 2013). In our study, *Lithobates areolatus* were detected calling between 9 March and 13 March 2016 and between 26 March and 21 April 2017. Thus, we did not include a date parameter in our models, instead focusing on a year effect. The difference in timing and length of the calling window between years, driven by the timing of spring rains, likely had a significant effect on detection probability. Our results indicate that call surveys immediately following a rain event and early in the breeding season are optimal for locating occupied sites with the least amount of survey effort.

In accordance with our hypotheses, *L. areolatus* occupancy probability increased with density of prairie mounds. Prairie mounds are dome-shaped soil structures that were likely formed by the selective erosion of soil deposits in Arkansas (Quinn 1961, Durre et al. 2019), but whose overall origins are contentious (Howarth and Johnson 2006). Prairie mounds are indicative of historic prairie habitat that has not been degraded by intensive agriculture (i.e., unplowed; Howarth and Johnson 2006), thus serving as a proxy for multiple aspects of prairie habitat quality. Habitat quality is important for *L. areolatus* due to their unique habitat needs (Williams et al. 2012a, Heemeyer et al. 2012). Large and connected habitats support *L. areolatus* juvenile dispersal and adult migration to and from breeding wetlands (Heemeyer and Lannoo 2012, Lannoo et al. 2017), and open-canopy habitat with high crayfish burrow density are important micro-habitat features (Williams et al. 2012a; Heemeyer et al. 2012). Areas with the

highest mound density in our study were also the least modified, primarily low-intensity agriculture (i.e. pasture and hayfields) and protected and/or restored conservation properties. One of our protected areas with high mound density and an *L. areolatus* population, Woolsey Wet Prairie Sanctuary (WWPS), was also a recent restoration and currently supports a diverse amphibian and reptile community, as well as an abundance of burrowing crayfish (Baecher et al. 2018). Thus, presence of prairie mounds can serve as a useful integrative proxy for habitat quality that can easily be used to assess new potential Crawfish Frog locations using remotely-sensed data, as well as identify potential habitats worth protecting.

Clay soils have been proposed as a potentially important predictor of *L. areolatus* occupancy (Busby and Brecheisen 1997). Our results lend some support to this idea, as soil PC5 had the second highest relative importance value of the covariates examined in our study. We also found some evidence for an occupancy relationship with chert loam and gravelly silt loam soils, as reflected by the positive effect of soil PC2 on occupancy. Prairie mounds are most commonly made-up of a claypan or compacted gravel layer (Cox 1984, Howarth and Johnson 2006). Thus, our prairie mound covariate may have also been an indicator of these soil conditions. However, the effects of soil covariates were weak, with wide confidence intervals. The lack of strong associations between occupancy and our soil covariates may in part reflect sites with suitable soils where populations have been extirpated by anthropogenic factors, such as urbanization, or where populations never occurred historically due to natural barriers or isolation of prairie remnants that prevented colonization. We also might have missed a key soil type association when we binned soil types into broader categories. However, Kwiatkowski et al. (2017) completed a fine-scale analysis of crayfish burrow soils used by *L. areolatus* and observed that individuals might simply occupy whatever crayfish burrows are available, regardless of soil

composition. Regardless, a more thorough evaluation of soil conditions preferred by *L. areolatus* would prove a valuable contribution towards understanding occupancy patterns.

Contrary to our hypotheses, historic prairie extent and urban land cover were not important predictors of *L. areolatus* occupancy. Much of the NWA landscape, especially large sections of the historic prairie, has been altered for human use rendering that habitat unsuitable for *L. areolatus*. Our site selection criteria might also have weakened the ability to detect effects of land cover covariates; we focused on open canopy habitats that were in proximity to historic prairie and high-intensity agricultural land use (i.e. row cropping) was rare in the region. Additionally, our exclusion of some covariates due to an inability to differentiate between restored prairie habitat and low-intensity agriculture likely limited our ability to detect relationships with land cover. Finally, lack of an effect of urban land cover might have been driven by low overall occupancy probability and the presence of *L. areolatus* in a few protected areas near urban edges. Nonetheless, the loss of *L. areolatus* population in urban areas suggests increasing urbanization throughout the NWA region is a threat to populations.

*Lithobates areolatus* is an imperiled amphibian throughout its range. We documented 18 breeding locations spread across two counties in NWA and found that *L. areolatus* occupancy was near 100% at sites with high densities of prairie mounds. However, we also documented extirpation of several populations near urban centers. Our research revealed that presence of prairie mounds can serve as an easily-assessed proxy for habitat suitability for *L. areaolatus* and that detectability of this species is high shortly after early spring rains. Thus, we recommend inventory and monitoring initiatives that employ auditory surveys following early spring rain events and that use prairie mound density to identify sites with a high potential for *L. areolatus* occupancy. We also recommend future research that includes collection of on-the-ground

covariates, such as fine-scale vegetation, soil, and crayfish burrow measurements, to fully understand the factors that are most strongly related to *L. areolatus* occupancy (Cruickshank et al. 2020). Finally, to ensure long-term persistence of *L. areolatus* populations within NWA, continued monitoring will be needed. Given that most populations currently occur on private property, establishing strong and mutually beneficial relationships with landowners is a critical aspect of *L. areolatus* conservation. Moreover, the species would greatly benefit from larger protected areas, centered on current metapopulations in western Benton county. Active habitat restoration has been shown to preserve and enhance *L. areolatus* populations (Lannoo et al. 2009, Baecher et al. 2018) and head-starting programs have been successful in Indiana (Stiles et al. 2016). With low overall occupancy indicating ample availability of apparently suitable habitat, restoration and reintroduction could help to secure *L. areolatus* population persistence within the NWA region.

### Acknowledgements

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**Table 1.** Model selection based on QAIC for covariates influencing *Lithobates areolatus* detection probability ($p_i$) at 81 sites across historic prairie regions of Northwest Arkansas, USA, 2016-2017. With the exception of the null model, all models included an effect of year on detection.

| Model | K | QAIC | ΔQAIC | QAIC wt | Cum. Wt | Quasi-LL |
|---|---|---|---|---|---|---|
| ψ(.) p(Rain + Temp + Year) | 6 | 99.03 | 0 | 0.54 | 0.55 | -43.52 |
| ψ(.) p(Rain + Year) | 5 | 99.4 | 0.45 | 0.45 | 0.99 | -44.7 |
| ψ(.) p(Year) | 4 | 108.1 | 9.09 | 0.01 | 1 | -50.06 |
| ψ(.) p(Temp + Year) | 5 | 109 | 10.01 | 0 | 1 | -49.52 |
| ψ(.) p(.) | 3 | 111.7 | 12.67 | 0 | 1 | -52.85 |

**Table 2.** Model selection based on QAIC for covariates influencing *Lithobates areolatus* occupancy probability ($\psi_i$) at 81 sites across historic prairie regions of Northwest Arkansas, USA, 2016-2017. The top-ranked models with a ΔQAIC ≤ 2 are listed, after removing covariates with 85% CI that included 0. The covariate set for detection was held constant and included days since rain, temperature, and year.

| Model | K | QAIC | ΔQAIC | QAIC wt | Cum Wt | Quasi-LL |
|---|---|---|---|---|---|---|
| ψ(mound density) | 7 | 92.67 | 0.00 | 0.47 | 0.47 | -39.33 |
| ψ(mound density + soilPC5) | 8 | 94.16 | 1.50 | 0.22 | 0.69 | -39.08 |
| ψ(mound density + soilPC2) | 8 | 94.50 | 1.83 | 0.19 | 0.88 | -39.25 |

**Table 3.** Cumulative QAIC weight of models that included each occupancy covariate. Higher weights indicate higher relative covariate importance (w+). Each covariate was listed in an equal number of candidate models (n=64 out of 128 total models).

| Site Covariate | w+ |
|---|---|
| Mound Density | 0.98 |
| Soil PC5 | 0.37 |
| Histoic Prairie | 0.36 |
| Urban Landuse | 0.35 |
| Soil PC2 | 0.33 |
| Soil PC1 | 0.31 |
| Number of Wetlands | 0.28 |
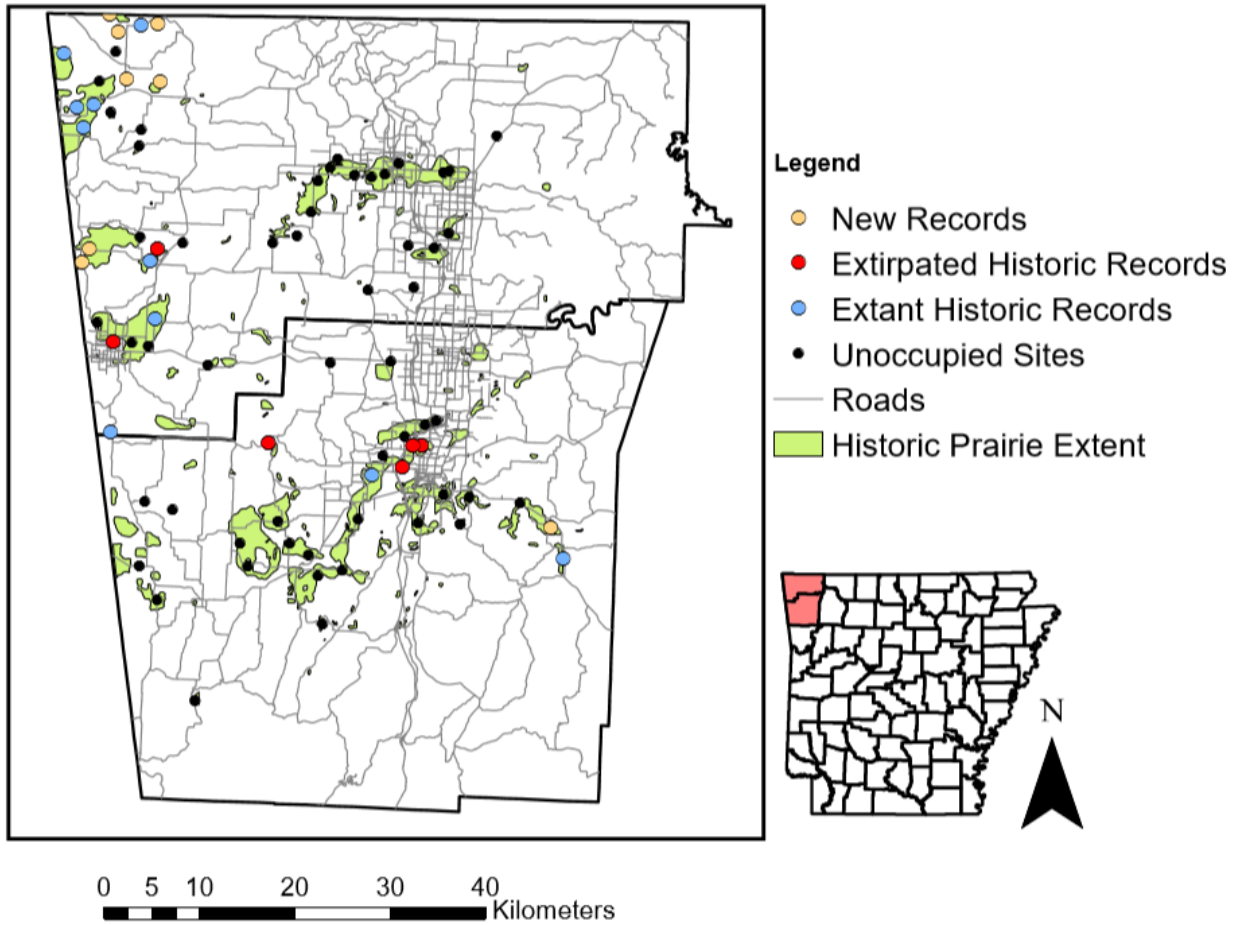
**Figure 1.** Sites (n=81) surveyed for *Lithobates areolatus* (Crawfish Frog) in Northwest Arkansas during the spring 2016 and 2017 breeding seasons. Sites were located within the Benton and Washington counties (shaded area of state inset). Historic and current occupancy records are indicated and in relation to historic prairie extent. Roads are included to portray urbanization.
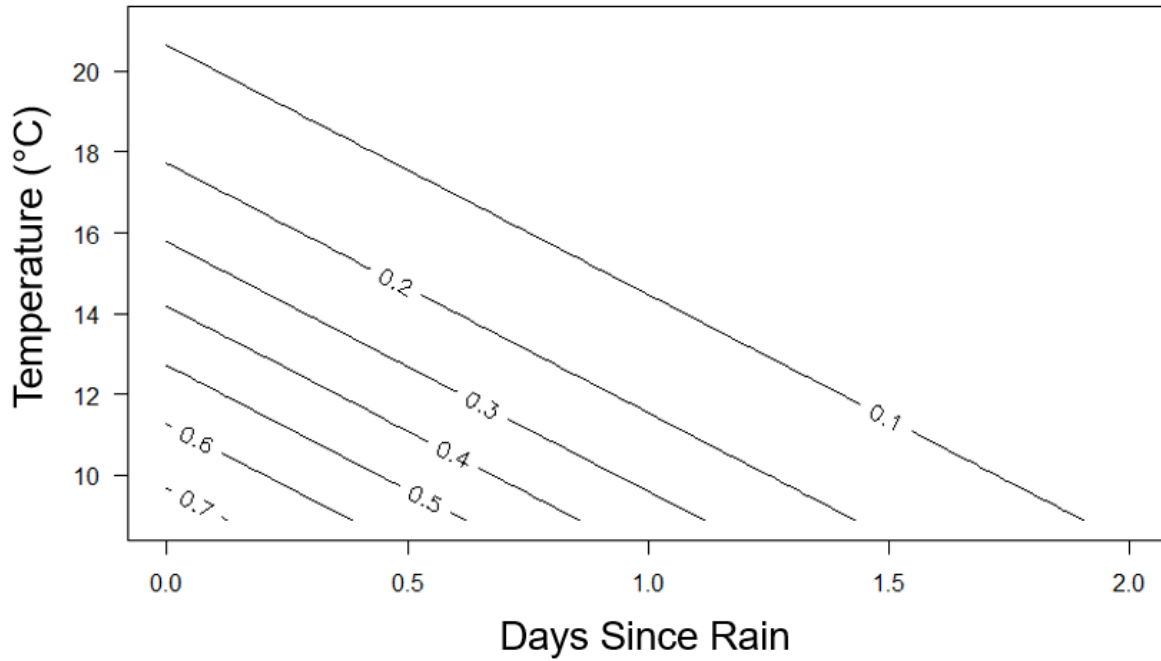
**Figure 2**. Mean detection probability of *Lithobates areolatus* (Crawfish Frog), showing a joint effect of temperature and days since rain.
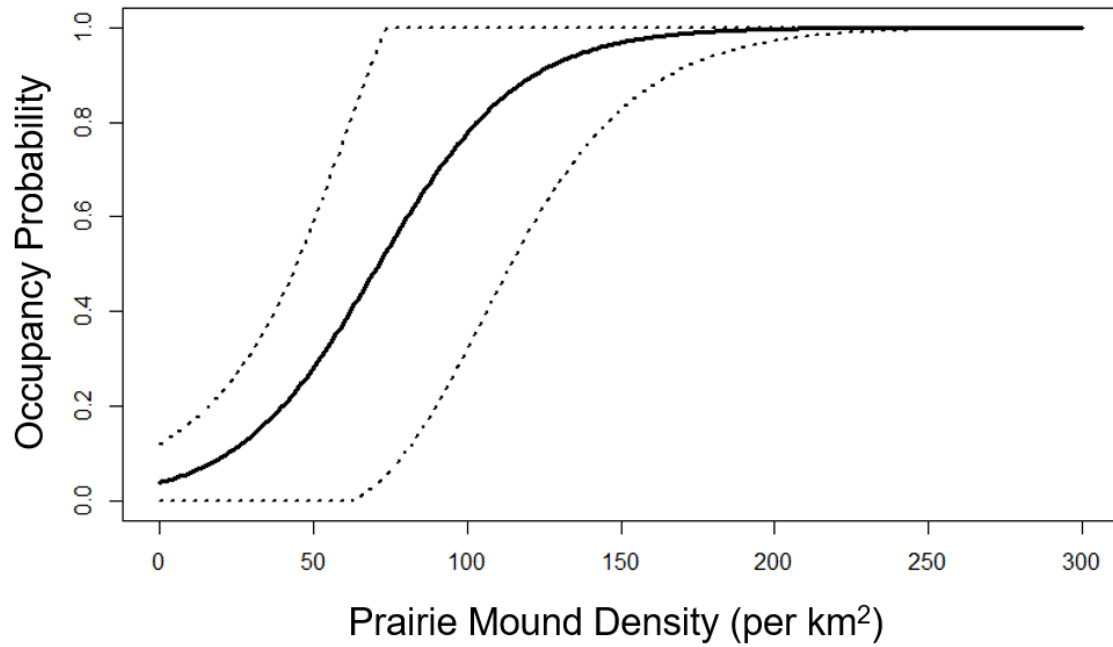
**Figure 3.** Model-averaged predictions (based on QAIC weights) of *Lithobates areolatus* (Crawfish Frog) occupancy probability based on prairie mound density within 1.2 km of a breeding wetland.

# Chapter 2

Timing of oviposition influences the effects of a non-native grass on amphibian development

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and

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**Abstract**

Land-use change can alter the energy dynamics in aquatic systems by changing the subsidies that form the nutrient base within them. However, experimental evaluations of subsidy change often fail to consider how effects, such as differences in individual growth and survival, may differ under varying ecological contexts experienced in the field. We used a mesocosm approach to investigate how litter (Native Prairie or Non-Native Tall-Fescue Grass) surrounding wetlands and timing of oviposition affected larval amphibian development. We found that survival differed between litter types in the Early-Oviposition treatment, with nearly 100% mortality in Fescue treatments. Conversely, survival rates were similar across litter types in the Late-Oviposition treatment (~43%), and larvae in Late-Fescue treatments metamorphosed more quickly and were larger post-metamorphosis than larvae in Prairie treatments. Follow-up experiments confirmed that low dissolved oxygen (DO) was responsible for high mortality in Early-Fescue treatments; high quantities of Fescue resulted in a microbial bloom that reduced DO to < 2 ppm for several days, resulting in low hatching success. This effect was eliminated in treatments with supplemental aeration. Finally, we confirmed that experimentally observed DO patterns also occurred in the field. Context (i.e., timing of inundation relative to amphibian breeding) is critical to understanding the effects of subsidies on amphibian populations; early and explosively breeding species may experience catastrophic mortality due to DO depletion, whereas species that breed later may experience enhanced fitness of recruits. Considering the effects of non-native species across different ecological contexts is necessary for elucidating the extent of their impacts.

**Introduction**

Land-use change can have profound effects on ecosystem function, either through direct physical impacts (e.g., habitat loss) or indirect consequences (e.g., climate change; Foster et al. 2003). Land-use can alter spatial subsidies, such as litter inputs, which often form the base of nutrients available for uptake and assimilation by consumers and detritivores in aquatic ecosystems (Stream Solute Workshop 1990, Polis et al. 1997, Pace et al. 2004, Leroux and Loreau 2008). Specifically, the quality of vegetation entering the recipient ecosystem (Rubbo and Kiesecker 2004, Maerz et al. 2010, Stephens et al. 2013), either through changes in labile properties or nutrient composition, can have strong cascading effects on food webs (Carpenter et al. 1985, 2001).

Vegetation entering the aquatic system from surrounding terrestrial habitat is the dominant type of spatial subsidy in temporary wetlands and can strongly influence microbial growth and algal production (Cohen et al. 2012, Earl et al. 2014). Oak leaves have been shown to support a less dense matrix of microbes compared to maple leaves in tree-hole ecosystems (Fish and Carpenter 1982). Microbial activity is the primary mechanism associated with litter (i.e., vegetation) conditioning, and litter type and quality can affect the rate of conditioning. For example, microbial production on oak leaves is low at the beginning of conditioning but increases 6-fold over time; in contrast, microbial production on maple leaves is 3-times higher at the beginning of conditioning, but remains relatively constant over time (Rubbo and Kiesecker 2004). The control litter quality exerts over microbial conditioning has potential ramifications throughout the food web, as microbial carbon is a critical source of nutrition for meiofauna and macroinvertebrates (Meyer 1994), representing a significant contribution toward aquatic secondary production (Hall et al. 2000). Reduced conditioning results in lowered food quality for

invertebrates which, in turn, negatively affects detritivore growth, survival, and production (Rubbo et al. 2006, Maerz et al. 2010). Overgrowth of microbial communities can result in hypoxic conditions (Mallin et al. 2006); thus, heterotrophic microbes are key players, albeit indirectly, in the population dynamics of aquatic vertebrate consumers.

Amphibians are often used as model organisms for testing the effects of spatial subsidies on population and community dynamics (Rubbo and Kiesecker 2004, Williams et al. 2011, Cohen et al. 2012) and litter quality has been shown to exert strong bottom-up effects on amphibian production (Maerz et al. 2005, Earl and Semlitsch 2013). Litter can affect amphibians through a variety of mechanisms including algal production, microbial production, tannin production, pH, and dissolved oxygen (DO). Additionally, human-induced changes in litter quality and composition, often resulting from the introduction of invasive plants and loss of native species, have been shown to strongly affect amphibian fitness (Stephens et al. 2013). For example, tannins produced from Eurasian Purple Loosestrife (*Lythrum salicaria*) reduced larval survival by 50% in a bufonid species (Maerz et al. 2005). Extracts from invasive Arum Honeysuckle (*Lonicera maackii*) also reduced larval amphibian survival up to 30% compared to native and control treatments (Watling et al. 2011a). Although several studies have evaluated the effects of non-native subsidies on amphibian larval development, these studies are usually conducted using a limited number of experimental treatments under controlled lab or mesocosm conditions, which avoid much of the variability in environmental conditions present in natural systems. Thus, these studies have limited ability to detect how consistent effects are across a variety of plausible ecological contexts, such as how litter effects might be influenced by the timing of wetland inundation and amphibian breeding. Additionally, most studies on the effects of litter subsidies on amphibian development have focused on vernal pool systems in forests;

comparatively little research on the role of subsidies has been conducted in prairie grassland ecosystems, despite their critical conservation status (Samson et al. 2004, Henwood 2010).

North America has lost more than 95% of its native grassland habitat (Samson and Knopf 1994, Comer et al. 2018), due largely to conversion of prairies to agricultural fields. One such crop is Tall-grass Fescue *(Lolium arundinaceum* [Schreb]), with over 86.5 million hectares grown across the United States for feeding livestock and as turf grass (Sleper and West 1996, Cherney 2007). Compared to prairie wetlands, fescue-dominated wetlands might provide a very different habitat for developing larval amphibians. While many studies have shown invasive and non-native vegetation negatively affect tadpole development (Rubbo and Kiesecker 2004, Maerz et al. 2005, Williams et al. 2011), other studies have found that non-native species can have positive effects on larval development (Watling et al. 2011b, Rogalski and Skelly 2012). Additionally, Cohen et al. (2012) suggest that vegetation quality (i.e. nutrient composition) and quantity might play a more important role in amphibian development than the specific vegetation species. Although, agricultural ponds have been shown to support amphibian populations (Knutson et al. 2004), as one of the most widely distributed grasses in the world (Miller 2003), the effects of Fescue grass on animal communities has remained understudied. Thus, determining whether Fescue has positive or negative effects on the survival and development of larvae under varying conditions (e.g., quantities, and environmental conditions) would provide important information for understanding declines in amphibian species, especially in prairie habitats.

The objective of this study was to evaluate the effects of non-native Fescue grass on the hatching success, larval development, and survival of prairie amphibians. Specifically, we used a series of mesocosm experiments and field studies to address the following questions: (1) What are the long-term effects of litter type on the survival and development of an imperiled frog? (2)

How important is litter type and quantity, and its effects on Dissolved Oxygen, in amphibian hatching success? (3) Does litter quality drive observed effects on amphibian development? (4) Do effects observed in mesocosms also occur in the field? We hypothesized that non-native Tall-grass Fescue would increase survival and growth of amphibians due to its expected labile (i.e. more readily broken down) properties.

## Methods

*Long-term effects of litter type on survival and development of an imperiled frog*

We used large mesocosms located at the University of Arkansas Uptown Campus, Fayetteville, AR, to test the effects of two litter treatments (non-native Fescue grass and mixed native Prairie vegetation) on the long-term development and survival of Crawfish Frog (*Lithobates areolatus*) larvae across two years. Native Prairie vegetation contained a mixture of sedges (*Carex* spp., *Pychnanthemum* spp., and *Eleocharis* spp.) and forbs (*Boltonia asteroids, B. diffusa,* and *Periscaria* spp.) collected from seasonally flooded areas within a restored prairie (Woolsey Wet Prairie Preserve [WWPS], Fayetteville, Arkansas; a known Crawfish Frog breeding site), and thus represents typical litter input for ephemeral wetlands in prairies. Fescue litter was collected from seasonally flooded wetlands within a hayfield adjacent to WWPS. We collected both litter types in late February and dried the litter for at least two weeks prior to experiments. The Crawfish Frog was chosen as a study species because it is currently experiencing precipitous declines throughout its range (Parris and Redmer 2005). The IUCN (Hammerson and Paris 2004) has listed the Crawfish Frog as near threatened; the species is listed as state endangered in Iowa and Illinois, and is a species of greatest conservation need in Arkansas, Oklahoma, Missouri, Kansas, Kentucky, Mississippi, and Louisiana. Additionally, Crawfish Frogs breed in both prairie and agricultural wetlands (Baecher et al. 2018). They are

often one of the first Ranid frogs to begin breeding in the spring and will move into a wetland following the first warm rains that inundate depressional wetlands, which in Arkansas usually occurs in early March (Trauth et al. 2004).

In 2015, we constructed an array of 12, 1,325 L polyethylene cattle tanks with six replicates of the two litter treatments and added Crawfish Frog larvae 7-days post-litter addition. In 2016, we constructed an array of 24 tanks with 12 replicates of each litter type. Based on the previous year's results (see below), we added Crawfish Frog larvae 22-days post-litter addition in 2016, resulting in two 'timing of larval introduction' treatments that were confounded with year (Early [2015] and Late [2016] Treatments). We filled tanks with 1000 L of tap water and allowed water to stand for five days to allow chlorine to dissipate. We added 1 kg (1g/L) of dried litter to each tank on 25-Mar-2015 and 28-Feb-2016. We selected 1 kg based on previously published experiments (Stoler and Relyea 2011), as well as field measurements of standing litter biomass (Kross et al. unpubl. data) from our litter collection location, which is a known Crawfish Frog breeding site. We inoculated each mesocosm with 0.5 L of concentrated zooplankton collected from Crawfish Frog breeding ponds by straining pond water through 150 μm mesh to create a concentration of zooplankton. Finally, we covered mesocosms with black 30% shade cloth (PAK unlimited, Inc. Conelia, GA) to prevent colonization by potential competitors (e.g., Gray Treefrog [*Hyla versicolor*]) and predators (e.g., Dragonfly nymphs).

We collected Crawfish Frog eggs at WWPS in March 2015 and 2016. Due to the conservation status of the Crawfish Frog, only two egg masses were collected in 2015 and one egg mass was collected in 2016. We hatched eggs in the lab. After hatching, we separated larvae (Gosner stage [GS] 25-26; Gosner 1960) haphazardly into groups of 40 and added them to each mesocosm on 31-Mar-2015 and 22-Mar-2016.

For both the Early and Late Treatment experiments, we measured Dissolved Oxygen (DO) using a YSI 556 MPS handheld meter (YSI Inc. Yellow Springs, OH). In 2015 (Early Treatment), we measured DO haphazardly 5, 8, 56, 71, and 77-days post-litter addition; in 2016 (Late Treatment), we measured DO between 0730 and 0930 h, regularly, but not daily, for two weeks, and then measured DO prior to tadpole introduction on day 22.

We checked on larvae every other day and removed larvae from mesocosms at emergence of the first forelimb (GS 42) and held them in the laboratory until full tail resorption occurred (GS 46). At stage 46, we measured snout-vent-length (SVL) to the nearest millimeter and mass to the nearest 0.01g (Mettler ML1502E balance; Mettler-Toledo International, Columbus, OH, USA). We also recorded the date of full tail resorption to determine time to metamorphosis (TTM). For survival, we calculated the proportion of individuals that survived through metamorphosis from each tank, and then calculated an average for each treatment. We used a one-way ANOVA with Timing/Year (i.e. Early vs. Late) as a block to compare their effect on survival. A Tukey's test for non-additivity was used to determine whether there was an interaction between Litter Type and Timing/Year. We analyzed these factors using an ANOVA with a Block due to the confounding of year with the Timing treatment. For body size (SVL and mass) and TTM, we used one-way ANOVAs followed by Tukey's tests for independent contrasts. We performed all data analyses using Program R (3.5.2; R development Core Team, 2018).

*Effects of litter type and quantity on amphibian hatching success*

Due to the confounding of year with the timing of litter addition, we conducted the following experiment to directly address the relationship between timing of litter addition, DO,

and amphibian survival. We conducted a mesocosm experiment, consisting of 48, 18.92 L

mesocosms (plastic buckets) with six replicates of eight treatments at the University of Arkansas

Uptown Campus in Fayetteville, AR, in spring 2017. Prairies and agricultural fields are managed

using a variety of mechanical techniques, such as mowing and fire, which can lower vegetation

quantity within the wetland area. Conversely, Fescue can achieve high biomass (500g –

1000g/m$^2$) in a small area (Kross et al. unpubl. data). Thus, to capture a range of plausible

scenarios of wetland provisioning, we used a 2 x 3 factorial combination of the two vegetation

type treatments (Prairie and Fescue) and three litter quantities: Low (0.5g/l), Moderate (1g/l),

High (2g/l). Additionally, we included two treatments with High Litter Quantity plus

supplemental aeration as a control for the low DO concentrations expected in High Quantity

treatments. We aerated mesocosms using standard aquarium air pumps and air stones. We

collected and dried litter as described in the previous experiment.

On 5-Apr-2017, we filled mesocosms with 13.25 L of tap water, allowed water to sit for

48 h for chlorine to evaporate, and then inoculated each with 120 ml of pond water that had been

strained through 150 μm mesh to concentrate zooplankton. On 8-Apr-2017, we collected three

Leopard Frog (*L. sphenocephalus*) egg masses from local ponds, and mixed and haphazardly

separated them into batches of 20 eggs. We checked all eggs to ensure they were successfully

fertilized. Within mesocosms, we placed eggs within a small sub-container (10 cm diameter PVC

tubing with mesh glued to the bottom and polyethylene foam glued to the top as a float) to

facilitate observations of hatching success and larval survival. We placed litter directly into the

mesocosms simultaneously with egg addition on 8 April to mimic wetland filling co-occurring

with breeding following a spring rain event.

We measured DO and water temperature in mesocosms each morning between 0530 h and 0730 h using a YSI 556 MPS handheld meter (YSI Inc. Yellow Springs, OH). We counted the number of larvae that successfully hatched 7-days post addition. By day 7, all unhatched eggs had become cloudy and embryos had become white, indicating mortality. We compared DO concentrations among litter types and quantities 2-days post addition using a two-way ANOVA. To determine if hatching success differed significantly between litter type and mass treatments, we performed a two-way ANOVA. Data did not meet assumptions of normality and homoscedasticity, but attempts to transform data did not alleviate this problem. More conservative non-parametric approaches yielded identical treatment differences, but still suffered from assumption violations. Thus, we interpreted ANOVA results conservatively and focused on differences between treatments that were the strongest.

*Litter quality and microbial activity*

Litter quality is an important driver of microbial activity and vertebrate production; thus, we determined initial quality for both litter types. We collected six subsamples of litter reserved from litter used in the hatching success experiment. We finely ground each subsample using a Wig-L-Bug ball mill (International Crystal Laboratories, Garfield, NJ) and combusted each at 550°C for two hours. We determined phosphorus (P) content by submitting ashed samples to a hydrochloric acid digest at 85°C before utilizing the ascorbic acid method to determine P (APHA 2005, Rosemond et al. 1993). We determined carbon (C) and nitrogen (N) content in samples with an Isotope Ratio Mass Spectrometer (Thermo Scientific, Waltham, MA).  We compared nutrient data between litter types using t-tests.

As microbial activity is an important driver of DO, we measured microbial respiration using 12, 1 L mesocosms with air tight lids. We filled each mesocosm with 1 L of dechlorinated tap water and recorded DO. We then added 1 g of Fescue litter to each of six mesocosms and 1 g of Prairie litter to the remaining six mesocosms. We inoculated each mesocosm with 1 mL of pond water to introduce microbes. We capped all bottles and placed them in an environmental chamber that cycled from 24°C for 12 h to 5°C for 12 h to mimic environmental temperatures in April. We measured DO at 24, 48, and 72 h to quantify net oxygen consumption by microbes. We report respiration values in $mgO_2 \cdot gAFDM^{-1} \cdot hr^{-1}$ (Entrekin et al. 2008, Fuell et al. 2013). We compared microbial respiration between litter treatments using a t-test.

*Field observations on the effect of litter type on dissolved oxygen in seasonal wetlands*

During the spring of 2017, we collected DO measurements in the field from five Fescue-dominated and five Prairie-dominated seasonally inundated wetlands. All wetlands are part of WWPS, a 48 ha prairie restoration and wetland mitigation site owned by the city of Fayetteville, AR. The Prairie wetlands were located in a restored section of the property, while the Fescue wetlands were located in hay fields adjacent to the restored section (see Baecher et al. [2018] for more site information). Wetlands were dry over the winter and filled on 27-Mar-2017, following the accumulation of 8.48 cm of rain over the previous four days (Drake Field Weather Station, Fayetteville, AR). Wetland areas varied from 0.1 to 0.21 ha and were less than 1 m in depth. We measured DO between 0730 h and 0930 h daily from 30-Mar to 02-Apr-2017 using a YSI 556 MPS handheld meter (YSI Inc. Yellow Springs, OH). During this time, Crawfish Frogs and other early spring breeding amphibians were actively breeding at some of the sampled wetlands and across Northwest Arkansas (Kross and Willson unpubl. data). We measured DO along the

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shallow edge of each wetland, in water deep enough to completely submerge the probe. We compared the 01 April 2017 DO concentrations between Fescue and Prairie wetlands using a t-test.

## Results

*Long-term effects of litter type on survival and development of an imperiled frog*

In the Early Treatment (2015) experiment, average DO concentration was less than 1 g/mL in both treatments when larvae were added to tanks, but lower in the Fescue treatment (Fig. 1A). DO concentration averaged above 3 mg/L towards the third month of the experiment in the Early Treatment and was similar between litter types. In the Late Treatment (2016) experiment, DO concentration decreased sharply after the introduction of Fescue and remained below an average of 3 mg/L for 14 days (Fig. 1B). Dissolved oxygen concentrations in the Prairie treatment decreased gradually and only averaged below 3 mg/L on day 8 post-litter introduction (Fig. 1B). By day 22, DO concentrations had risen, and were similar across both litter types (average Fescue DO = 6.3 mg/L and average Prairie DO = 5.9 mg/L; Fig. 1B).

Litter type and Timing/Year interacted to significantly affect larval survival ($F_{1,33}$=9.36, p <0.01; Fig. 2A). Only one individual larva completed metamorphosis from the Early Fescue Treatment. Larval survival was similar (~ 43%) across Early and Late Prairie, and Late Fescue Treatments. Metamorphs from the Late Fescue Treatment averaged 6 mm longer in SVL ($F_{2,26}$=16.74, p<0.001) and almost twice the mass ($F_{2,26}$=17.26, p<0.001) of metamorphs from the Early and Late Prairie Treatments (Fig. 2B, 2C). Additionally, larvae reached metamorphosis 5 days earlier, on average, in the Late Fescue Treatment compared to the Early and Late Prairie treatments ($F_{2,26}$=23.39, p<0.001; Fig. 2D).

*Effects of litter type and biomass on amphibian hatching success*

Our follow-up experiment on hatching success confirmed that low DO early in development was the driver of low survival observed in the Early Fescue Treatment. Overall, DO concentrations were lower across all Fescue Quantity Treatments and decreased as Fescue Quantity increased (Fig. 3). Litter type and quantity interacted significantly to affect DO two days after litter addition ($F_{3,40}$=37.95, p<0.001). Prior to litter addition, DO concentrations were similar and above 4 mg/L across all treatments. Twenty-four hours following litter addition, DO of the High Fescue Treatment averaged 0.27 mg/L. In contrast, the High Prairie Treatment averaged 1.47 mg/L. The Moderate Fescue Treatment averaged 0.96 mg/L, while the Moderate Prairie Treatment averaged 4.84 mg/L. Low Fescue and Low Prairie treatments averaged 3.95 and 6.22 mg/L, respectively. For all Low and Moderate treatments, DO concentrations remained relatively stable 24-hours post-addition and began to increase 6-days post-addition. For the High (non-aerated) Treatments, DO concentration remained low, but consistent, during the entirety of the experiment. DO concentrations remained high (> 6 mg/L) in the High Aerated Treatments throughout the experiment.

Litter type and quantity interacted to affect Leopard Frog hatching success ($F_{3,40}$= 13.882, p< 0.001). Overall hatching success was highest across Prairie Treatments, compared to Fescue Treatments, and decreased as quantity increased (Fig. 4). Zero eggs hatched in the High Fescue Treatment. In the Moderate Fescue Treatment, an average of 35% of eggs hatched. In contrast, the High and Moderate Prairie Treatments had an average hatching success of 53% and 95%, respectively. Hatching success was similar between the Low Prairie (96%) and Fescue (90%) Treatments. High Aerated Fescue and High Aerated Prairie Treatments had an average hatching success of 93%.

*Vegetation quality and microbial activity*

Both N and P comprised roughly twice as much of initial dry biomass in Fescue than Prairie litter (N: p<0.001; P: p=0.007; Table 1). Carbon accounted for significantly greater biomass in Prairie (45%) compared to Fescue (42%) litter (p<0.001; Table 1). Microbial activity was nearly three times higher in the Fescue Treatment compared to the Prairie treatment after 72 hours (p<0.001; Fig. 5).

*Field observations on the effect of litter type on dissolved oxygen*

Following a heavy rain event in late March 2017, average DO concentration in Fescue Wetlands was lower than that of Prairie Wetlands ($F_{1,8}$= 10.38, p= 0.01) and followed a similar pattern of decrease observed in mesocosms (Fig. 6). In Fescue Wetlands, DO decreased by 2 mg/L over the sampling period and averaged 2.05 mg/L 72-hours post-wetland filling. In Prairie Wetlands, DO decreased by 3 mg/L and averaged 4.5 mg/L 72-hours post-wetland filling.

**Discussion**

Changes in vegetation community composition and the alteration of subsidies forming the base of ephemeral aquatic systems can have mixed effects on vertebrates, making it difficult to determine how land-use change might affect wildlife populations. Our experiments demonstrate that non-native Fescue vegetation can have either positive or negative effects on anuran hatching success and larval development, depending on when wetlands are filled and breeding occurs. When eggs and larvae were reared in adequately oxygenated Fescue Treatments, eggs hatched successfully (Low Quantity and Aeration Treatments) and larvae metamorphosed 7-days earlier and had twice the mass than larvae reared in Prairie Treatments (Late Oviposition Treatment). In contrast when eggs and larvae were introduced to mesocosms at

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the same time as litter, DO concentrations crashed and resulted in fewer or no eggs hatched (Moderate and High Quantity treatments) and larvae did not survive to metamorphosis (Early Fescue Treatment). Low DO conditions were driven by the higher nutrient concentrations found in Fescue Treatments, which increased microbial activity. The pattern of the short-term DO depletion in Fescue was observed in wetlands in the field. Collectively, our data suggest that Fescue could have positive effects on the development of some amphibians, but could cause mass mortality for species that exhibit explosive breeding or early-spring breeding coincident with wetland inundation.

The results of the Late litter type and timing experiment and litter quality comparison generally supported our hypothesis that Fescue would result in increased growth of developing larvae. Larvae in the Late Fescue Treatment reached metamorphosis 7-days faster than individuals from the Late Prairie Treatments, but had similar survival. Metamorphs in the Late Fescue Treatment were nearly twice as large as metamorphs from the Early and Late Prairie Treatments. In the microbial activity experiment, we observed higher microbial respiration rates in the Fescue Treatment, which suggests that Fescue is more labile than Prairie grass (Wilcox et al. 2005). Metamorph size differences were likely due to the higher N and P content found in Fescue litter, which makes it more labile for conditioning. Labile litter is more readily broken down and used by microbes and algae, which can then transfer those nutrients to higher order consumers (Webster and Benfield 1995, Hall and Meyer 1998). However, these same attributes that benefit development might also reduce survival under certain conditions.

In the Early litter type and timing experiment, we observed near complete mortality of Crawfish Frog larvae reared in Fescue, which was unexpected. We also observed that DO was below 1 mg/L after larvae were added, which likely caused the mortality. In the hatching success

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experiment, we found that Fescue in Moderate and High Quantities caused a DO crash that lasted for multiple days, resulting in reduced hatching success or total mortality in those treatments. However, mortality was completely ameliorated by adding supplemental aeration. These results are similar to a study that found high quantities (2 and 4 g/L) of Chinese Tallow (*Triadica sebifera*), an invasive species, reduced DO to below 2 mg/L and resulted in complete mortality of Leopard Frog eggs (Adams and Saenz 2012).

Litter quality (i.e. Nitrogen [N], Phosphorus [P], Carbon [C] content) is an important factor associated with amphibian production (Cohen et al. 2012). Stephens et al. (2013) observed that amphibians reared in mesocosms with litter that had higher percent N were larger (i.e. increased SVL and mass) than those reared in litter with low percent N. Others have found that litter quality influences microbial activity, and phosphorus content can exert strong controls on the early stages of decomposition (Aerts and de Caluwe 1997). While we did not manipulate nutrient levels to determine the specific nutrient controlling microbial activity, our results do indicate that higher litter quality may influence microbial oxygen consumption. Heterotrophic microbes are key players in the breakdown of decomposing organic matter such as leaves and grasses (Battle and Golladay 2001). Their primary role is in mineralization and conditioning, which aids in the breakdown of recalcitrant compounds and creates a more suitable, nutritious substrate for higher trophic levels. Given the higher nutrient content of Fescue, areas dominated by Fescue may be more susceptible to DO crashes, as high-quality food supports greater microbial production and, thus, respiration. We conclude that high N content and the labile properties of Fescue results in a microbial bloom that depletes DO and can cause mass mortality of eggs and early-stage amphibian larvae. The particular attributes of Fescue (i.e., labile, high

nutrient composition) that are beneficial for larval development can also drastically reduce egg and larval survival by inducing a DO crash.

While many amphibian studies record DO, most only report that DO varied across experimental treatments (e.g., Maerz et al. 2010; Earl and Semlitsch 2013; Stephens et al. 2013). As a result, the effects of litter inputs on DO and larval anuran survival have not received much attention (but see Adams and Saenz 2012). This is largely due to the fact that most larvae of most anuran species have lungs. However, lungs are not fully functional immediately after hatching or during early larval stages for many species (Burggren and West 1982). Although the effect of decreased access to oxygen on larval amphibian respiration has been examined (Feder 1983a, 1983b), few studies have reviewed the effect of low DO on anuran hatchling and early larval (GS 26-27) survival. Additionally, the duration of low DO conditions may influence hatching success and larval survival. In our experiment, low DO (< 2.0 mg/L) persisted for up to 7 days and at levels below 4.0 mg/L for up to 14 days. Additionally, we observed low DO in recently inundated Fescue-dominated wetlands in the field. A prolonged effect of low DO immediately following inundation might disproportionately affect amphibians that breed explosively or early in the spring.

Amphibian populations are declining due to anthropogenic habitat changes; our field and experimental results highlight yet another pathway that could contribute to population declines. Low DO concentrations in wetlands have been shown to have negative effects on amphibian egg and larval survival (Bradford 1983, Sacerdote and King 2009). We were able to confirm that the DO reductions we observed in our experiments also occur in nature. Thus, there is an increased risk of reproductive failure for the many amphibian species that breed concurrently with wetland filling in grassland habitats, such as the Crawfish Frog, Gopher Frog (*L. capito*), Spadefoot

Toads (*Spea spp.* and *Scaphiopus spp.*), and the Small-mouthed Salamander (*Ambystoma texanum*). Salamander species may be particularly sensitive to the low DO conditions we observed in Fescue wetlands. Sacerdote and King (2009) found that Spotted Salamander (*A. maculatum*) eggs did not hatch if DO was below 4.0 mg/L. Many salamanders, including all pond-breeding species, have gills throughout their larval period; thus, they require high concentrations of DO in order to meet their metabolic needs. In contrast, species with prolonged breeding activity or those that breed after DO has returned to levels above 4.0 mg/L might benefit from larger body sizes and shorter times to metamorphosis observed in Late Fescue treatments. Larger body sizes at metamorphosis have been linked to increased survival to reproduction (Smith 1987), reduced time to first reproduction (Semlitsch et al. 1988), and improved juvenile foraging ability (Cabrera-Guzmán et al. 2013). A reduction in time to metamorphosis can also be important for species that breed in temporary wetlands, where hydroperiod can be an important factor associated with survival. If a pond dries prior to metamorphosis, larvae will desiccate, sometimes resulting in complete reproductive failure (Semlitsch 1983, 1987). Our results suggest that interpreting the effects of non-native vegetation on amphibian survival and development is complex and dependent upon timing of wetland inundation, oviposition, and vegetation quality and quantity.

Ecosystems have been substantially modified by anthropogenic activities and understanding the implications of such changes is one of the greatest challenges facing ecologists. Non-native species introductions and propagation can have unforeseen effects on native species, such as positive and negative effects of Fescue on tadpole development, which serve to further complicate our understanding of ecosystem function and conservation. Investigating the effects of non-native species introductions and abiotic factors under different

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ecologically relevant contexts (e.g. breeding, migration, growth) and the mechanisms that drive those effects is critical for understanding population and community ecology in changing landscapes (Agrawal et al. 2007). Here, we described a potential mechanism that may exacerbate current amphibian population declines by reducing reproductive success through increased variation in breeding wetland suitability. Further work modeling the population-level consequences of these effects are needed, especially for species that are explosive or early spring breeders. More broadly, our results suggest that realistic assessment of the effects of non-native species should incorporate treatments that consider a variety of plausible ecological interactions, as well as comparison with field data to assess consistency of effects observed in experimental mesocosms.

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**Tables**

**Table 1.** Nutrient content of Prairie and Fescue litter collected in Arkansas, USA.

| Plant Species | Mean ± 1 SE | | |
| --- | --- | --- | --- |
| | %C | %N | %P |
| Native Prairie Grass Mix | 45.8 ± 0.06 | 0.62 ± 0.02 | 0.07 ± 0.03 |
| Non-native Fescue | 42.5 ± 0.07 | 1.42 ± 0.04 | 0.18 ± 0.02 |

**Figures**
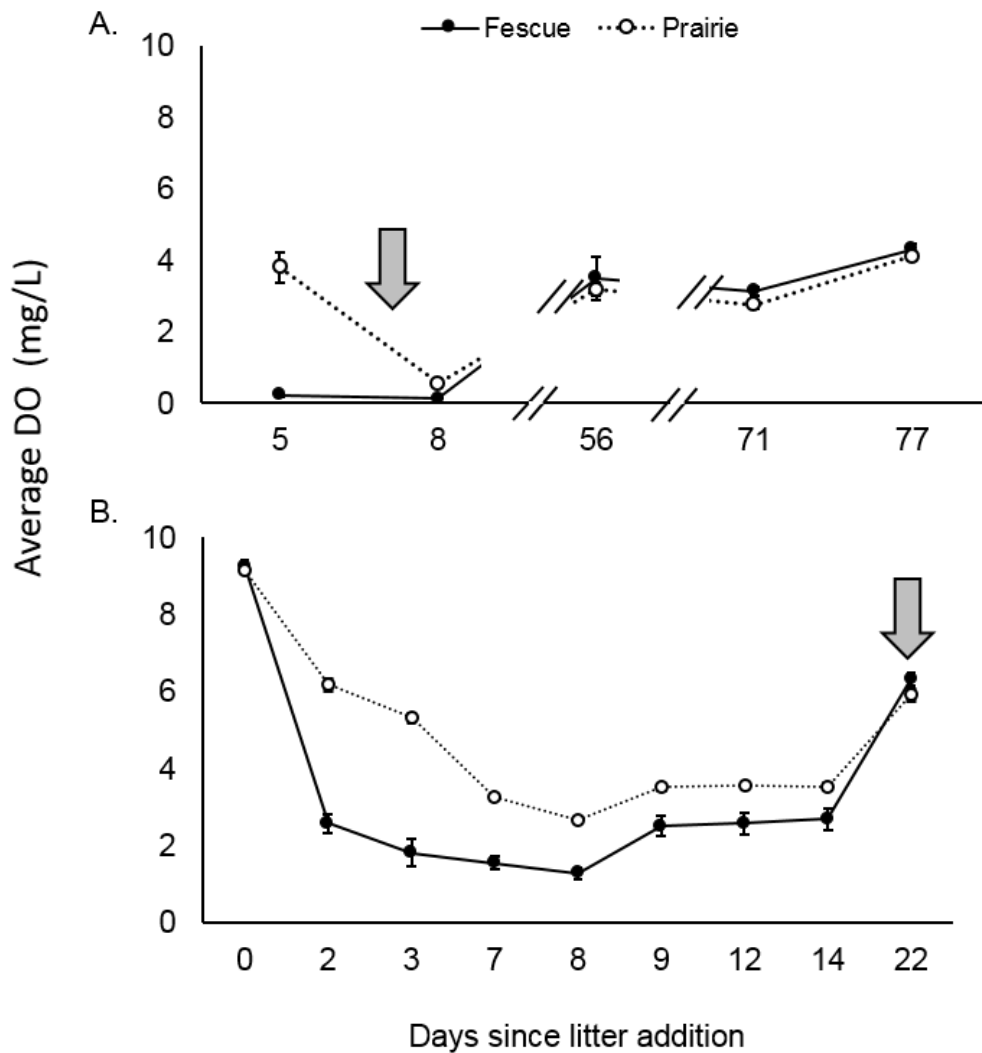


**Figure 1.** Average dissolved oxygen (DO) concentration (± 1 SE) in the (A) Early and (B) Late Oviposition Treatments. Arrows indicate when larvae were added to tanks. Solid lines indicate Fescue (F) Treatments. Dashed lines indicate Prairie (P) Treatments.
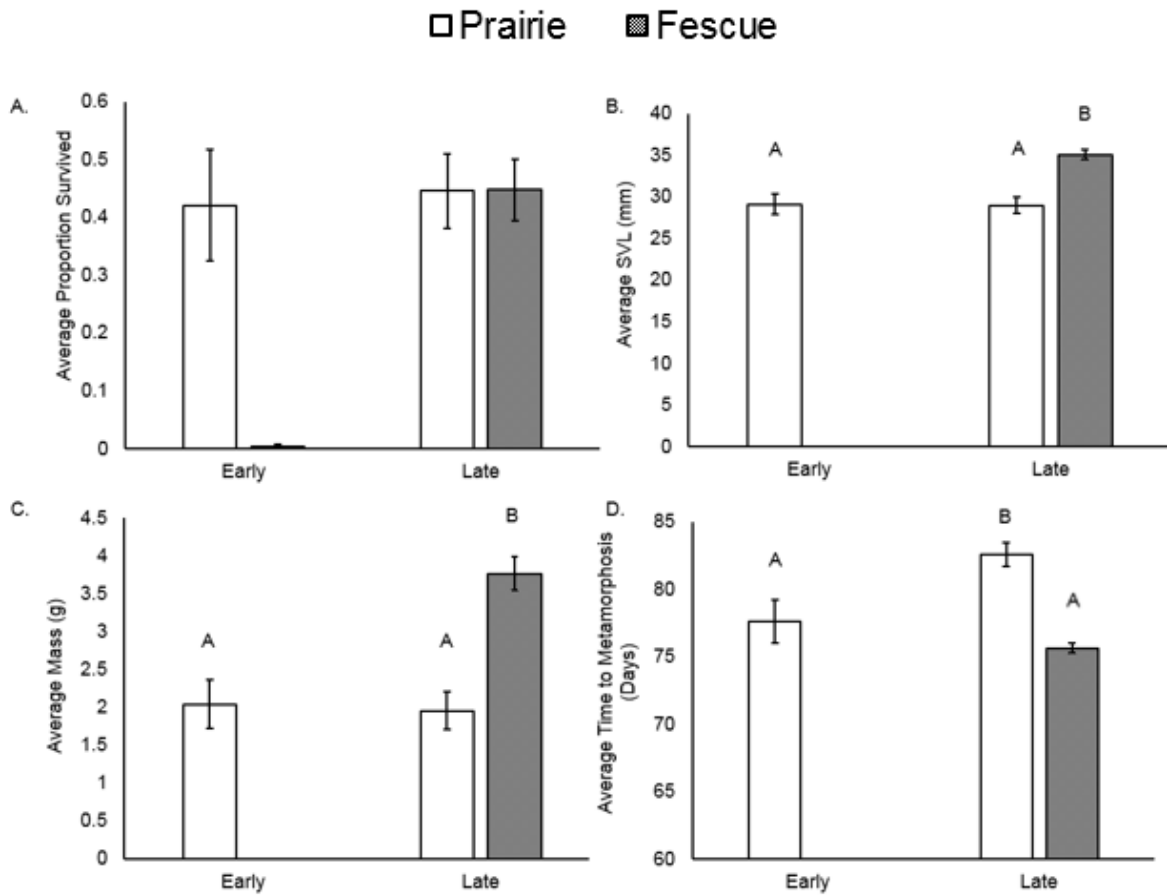
Figure 2. Average (A) Survivorship to metamorphosis (± 1 SE), (B) Snout-Vent-Length at metamorphosis (± 1 SE), (C) Mass at metamorphosis (± 1 SE), and (D) Time to metamorphosis (± 1 SE) of Crawfish Frog (*Lithobates areolatus*) larvae introduced to Fescue and Prairie mesocosms 7-days post-litter addition (Early) and 22-days post-litter addition (Late). Letters indicate significant differences based on an ANOVA and Tukey's HSD test. Figure 2A does not include letters due a significant interaction effect.
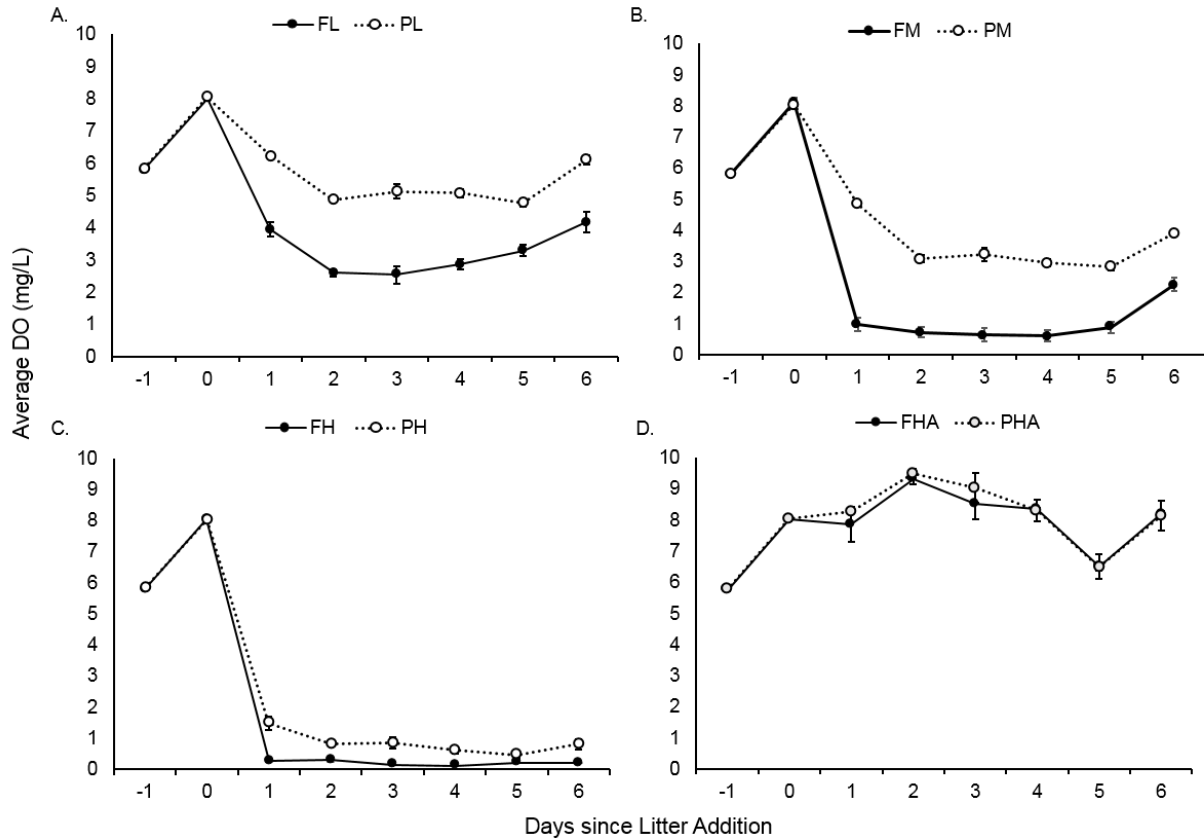
**Figure 3.** The effect of litter type and quantity (H = High; M = Moderate; L = Low) on average dissolved oxygen (DO) concentration (± 1 SE) over six days in 18.92 L mesocosms. (A) Low Quantity (0.5 mg/L), (B) Moderate Quantity (1.0 mg/L), (C) High Quantity (2.0 mg/L), (D) High Quantity with Aeration.  Solid lines indicate Fescue (F) Treatments. Dashed lines indicate Prairie (P) Treatments.
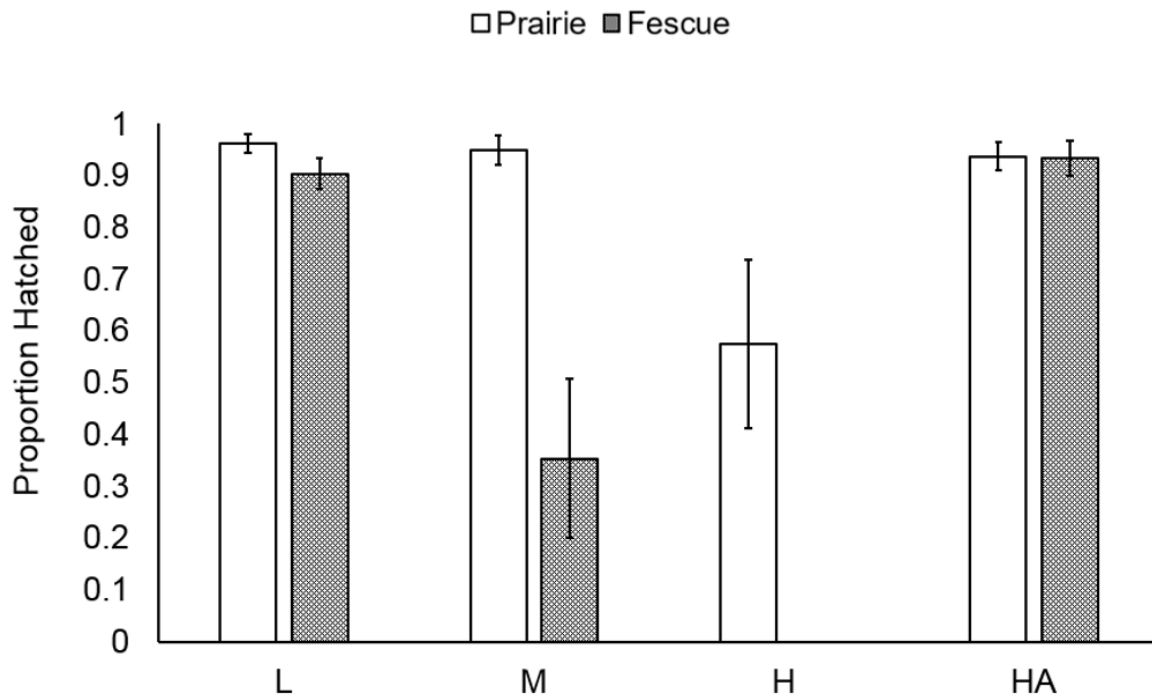
**Figure 4.** Average hatching success (± 1 SE) of Leopard Frog (*Lithobates sphenocephalus*) eggs among eight litter type and quantity treatments. Vegetation quantities are indicated by an L (low), M (moderate), H (high), HA (high with aeration).
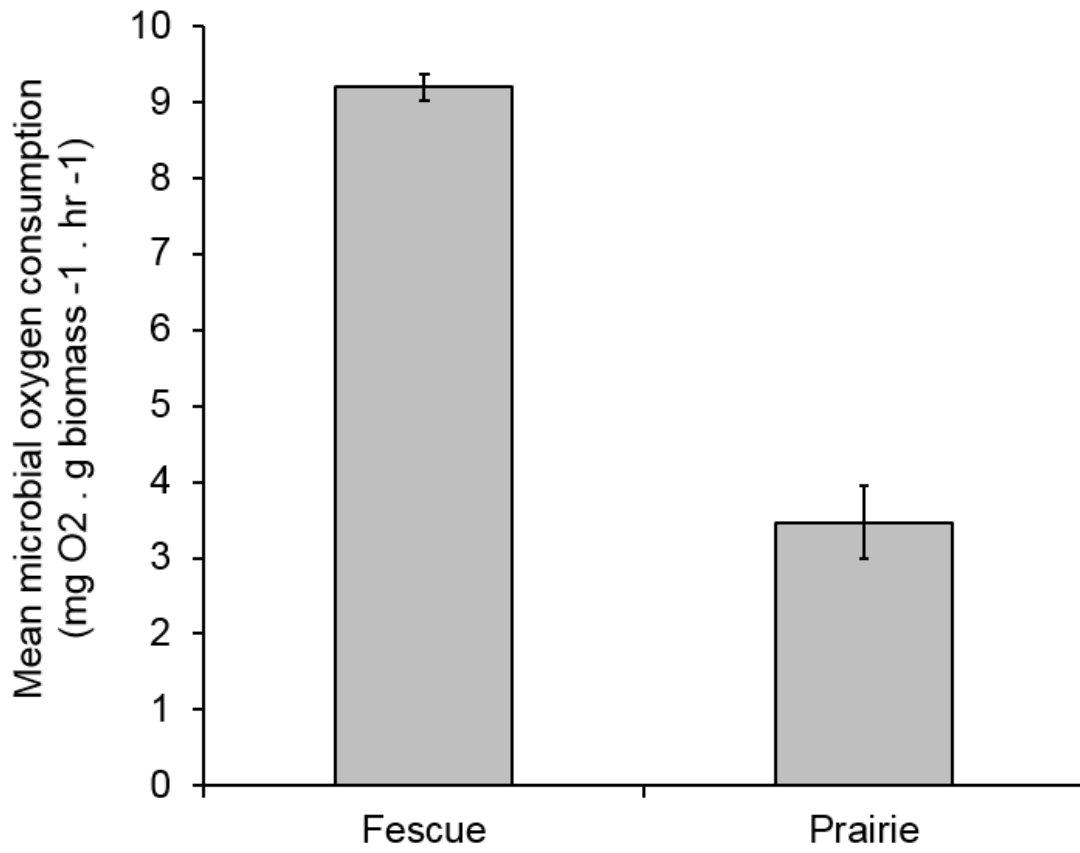
**Figure 5.** Average microbial respiration (± 1 SE) in mesocosms containing Fescue or Prairie litter.
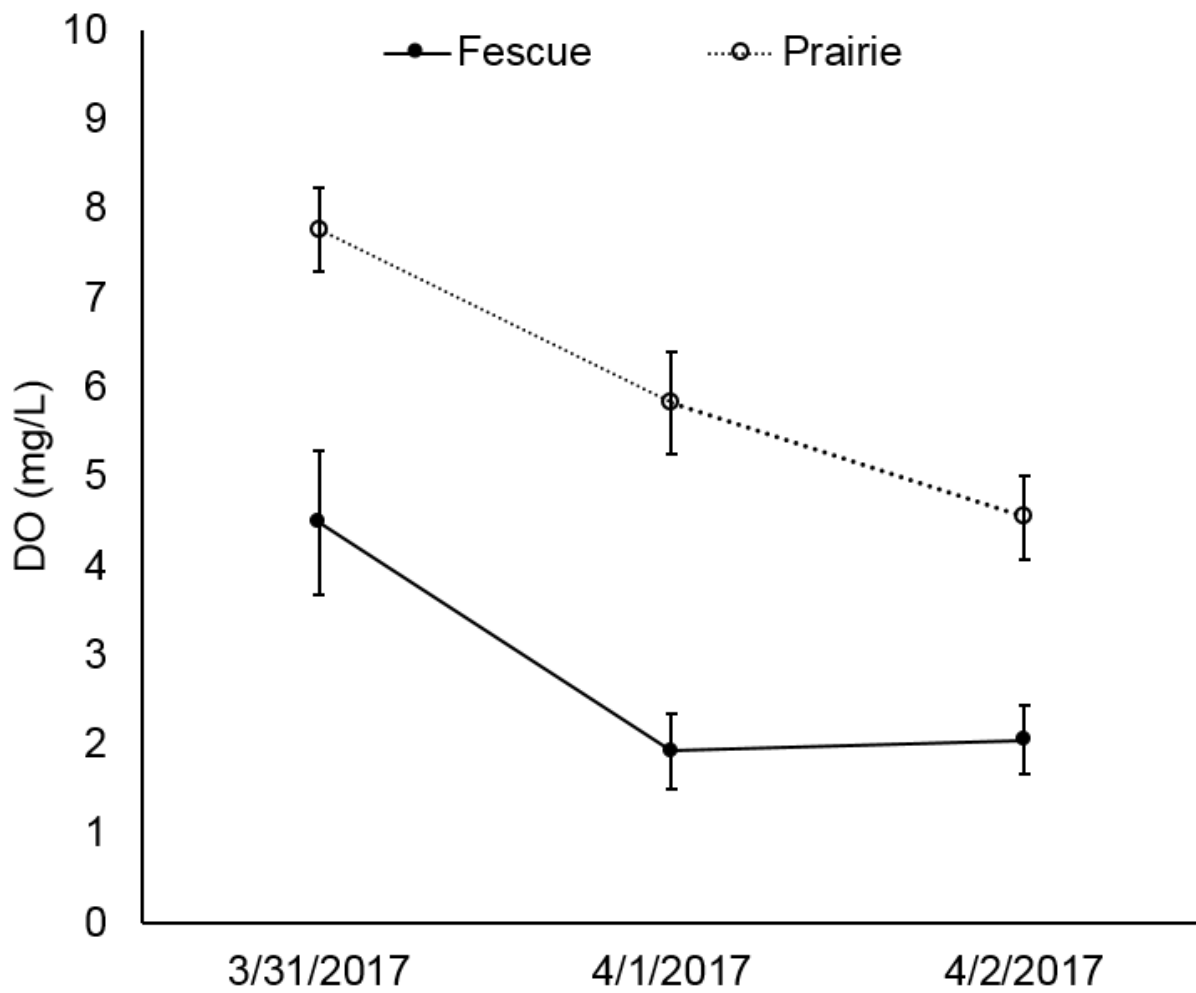
**Figure 6.** Average Dissolved Oxygen (DO) concentration (± 1 SE) in Fescue- (solid line) and Prairie-dominated (dashed line) wetlands (n=5 per treatment) in Northwest Arkansas following a heavy (8.48 cm) rain event in April 2017.

# IACUC Approval Documents

UNIVERSITY OF ARKANSAS

MEMORANDUM

TO:             J.D. Willson

FROM:         Craig N. Coon, Chairman

DATE:          Feb 6, 2015

SUBJECT:     IACUC Approval

Expiration Date:   Feb 28, 2018

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your
Protocol:     15033          Effects of litter type on development of larval Crawfish
Frogs (Lithobates areolatus) to begin March 1, 2015

In granting its approval, the IACUC has approved only the information provided. Should
there be any further changes to the protocol during the research, please notify the  IACUC
in writing (via the Modification form) prior to initiating the changes. If the study period is
expected to extend beyond Feb 28, 2018   you must submit a **newly drafted protocol**
prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for
more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal
guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

UNIVERSITY OF ARKANSAS.

Office of Research Compliance

To:             John Willson
Fr:             Craig Coon
Date:           May 4th, 2017
Subject:        IACUC Approval
Expiration Date: May 3rd, 2020

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **17079**: *Effect of non-native vegetation development of amphibian larvae.*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond May 3rd, 2020  you must submit a newly drafted protocol prior to that date to avoid any interruption.  By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study:  .   Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.


CNC/aem

I, Dr. John D. Willson, certify that Chelsea S. Kross completed the required training as required by the Institutional Animal Care and Use Committee (IACUC) for work completed in Chapter 2.

*J. D. Willson*
J. D. Willson (Jul 16, 2020 20:23 CDT)          7/16/2020

John D. Willson                                        Date
Associate Professor
Department of Biological Sciences

Chapter 2, "Timing of oviposition influences the effects of a non-native grass on amphibian development" of C.S. Kross's dissertation is accepted for publication in the journal Oecologia with three coauthors, A.K. Dodd, P.L. Mariage, and J.D. Willson.

I, Dr. John D. Willson, advisor of Chelsea S. Kross, confirm Chelsea S. Kross will be first author and completed at least 51% of the work for this manuscript.

*J. D. Willson*
J. D. Willson (Jul 16, 2020 20:23 CDT)          7/16/2020

John D. Willson                                        Date
Associate Professor
Department of Biological Sciences

# CHAPTER 3

Land-use and density-dependence interact to increase extinction risk in a grassland amphibian

Chelsea S. Kross

and

John D. Willson

**Abstract**

An understanding of interactions among anthropogenic stressors and intrinsic population drivers is needed to fully understand wildlife population fluctuations. Density-dependence is a key aspect of population regulation for many species, especially for species that have high reproductive potential. For this reason, amphibians have often been used as model organisms for addressing questions related to density-dependence. However, patterns of density-dependence have been characterized for only a few species and little work has evaluated how density-dependence may be altered by anthropogenic habitat modifications. Specifically, changes in land-use surrounding breeding habitats might alter density-dependent relationships through changes in vegetation used as food and cover for developing larvae. We combined the results of a mesocosm experiment and demographic modeling to investigate how vegetation composition (native prairie or non-native agriculture-associated vegetation [tall fescue grass; *Lolium arundinacea*]), surrounding breeding wetlands affected larval density-dependence and adult population size of an imperiled amphibian, the Crawfish Frog (*Lithobates areolatus*). Overall, density-dependence was overcompensatory, suggesting that *L. areolatus* exhibit scramble competition as larvae. Both vegetation treatments had low survival at high densities, but more individuals survived to metamorphosis at moderate densities in Fescue treatments compared to Prairie treatments. We evaluated the implications of our experimental results using a stochastic density-dependent matrix population model to estimate adult population size and long-term population dynamics. Simulated populations breeding in Fescue-dominated wetlands had a more variable population size and had a 100-500% higher probability of quasi-extinction within 200 years, compared to populations breeding in ponds with native prairie vegetation. Without incorporating multiple densities and mathematical modeling, our mesocosm experiment results

would have suggested a slightly positive effect of Fescue grass on amphibian development and survival. Thus, models are useful for examining the population-level consequences of individual-level effects typically measured in experiments.

## Introduction

To understand animal populations during the Anthropocene, scientists need to consider interactions among effects of anthropogenic stressors and intrinsic drivers of population dynamics. Experimental studies offer powerful opportunities to isolate effects of anthropogenic stressors, but these effects are seldom placed within the context of natural population drivers, such as density-dependent interactions and responses to environmental stochasticity (O'Gorman et al. 2012, Stephens et al. 2013, Injaian et al. 2018). Population models provide a framework for integrating experimental results with demographic and life-history information to assess emergent dynamics of populations and communities (Caswell 2001, Willson et al 2012).

Density-dependent competition for resources is a key aspect of population regulation for many species (Turchin 1999), especially for species with complex life cycles that lead to high densities within particular life stages (Wilbur 1980). For this reason, amphibians have classically served as model organisms for addressing questions related to the role density-dependence plays in population regulation (Wilbur 1976, 1977, Skelly and Kiesecker 2001, Vonesh and De la Cruz 2002). Due to the high fecundity of amphibians, there is often a strong effect of larval density on growth (Skelly and Kiesecker 2001) and survival (Van Buskirk and Smith 1991). High larval densities can result in reduced growth rates, small juvenile body sizes, and increased time required to attain reproductive maturity (Van Buskirk and Smith 1991, Altwegg 2003). In extreme cases, high density can result in complete reproductive failure due to increased development times and failure to metamorphose prior to drying of an ephemeral breeding

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wetland (Taylor et al. 2005). Although amphibians are among the most threatened vertebrate groups (IUCN 2019), few studies have examined interactions between density-dependence and the effects of anthropogenic stressors. Studies that have incorporated density-dependence in stessor related research (e.g., Vonesh and De la Cruz 2002, Willson et al. 2012) have found that experimental studies can be misleading, if not considered within the context of density-dependence. For example, although UV-B radiation can cause sharp decreases in survival of anuran and salamander eggs, this mortality can be compensated for by competitive release in the larval stage, resulting in increased survival of larvae to metamorphosis (Vonesh and De la Cruz 2002).

Land-use change and habitat loss are primary drivers of global amphibian population declines (Stuart et al. 2004, Zamberletti et al. 2018, Powers and Jetz 2019). Land-use change can have profound effects on the dynamics of amphibian populations through direct habitat loss and destruction, or through indirect pathways (Foster et al. 2003). For example, natural landscapes can be altered via changes in producers (algae or vegetation) or trophic down-grading (loss of predators), which have been shown to have strong bottom-up and top-down effects on native species, respectively (Fish and Carpenter 1982, Estes et al. 2011). Changes in the quality or quantity of vegetation that forms the nutrient base in amphibian breeding wetlands can affect larval development and recruitment into the terrestrial adult population (Maerz et al. 2010, Stephens et al. 2013, Kross et al. Accepted). However, little work has evaluated how rearing habitat and density-dependence interact to affect recruitment and subsequent population dynamics.

Grasslands and their associated wetlands have become one of the most endangered ecosystems worldwide (Samson and Knopf 1994, Curtin and Western 2008, Ceballos et al.

2010). Over 95% of the original tallgrass prairies in the United States have been destroyed (Gleason et al. 2008), and many have had their wetlands drained and uplands tilled for agricultural use (Knutson and Euliss 2002). Vegetation that dominates seasonally inundated prairie wetlands has changed from a diverse array of grasses, sedges, and other herbaceous species to monocultures of non-native agricultural vegetation, such as Tall-grass Fescue (*Lolium arundinaceum* [Schreb. 2017]). As a result, agricultural wetlands are often some of the only suitable amphibian breeding habitats in severely altered landscapes (Knutson et al. 2004). Previous work has shown that Fescue grass can positively affect larval amphibian development under well oxygenated, low-density conditions (Kross et al. Accepted). Specifically, Nitrogen (N) and Phosphorous (P) content is higher in Fescue compared to native prairie vegetation, resulting in increased primary production and increased body size of larvae in Fescue-dominated habitats (Kross et al. Accepted). However, it is unclear how this effect might change based on larval density or the ultimate consequences of altered recruitment on emergent dynamics of amphibian populations.

We combined a mesocosm experiment with mechanistic population modeling to investigate how vegetation composition (native prairie or non-native agriculture-associated vegetation [Tall-grass Fescue; *L. arundinaceum*]) surrounding breeding wetlands affected density-dependent relationships in larvae of an imperiled prairie amphibian, the Crawfish Frog (*Lithobates areolatus*), and the implications of these differences for emergent population dynamics, including adult population size and extinction risk. We hypothesized that survival of larvae would decrease at high densities, leading to overcompensation (scramble dynamics), but that the form of the density-dependent effects on larval survival would differ based on vegetation type. Specifically, we predicted that larvae reared in Fescue would have increased survival at

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moderate densities due to nutrient composition (Kross et al. Accepted), leading to stronger (i.e. more overcompensatory) density-dependence. Ultimately, we predicted higher variability in population size and greater stochastic extinction risk in Fescue simulations due to increased survival at moderate densities.

## Methods

<u>Study Species</u>

*Lithobates areolatus* (Crawfish Frog) occurs throughout much of the historic tall-grass prairie extent of the central United States and has recently experienced precipitous declines throughout its range (Parris and Redmer 2005). Declines in *L. areolatus* are largely associated with the loss of suitable terrestrial and aquatic habitat (Parris and Redmer 2005). The IUCN (Hammerson and Parris 2004) has listed *L. areolatus* as near threatened; the species is listed as state endangered in Iowa, Illinois, and Indiana, and is a species of greatest conservation need in Arkansas, Oklahoma, Missouri, Kansas, Kentucky, Mississippi, and Louisiana. *Lithobates areolatus* are known to use both prairie and agricultural wetlands, but have shown a preference for prairie habitats (Baecher et al. 2018). Thus, understanding how vegetation changes surrounding breeding wetlands affect *L. areolatus* and their population dynamics could provide important implications for amphibian conservation and management.

<u>Mesocosm Experiment</u>

*Experiment Design*

We conducted a mesocosm experiment to evaluate the interactive effects of habitat and density-dependence on larval survival of *L. areolatus* at the University of Arkansas Uptown Campus (Fayetteville, Arkansas, USA) following methods similar to those used by Kross et al. (Accepted). In 2018, we constructed an array of fifty 379 L cattle tanks, each with a surface area

of 0.42 m$^2$, and randomly assigned each tank to one of 10 treatments (N = 5 tanks per treatment): five densities (5, 15, 45, 135, 405) and two vegetation types (Prairie and Fescue). These densities represent larval densities produced by 1 to 20 female *L. areaolatus* ovipositing (mean clutch size = 5,000 [Parris and Redmer 2004]) within a single 100 m$^2$ wetland, which are typical densities for our region (Kross, upublished data). We collected vegetation for the Fescue and Prairie treatments from local hay fields and a restored prairie (Woosley Wet Prairie Preserve [WWPS], Fayetteville, Arkansas; see Baecher et al. 2018 for more detailed site information). Agricultural wetlands are often surrounded by a monoculture of a single grass species. Therefore, the agriculture vegetation treatment was made up of a single grass species, Tall-grass Fescue (*L. arundinaceum*). Native prairies have a variety of herbaceous species, which serve as the primary resource base in ephemeral pools used by breeding amphibians (Baecher et al. 2018). Thus, the prairie vegetation included a combination of grasses, sedges (*Carex spp.*, *Pychnanthemum spp.*, and *Eleocharis spp.*) and forbs (*Boltonia asteroids, B. diffusa,* and *Periscaria spp.*). We cut and raked vegetation from the vicinity of known *L. areolatus* breeding wetlands in February 2018 and allowed it to dry to a constant mass for two weeks prior to allocation to mesocosms. We filled each mesocosm with 300 L of city water between 19 Feb and 23 Feb 2018. We added 300 g (1g/L) of dry vegetation to tanks on 2 March 2018 after allowing chlorine to dissipate from tanks. We inoculated each tank with a zooplankton/phytoplankton slurry collected from a local wetland. To prevent colonization by potential competitors (e.g., *Hyla versicolor* [Gray Treefrog]) and predators (e.g., Odonates), we covered mesocosms with black 30% shade cloth (PAK unlimited, Inc. Conelia, GA).

In April 2018, we collected three *L. areolatus* egg masses at wetlands in prairie remnants in Northwest Arkansas. We maintained egg masses in aerated pond water in the laboratory until

they hatched (~ 7 d). Once larvae reached Gosner Stage (GS; Gosner 1960) 25, we haphazardly

mixed them, allocated them to density treatments, and transferred them to mesocosms on 22

April 2019. We monitored larvae every 1-3 days throughout development, removed them at the

emergence of at least one forelimb (GS 42) and transferred them to the lab until full tail

resorption (GS 46). We began a draw-down of mesocosms 15 August 2018 and removed 50 L of

water per week until tanks were emptied on 24 September 2018. Water drawdown mimics

natural mid- to late-summer drying of ephemeral breeding habitats, which is typical of *L.*

*areolatus* breeding wetlands in our region and is common in experimental studies of amphibian

population and community dynamics (e.g., Wilbur 1976, Roe et al. 2006). We captured all

individuals that were at least GS 42 and held them in the lab until full tail resorption. Any larvae

that had not reached GS 39 by the final drawdown were considered mortalities. We analyzed the

effects of density and vegetation on larval *L. areolatus* survival using a  Two-Factor ANOVA

with main and interactive effects of Vegetation Type (Fescue vs. Prairie) and Density

(categorical) using the Car (Fox and Weisburg 2019) and Performance (Lüdecke et al. 2020)

packages in R v 3.6.3 (R Core Team 2020). We applied a White adjustment and arcsin

transformed survival probabilities, due to violation of the homogeneity of variances assumption

of ANOVA.

Modeling Population Dynamics

Density-dependence at the larval stage is an important factor regulating amphibian

populations (Wilbur 1980, Willson et al. 2012) and stochastic reproductive failure due to a

variety of mechanisms (e.g., wetland hydroperiod, fish invasions, disease outbreak) can affect

long-term population viability (Semlitsch et al. 1996, Salice et al. 2011, Willson and Hopkins

2013). We included density-dependent larval survival and varied frequency of stochastic

reproductive failure to model the population dynamics of *L. areolatus* in agricultural and prairie wetlands. Additionally, we ran a deterministic model, where no reproductive failure occurred, to examine underlying population dynamics in the absence of stochastic forcing.

*Model Description*

We developed a stochastic stage-based matrix model for *L. areolatus* populations and parameterized the model to represent populations that breed in either Fescue-dominated or Prairie wetlands. Our model was based on the general amphibian population model proposed by Vonesh and De La Cruz (2002). Briefly, this pre-breeding matrix model included two stages: Juvenile (J) and Adult (A). Adult *L. areolatus* emerge in the spring to reproduce and lay eggs. The eggs hatch, becoming larvae that metamorphose into juveniles within 3-4 months. Juvenile females mature within 3-5 years in the northern portion of their range (Illinois; Parris and Redmer 2005), but maturity times are unknown throughout most of their range. A closely related species, *L. capito* (Gopher Frog), has been extensively studied in the southeast and reach maturity at two years (Jensen and Richter 2005). Due to the geographic location of our study, we assumed *L. areolatus* reached reproductive maturity at age two and reproduce annually thereafter. In our matrix, juveniles do not reproduce in their first year and their survival at each time step is $\sigma_j$. Adults have an annual survival rate of $\sigma_a$ and reproduce annually following the formula F[A]. The model can be expressed in matrix form as:

$$\begin{bmatrix} J \\ A \end{bmatrix}^{t+1} = \begin{bmatrix} 0 & F[A] \\ \sigma_j & \sigma_a \end{bmatrix} \begin{bmatrix} J \\ A \end{bmatrix}_t,$$

The function F[A] represents the production of juveniles per adult at each yearly time step (Vonesh and De la Cruz 2002). The F[A] function is the product of adult sex ratio ($\rho$), annual per capita egg production ($\phi$), egg survival ($\sigma_e$), density-dependent larval survival at that

time step ($\sigma_t$), and metamorph survival for the remainder of their first year ($\sigma_m$) (Vonesh and De la Cruz 2002):

$$F[A] = \rho\phi\sigma_e\sigma_t\sigma_m \qquad \text{(eqn 1)}$$

Within the F[A] function, density-dependent larval survival ($\sigma_t$) is calculated for each time step using a density-dependent function based on the Beverton-Holt fisheries recruitment model (Beverton and Holt 1975):

$$\sigma_t = \sigma_{tmax}/(1+dT)^\gamma \qquad \text{(eqn 2)}$$

where $\sigma_{tmax}$ is maximum larval survival at very low density, d is the density-dependent coefficient, gamma ($\gamma$) is the density-dependent exponent, and T is initial tadpole density at that timestep, calculated as:

$$T=\rho\phi\sigma_e A \qquad \text{(eqn 3)}$$

The density-dependent exponent ($\gamma$) in eqn 2 determines the form of larval density-dependence, with density-independence (linear relationship between initial density and recruitment) when $\gamma=0$, perfect compensation when $\gamma=1$, and overcompensation when $\gamma>1$. The density-dependent coefficient (d) acts as a scaling factor.

*Model Parameterization*

We parameterized our model using demographic data from our study and the literature (Table 1). *Lithobates areolatus* exhibit a 1:1 sex ratio as adults (Kinney 2011), so we assumed a 1:1 offspring sex ratio by setting $\rho = 0.5$. Heemeyer and Lannoo (2012) documented individual adult survival over a 21-month period was estimated to be approximately 42%, based on within year mortality from the same study, we set annual adult survival probability to 0.75. Larval survival was based on the $\gamma$ and d estimates from our mesocosm experiment (see below). Published data on the survival of juvenile *L. areolatus* are sparse, but Lannoo et al. (2017) state

that the probability of a recently metamorphosed juvenile surviving to its first breeding event is

4%, based on unpublished mark-recapture data. We therefore assigned $\sigma_m$ and $\sigma_j$ values of 0.2,

for a cumulative probability of 0.04 for survival from metamorphosis to first reproduction at age

2.

Prior to parameterizing our model, we transformed our survival data from our mesocosm

experiment to survival densities (# surviving per m$^2$; Figure 1) and used eqn 2 to determine the $\gamma$

and d value that best fit our experimental data for each vegetation treatment. To fit $\gamma$ and d, we

used eqn 2 to calculate density of surviving larvae for a range of $\gamma$ (1000 increments from 0 to

100) and d (1000 increments from 0 to 0.1) combinations, holding $\sigma_{tmax}$ constant. For each $\gamma$ and

d combination, we summed the absolute value of the difference between each observed survival

density and estimated survival density for each combination of $\gamma$ and d (i.e., residuals). We then

used the $\gamma$ and d combination with the smallest summed residual value (i.e., best fit) to

parameterize simulations for each vegetation type.

We first examined underlying deterministic dynamics of the "Fescue" and "Prairie"

parameterizations of our model described above by projecting a 200 year time series and visually

examining transient and equilibrium dynamics in the time series. We then conducted stochastic

simulations to capture the stochastic nature of amphibian populations in response to annual

variation in precipitation and temperature by varying the frequency of reproductive failure in

models parameterized to represent *L. areolatus* populations breeding in a wetland dominated by

Fescue or Prairie vegetation. For each simulation we generated a vector of 200 random values

evenly distributed between 1 and 100, each corresponding to a simulation year. When values fell

below the reproductive failure frequency threshold (e.g., 5 for 5% probability of failure),

recruitment into the juvenile class (F[A]) in the corresponding year was set to 0. In a 16-year

study, Semlitsch et al. (1996) found that pond-breeding amphibians experienced catastrophic reproductive failure up to 25% of years due to drying of wetlands prior to metamorphosis. Since frequency of catastrophic reproductive failure is not known for *L. areolatus,* we simulated a range of frequencies: 5%, 10%, 15%, 20%. Since most breeding wetlands used by *L. areolatus* in our study region are ephemeral, the frequencies we used are likely representative of what they experience in nature. All models were constructed and simulated in program R v.3.6.3 (R Core Team 2020).

*Simulations*

We ran 200 stochastic simulations of a 200-year time series each for a population breeding in a Fescue-dominated wetland and a Prairie wetland under four different frequencies of stochastic reproductive failure (5%, 10%, 15%, and 20% of years). During each simulation, we monitored average population size and quasi-extinction probability. For our simulations, we set starting adult population size at 1 individual/m$^2$, which is near the long-term average for both treatments (see below). Finally, we set a quasi-extinction threshold of <0.1 individuals/m$^2$, which equates to 10 individuals in a 100 m$^2$ wetland. If a population dropped below this value, the population was considered permanently extinct.

<div align="center">

**Results**

</div>

Mesocosm Experiment

Density and Litter type interacted to significantly affect larval survival ($F_{4,40}$ = 3.34, p = 0.019; Figure 1). Survival was lowest in the highest density treatments and, generally, survival was higher in Fescue treatments (Figure 1). Larval survival was high (>50%) in low to moderate density treatments (5, 15, 45), ranging from 57 to 88% in Fescue treatments and from 57 to 69% in Prairie treatments. The largest difference in survival between litter type was observed in the

moderately-high density (135) with 4% surviving in Prairie treatments compared to an average

of 30% in Fescue treatments. Less than 2% of larvae survived to metamorphosis in the highest

density (405) in Fescue treatments and less than 3% survived in Prairie treatments. The best-fit

curves relating initial density to density of surviving metamorphs in the Prairie and Fescue

treatments had a $\gamma=2.102$ and d=0.005, and $\gamma=37.73$ and d=0.0001, respectively (Figure 2),

suggesting that the larvae within the Fescue treatment exhibited stronger overcompensatory

dynamics.

<u>Population Simulations</u>

In deterministic simulations that did not include stochastic reproductive failure, average

adult population density in Prairie wetland simulations initially overshot carrying capacity, but

rapidly returned to a stable equilibrium density of 1.67 per $m^2$ of wetland (Figure 3A).

Conversely, Fescue wetland simulations exhibited stable limit cycles in the deterministic

simulation, oscillating around a lower carrying capacity (0.92 - 1.19 per $m^2$ of wetland ) (Figure

3A). In stochastic simulations, average adult density was higher in Fescue wetland simulations,

but was much more variable compared to average population size in Prairie wetland simulations

(Figure 3B). Average adult population density was similar across reproductive failure rates for

both Fescue (1.40-1.49 per $m^2$ of wetland) and Prairie (1.11-1.15 per $m^2$ of wetland) simulations,

but variance increased as failure rates increased from 0.99 to 1.19 SD in Fescue simulations, and

from 0.18 to 0.37 SD in Prairie simulations.

Generally, as frequency of reproductive failure events increased, quasi-extinction

probability also increased. However, *L. areolatus* populations in the Fescue simulations were

100% to 500% more likely to go extinct compared to populations in Prairie (Figure 4). At the

lowest reproductive failure frequency quasi-extinction probability was 1.10% for Fescue and

0.20% for prairie population simulations. At the 10% at 15% reproductive failure frequencies, quasi-extinction probability was 7% and 19% for Fescue simulations, and 2.3% and 8% for Prairie population simulations, respectively. At the highest frequency of reproductive failure, quasi-extinction probability was 40% and 20% for Fescue and Prairie populations, respectively.

## Discussion

We combined the results of a mesocosm experiment and demographic modeling to demonstrate that density and rearing habitat (vegetation type) interact to affect amphibian population dynamics. Overall, as density increased, survival decreased, indicating strong density-dependence in the larval stage. Average survival was similar between vegetation treatments at low and high densities, but survival was much higher in Fescue treatments at the moderate and moderately-high densities. The decline in survival between the moderately-high and high density Fescue treatments indicates a stronger effect of density-dependence, which led to stronger overcompensation when compared to the Prairie treatments. Models parameterized using our experimental data revealed that population size was more variable in Fescue simulations, leading to increased extinction risk compared to Prairie simulations. Additionally, increasing frequency of reproductive failure greatly increased quasi-extinction probability in Fescue simulations when compared to Prairie simulations, suggesting that an apparently positive effect on recruitment can destabilize population dynamics and increase local extinction risk. Our results highlight that while rearing habitat can differentially affect survival, these data need to be considered within the context of natural population drivers, such as density-dependent larval survival, to infer population-level effects.

Larval survival in our mesocosm experiment was similar at the lowest and highest densities, but was higher in Fescue treatments at moderate and moderately-high (45 and 135)

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densities, which had important biological consequences. Fitting curves to our survival data revealed a distinct difference in the form of density-dependence between the two vegetation treatments; the strength of density-dependence was higher within the Fescue treatment, indicating stronger overcompensation. Greater survival of larvae at the moderate and moderately-high densities within Fescue treatments was likely driven by the higher quality of Fescue vegetation. Fescue has a lower C:N ratio than Prairie grass, and is more readily broken down by microbes (Kross et al. Accepted). As a result, larvae can experience enhanced growth (Kross et al. Accepted) and survival, under well oxygenated conditions.

The results of our mesocosm experiment and population simulations supported our hypotheses that density-dependence would be stronger and lead to higher extinction probability in Fescue treatments. While larval survival was generally higher at higher densities in Fescue treatments, density-dependence was also stronger, which led to stable limit cycles (Figure 3A) in the simulated population. Stable limit population cycles are an indication of unstable dynamics, driven by high intrinsic rates of population increase (Hassell et al. 1976, May and Oster 1976). Stochastic demographic and environmental forcing (i.e. reproductive failure; May 1975, Melbourne and Hastings 2008) can further destabilize population fluctuations, potentially leading to local extinction. Amphibian dynamics are often boom-and-bust; populations can go years without any recruitment and then have a year where favorable conditions where hundreds or thousands of juveniles are recruited into the population (Pechman et al. 1991; Whiteman and Wissinger 2005). Strong overcompensation as a result of density-dependence can exacerbate the boom-and-bust nature of amphibian populations, increasing extinction risk, as observed in our simulations.

Populations in Fescue simulations were more likely to go extinct due to the increased overcompensation compared to the Prairie populations and extinction risk increased as stochastic reproductive failure increased. While not incorporated in our model, stochastic variation may also be higher in agricultural wetlands. Amphibians breeding in Fescue wetlands are at a higher risk of reproductive failure, due to a dissolved oxygen crash immediately following inundation, caused by increased microbial activity (Kross et al. Accepted). If this crash coincides with oviposition, complete mortality of eggs or larvae can occur (Kross et al. Accepted) Additionally, many agricultural wetlands have been modified to maintain longer hydroperiods, which can facilitate colonization by fish, making the wetlands unsuitable for amphibian reproduction (Semlitsch 2000, Boone et al. 2007). Agricultural ponds are also often used by cattle, which can destroy shoreline habitat, decrease water quality, and have been correlated with increased disease prevalence (e.g., Ranavirus; Burton et al. 2009, Schmutzer et al. 2008, Hoverman et al. 2012). Any or all of these factors could lead to increased frequency of reproductive failure in agricultural fields, further increasing extinction probability relative to natural wetlands.

Density-dependence is an important aspect of animal populations that is often neglected in experimental studies that attempt to link anthropogenic habitat alteration to population decline. The results of our study highlight why consideration of density-dependent processes can be critical to understanding population declines. Most experimental studies focus on low or average density conditions (Williams et al. 2008, Cotton et al. 2012, Stephens et al. 2013). If we had simply compared survival metrics between our vegetation treatments at moderate densities, we would have found that larvae reared in Fescue have higher survival and concluded that Fescue was beneficial for amphibian populations. Manipulating density in conjunction with vegetation treatments allowed us to link the results of our experiment to a population model,

which demonstrated that the opposite was true. Our results corroborate previous research demonstrating that population models are needed to fully interpret results of experimental studies focusing on amphibians (Vonesh and De La Cruz 2002, Willson et al. 2012).Models from Willson et al. (2012) found that some strong effects measured in the lab had minor population-level consequences, while others were strong drivers of populations dynamics. Integrating the results from manipulative experiments with population models provides a more complete understanding of how individual-level effects scale-up to affect population dynamics, ultimately improving our ability to manage and conserve species.

Although our models revealed a destabilizing effect of Fescue vegetation on anuran population dynamics, there may still be potential for positive effects at larger spatial scales. When considered at the metapopulation scale, the higher population sizes achieved in Fescue simulations might provide a benefit through an increase in the number of dispersing individuals, if there is connectivity among a network of breeding wetlands. For amphibians, most interpopulation dispersal occurs during the juvenile stage (Funk et al. 2005, Griffiths et al. 2010, Pittman et al. 2014) and when reproductive success is high, hundreds or thousands of juveniles can disperse to the surrounding area, as well as other sub-populations. While density-dependence at the post-metamorphic juvenile stage has been linked to decreased survival at a single pond (Berven 2009), high juvenile densities at natal ponds might increase the probability of juvenile dispersal (Semlitsch 2008). As a result, the high number of juveniles produced during "boom" years in agricultural wetlands might have the capacity to serve as 'sources' for nearby 'sink' populations or rescue nearby populations that have gone locally extinct (Griffiths et al. 2010, Willson and Hopkins 2013). More complex modeling efforts (e.g., Willson and Hopkins 2013)

and a better understanding of juvenile dispersal parameters are needed to fully explore these hypotheses.

Considering the effect of habitat degradation (i.e. changes in vegetation) within the context of density-dependence might provide a better understanding of population declines and local extinction. Habitat alteration is associated with the decline of multiple species populations across taxonomic groups (Gaston and Fuller 2008, Brewster et al. 2018, Kentie et al. 2018). In our system, replacing the vegetation forming the nutrient base of breeding wetlands increases the risk of reproductive failure (Kross et al. Accepted) and alters the strength of larval density-dependence. The conversion of grassland habitats for agriculture has changed breeding wetland conditions making them less suitable for grassland-associated species (Balas et al. 2012). Our results suggest that vegetation changes surrounding breeding wetlands might play an important role in the decline of open canopy and prairie-associated amphibian populations in low-intensity agricultural areas.

Anthropogenic enrichment is a consequence of land-use change and has the potential to greatly affect the dynamics of populations and communities (Porter et al 2012). We found that enrichment (i.e., increased N and P from Fescue vegetation) can exacerbate boom-and-bust dynamics by increasing the amplitude of population oscillations, leading to stronger overcompensation and destabilization of population dynamics. Our results align well with other studies that have demonstrated destabilizing effects of enrichment through what is known as the paradox of enrichment (i.e., Loss of stability in consumer-resource dynamics after resource enrichment; Rosenzweig 1971, Holyoak 2000, Johnson et al. 2010). Forms of enrichment can include direct pollution of terrestrial and aquatic habitats with nutrients (e.g., Nitrogen and Phosphorus), however, less obvious forms of enrichment such as altering the dominant

vegetation within an ecosystem can have similar effects. Further research combining

experimental manipulation and population modeling is needed to understand how anthropogenic

enrichment and other stressors interact with animal population dynamics to affect extinction risk.

## Acknowledgements

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**Table 1.** Parameter values used to evaluate *Lithobates areolatus* population dynamics under varying frequencies of stochastic reproductive failure.

| Parameter | Fixed Value | Source |
|---|---|---|
| Clutch size ($\phi$) | 5000 | Parris and Redmer 2004 |
| Egg Survival ($\sigma_e$) | 0.6 | Stiles et al. 2016 |
| Metamorph Survival ($\sigma_m$) | 0.2 | Lannoo et al. 2017 (see description in the methods) |
| Juvenile Survival ($\sigma_j$) | 0.2 | Lannoo et al. 2017 (see description in the methods) |
| Adult Survival ($\sigma_a$) | 0.75 | Heemeyer and Lannoo 2012 (see description in the methods) |
| Maximum Tadpole Survival ($\sigma_{tmax}$) | 0.96 | Kinney 2011 |
| Prairie Density-Dependent Coefficient (d) | 0.005 | This Study |
| Prairie Density-Dependent Exponent ($\gamma$) | 2.102 | This Study |
| Fescue Density-Dependent Coefficient (d) | 0.0001 | This Study |
| Fescue Density-Dependent Exponent ($\gamma$) | 37.73 | This Study |
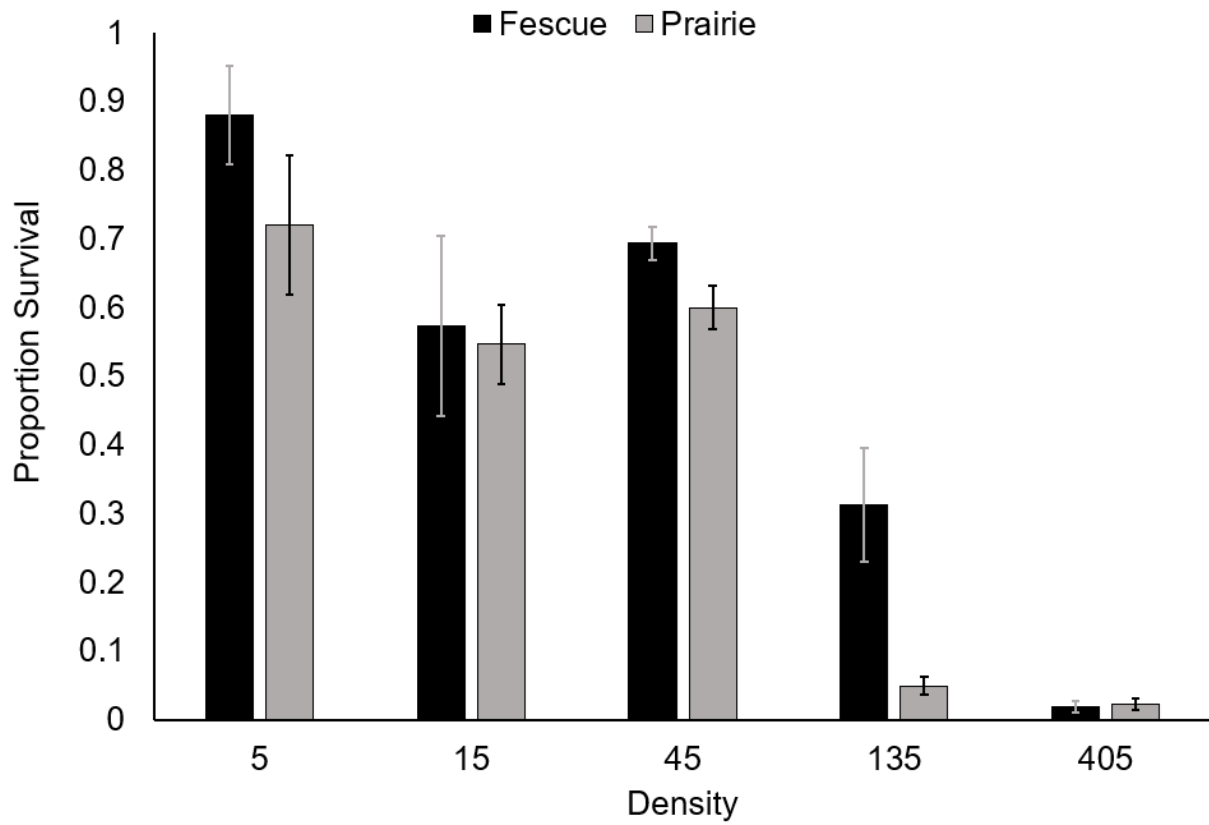
**Figure 1.** Average larval survival (±SE) at each of five stocking densities in Fescue and Prairie treatments.
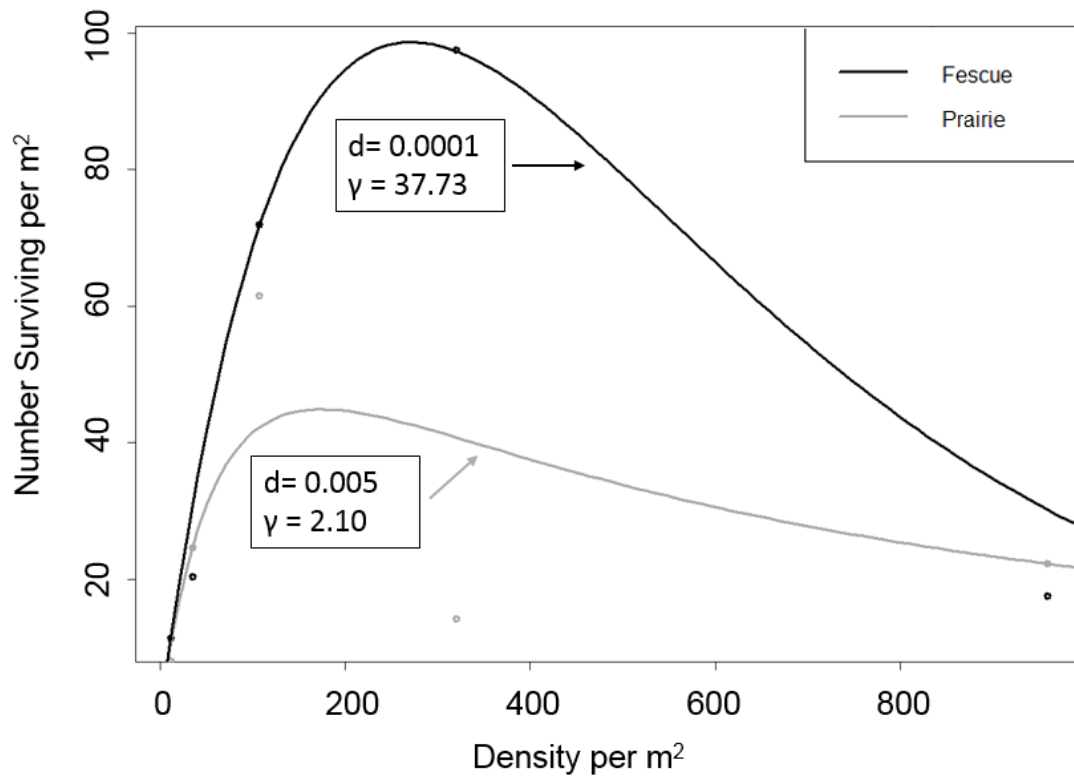
**Figure 2.** Best fit density-dependence curves for larval *Lithobates areolatus* (Crawfish Frogs) from Fescue and Prairie mesocosms. Gamma (γ) and (d) represent the density-dependent exponent and coefficient, respectively, from Equation 2 (see methods). Densities were transformed to represent density per $m^2$.
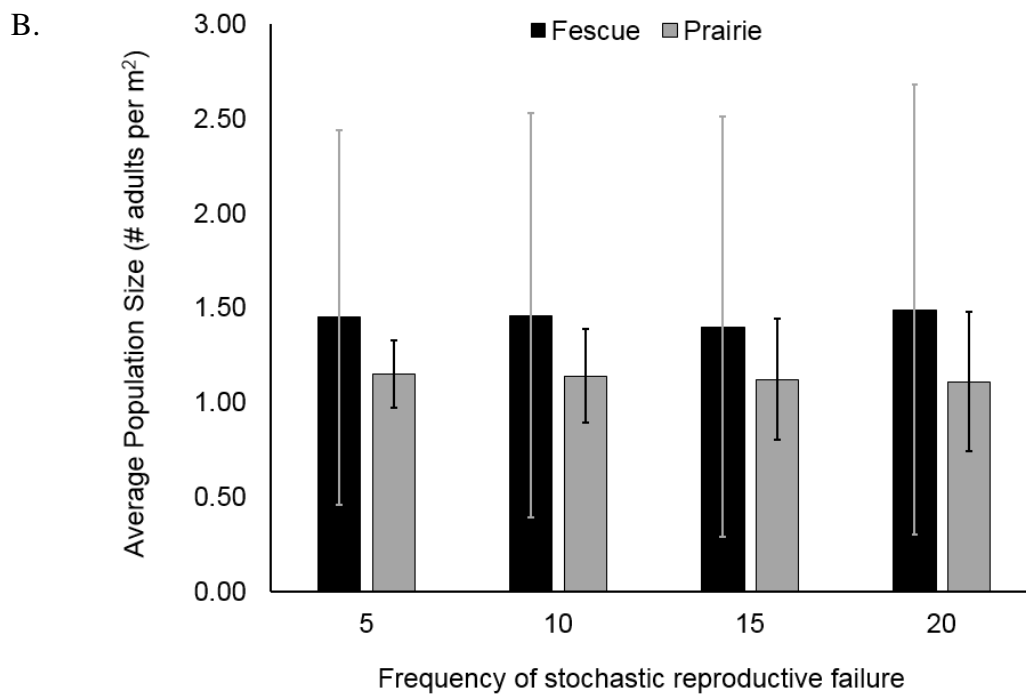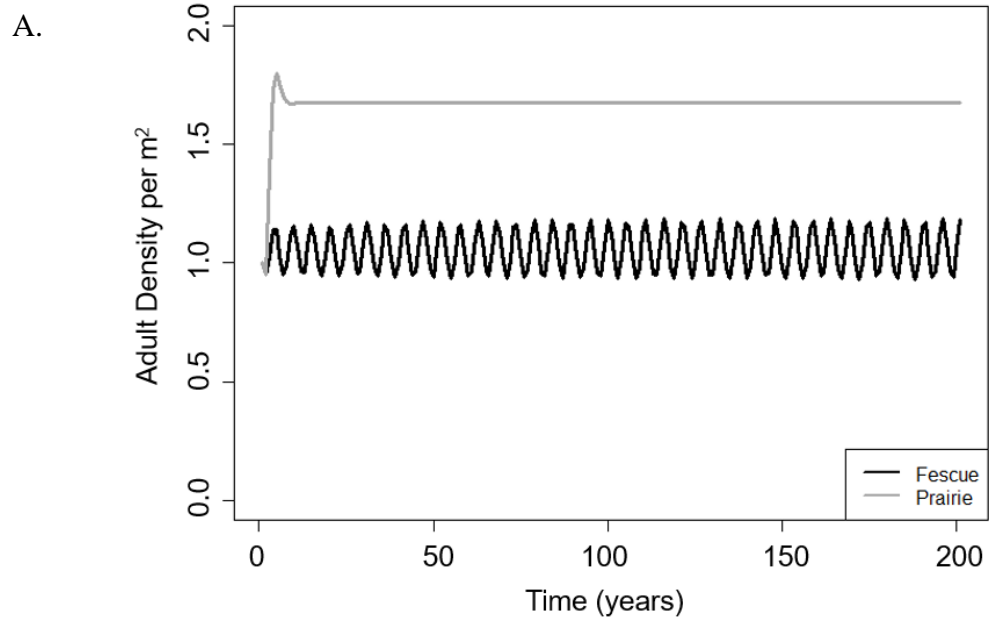
**Figure 3.** A) Adult density of *Lithobates areolatus* (Crawfish Frogs) per m$^2$ of a Fescue and Prairie wetland from a single 200-year deterministic time-series. B) Average density (±SD) of adult *L. areolatus* per m$^2$ of a Fescue and Prairie wetland in stochastic simulations under four stochastic reproductive failure frequencies (5%, 10%, 15%, 20%).
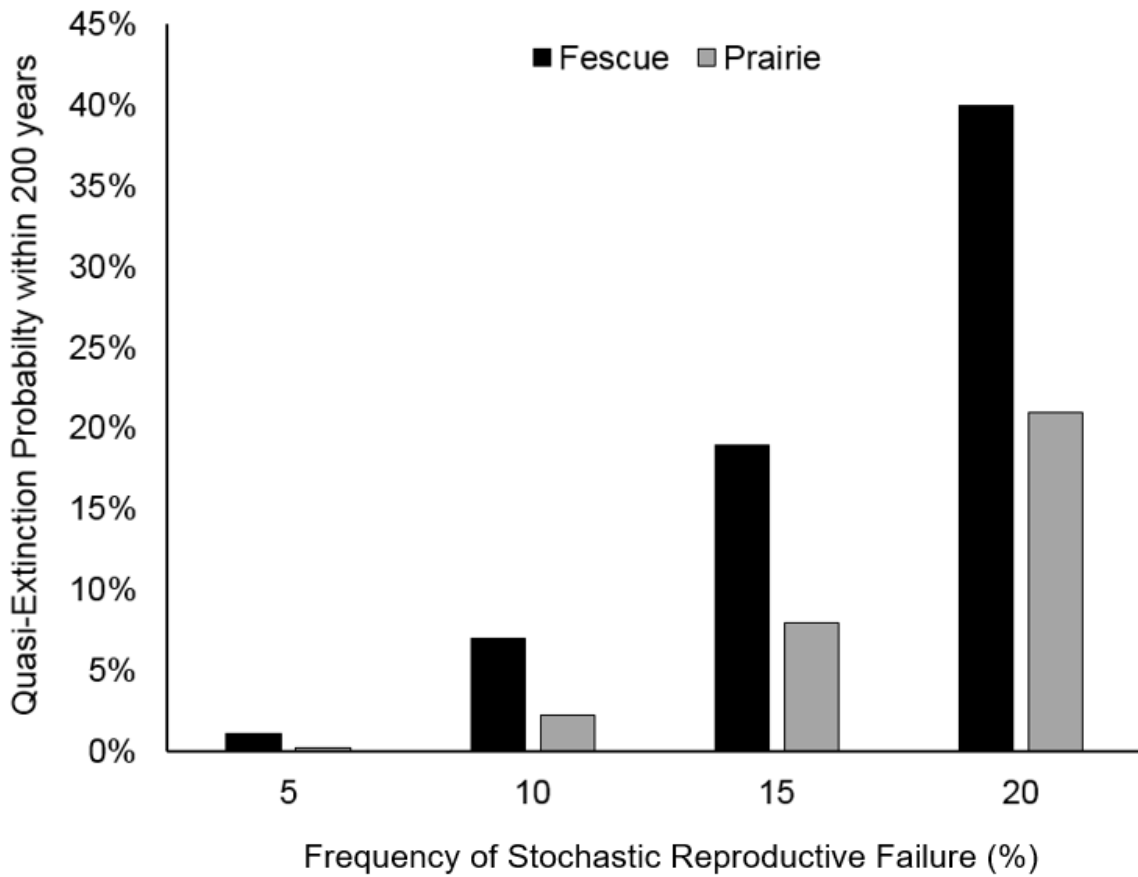
**Figure 4.** Quasi-extinction (N<10 individuals) probability within 200 years under four frequencies of stochastic reproductive failure (5%, 10%, 15%, and 20%) for a *Lithobates areolatus* population breeding within a Fescue-dominated wetland and a wetland dominated by mixed Prairie vegetation.

# IACUC Approval Documents

**UNIVERSITY OF ARKANSAS**

Office of Research Compliance

To:          John David Willson
Fr:           Craig Coon
Date:        March 8th, 2018
Subject:     IACUC Approval
Expiration Date:  March 1st, 2021

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **18097**: *Density-dependence in a larval amphibian.*

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond March 1st, 2021 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: John Willson and Chelsea Kross. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

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# Chapter 4

Indirect Effects of Fire on Crawfish Frogs (*Lithobates areolatus*): Deposition of ash and

vegetation biomass

Chelsea S. Kross

and

John D. Willson

## Abstract

Prescribed fire is an important management tool used in the restoration and management of disturbance-adapted habitats (e.g. grasslands, pine savannahs, glades). Seasonal prairie wetlands can be affected by prescribed fire through a reduction in vegetative biomass, which serves as an important nutrient base, or through the deposition of ash from surrounding terrestrial habitat. While it has been well-documented that terrestrial amphibians can experience mortality when prescribed fire occurs, few studies have assessed the effects of fire on larval amphibians. We used a mesocosm approach to investigate the effects of prescribed fire on larval amphibian development. We had treatments representing three scenarios: (1) Control – unburned prairie vegetation, (2) Ash Addition – unburned prairie vegetation, with added ash to simulate deposition from surrounding terrestrial habitat, and (3) Vegetation Reduction – 50% of vegetation burned, with ash retained. We compared average time to metamorphosis, snout-vent-length, mass, total tadpole biomass, and survival of larval Crawfish Frogs (*Lithobates areolatus*) among treatments. Larval survival was lowest in the Vegetation Reduction treatment, but other developmental metrics were similar among treatments. Additionally, overall biomass of successful metamorphs in the Vegetation Reduction treatment was significantly lower than the control treatment. Our results suggest that prescribed fire has negligible effects on the development of aquatic amphibian larvae but might result in reduced survival and biomass production if burning occurs prior to wetlands filling. Mechanisms that explain the potential indirect effects of prescribed fire on larval amphibian development are needed to develop adequate management plans in fire-maintained ecosystems.

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**Introduction**

Prescribed fire is an important management tool for many disturbance-adapted terrestrial systems and their associated wetlands (Gilliam and Platt 1999, Kirkman 1995, Lugo 1995, Gray et al. 2013, Swan et al. 2015). Historically, many of the most imperiled ecosystems relied upon disturbances such as fire and grazing to prevent succession, and to maintain habitat heterogeneity and community structure (Mushinsky and Gibson 1991, Lettow et al. 2014, Swan et al. 2015). Fire-suppression and land-use change have resulted in the degradation of habitat, loss of biodiversity, and an increase in high-intensity wildfires (MacDougall et al. 2004, Hautier et al. 2015, Durigan and Ratter 2016). Conservation efforts within fire-adapted systems have included determining the optimal prescribed fire regimes for different regions and habitats, and evaluating how prescribed fire effects floral and faunal diversity and persistence (Lyet et al. 2009, Rooney and Leach 2010, Price et al. 2015).

North American grasslands and their associated wetlands are among the most imperiled ecosystems world-wide (Nature Conservancy 2016). Their flat topography and rich soils made grasslands ideal for agricultural and urban development within the United States, and as a result, an estimated 95% of historic prairie habitat has been altered or destroyed (Gleason et al. 2008). The loss of grasslands and their associated wetlands has negatively affected a wide variety of species (e.g. Lannoo et al. 1994), and several grassland species are listed by state and federal governments (e.g. Black-footed Ferret [*Mustela nigripes*], Crawfish Frog [*Lithobates areolatus*], Greater Prairie Chicken [*Tympanuchus cupido*]; IUCN 2019). As a result, grassland restoration and protection has been a major focus of conservation aimed at maintaining and recovering populations of imperiled species (Collins et al. 1998, Brennan and Kuvlesky 2005).

Habitat degradation is a primary driver of the amphibian diversity crisis (Stuart et al. 2004, Powers and Jetz 2019). Many amphibian species inhabit fire-adapted and fire-maintained landscapes, including critically endangered species, such as the Dusky Gopher Frog (*L. sevosa*; Thurgate and Pechmann 2007) and the Reticulated Flatwoods Salamander (*Ambystoma bishopi;* Gorman et al. 2013). When properly applied, prescribed fire can be used to maintain a diverse assemblage of amphibians. For example, Mushinsky (1985) found that amphibian species richness was higher in habitats with higher habitat complexity due to a 5-7 year burn cycle compared to a control treatment plot where a burn had not occurred in over 20 years. However, most research on fire and amphibians has been focused within hardwood forests and stream habitats and has found mixed effects on amphibian occupancy patterns (Hossack and Corn 2007, Perry et al. 2009, Chelgren et al. 2011) and negative effects on larvae in streams (Gamradt and Kats 1997, Hossack et al. 2006). Effects of fire might be very different in grasslands, where many amphibians are adapted to a drier climate and primarily breed in wetlands.

Although some studies have addressed the effect of fire on terrestrial life stages of amphibians (Russell et al. 1999, Chelgren et al. 2011), there is a paucity of studies examining how fire might affect wetland communities and amphibian larvae within fire-maintained ecosystems (Pilliod et al. 2003). Prescribed fire is often implemented during the dormant (Late Fall - Early Spring) season (Knapp et al. 2009), when many amphibian species are breeding. Prescribed fires completed during the dormant season could affect amphibian development in seasonal wetlands through two primary pathways: (1) reduction of vegetative biomass, which serves as an important nutrient base, and (2) deposition of ash from surrounding terrestrial habitat. Reducing wetland vegetation biomass might reduce epiphyton production, which could reduce food availability during the early stages of larval development, leading to increased

competition for food resources (Morin and Johnson 1988, Rubbo et al. 2008). The deposition of ash might alter water chemistry (Battle and Golladay 2003), which can affect larval development and survival (e.g. Cohen et al. 2012, Kross et al. Accepted), as well as nutrient availability in wetlands (Pilliod et al. 2003). Due to the prevalent use of fire in grassland management, studies are needed to determine how fires might directly and indirectly affect larval amphibians.

We conducted a mesocosm experiment to investigate the effects of prescribed fire on the development of larval Crawfish Frogs (*L. areolatus*), a grassland amphibian that has declined across its range (Hammerson and Parris 2004). Specifically, we manipulated vegetation biomass and the amount of ash from burned vegetation within experimental mesocosms and measured larval survival and development. We hypothesized larval development and survival would be slowed or reduced in treatments where vegetation biomass had been reduced compared to treatments where there was no change in vegetation biomass or in treatments that received ash.

**Methods**

Species Description

The Crawfish Frog is an obligate burrow-dwelling amphibian that inhabits grassland habitats of the eastern Great Plains, but is in steep decline throughout its range (Parris and Redmer 2005). The IUCN has listed the Crawfish Frog as Near-threatened, however the species was last assessed in 2004 and needs updating (Hammerson and Parris 2004, IUCN 2019). Crawfish Frog populations are likely to experience prescribed burns and adults have been shown to exhibit different movement behaviors post-burn compared to unburned control habitats (Engbrecht et al. 2012). During a prescribed fire event, adult Crawfish Frogs will escape down their burrow to avoid burning and desiccation (Engbrecht et al. 2012). However, nothing is

known about how larval Crawfish Frogs developing within prairie wetlands might be indirectly affected by fire.

<u>Experimental Design and Data Analysis</u>

We used an array of 1325 L cattle tank mesocosms located at the University of Arkansas Uptown Campus, Fayetteville, to test the effects of vegetation biomass and ash deposition on the development and survival of larval Crawfish Frogs in 2015 and 2016. We had three treatments: 1) Vegetation Reduction; 2) Ash Addition; and 3) Control. The Vegetation Reduction treatment consisted of 1 kg of grassland vegetation; 50% of which was burned prior to addition (i.e. 500 g unburned vegetation, plus 83 g ash), to simulate the loss of wetland vegetation biomass from burns completed when wetlands were dry or partially filled. The Ash Addition treatment consisted of 1 kg of unburned vegetation and 83 g of ash to simulate deposition of ash from surrounding terrestrial habitat. Thus, the Ash Addition treatment was representative of fire that occurs after wetland filling. After burning vegetation we divided all ash equally, so each replicate within the Vegetation Reduction and Ash Addition treatments received 83 g of ash. The Control treatment consisted of 1 kg of grassland vegetation and represented a habitat without a fire event leading up to or during the Crawfish Frog breeding season. Vegetation consisted of native grass and herbaceous vegetation collected from seasonally flooded areas within a restored prairie (Woolsey Wet Prairie Preserve [WWPS], Fayetteville, AR; a known Crawfish Frog breeding site) managed with annual prescribed fire. One kg of vegetation was used based on previous vegetation studies and measurements of standing vegetation biomass from our collection location (Stoler and Relyea 2011, Kross et al. Accepted). We collected vegetation in late February of both years and allowed it to dry for at least two weeks prior to initiation of the experiment.

In 2015, we assembled 12 mesocosms with six replicates each of the Vegetation Reduction and Control treatments. In 2016, we assembled 24 mesocosms with 12 replicates of the Control treatment, 9 replicates of the Ash Addition treatment, and 3 replicates of the Vegetation Reduction treatment (total N=18 control, 9 Ash Addition, 9 Vegetation Reduction). We filled mesocosms with 1000 L of tap water and allowed them to sit for one week prior to adding vegetation and ash. We inoculated each tank with 0.5 L of a zooplankton concentration collected from a wetland within WWPS. We added vegetation and ash to mesocosms on 25-Mar-2015 and 28-Feb-2016. We collected Crawfish Frog eggs at WWPS in March 2015 (two egg masses) and 2016 (one egg mass) and allowed the eggs to hatch in the lab. After hatching, we mixed clutches and haphazardly allocated larvae (Gosner stage [GS] 25-26; Gosner 1960) into groups of 40 and added them to each mesocosm on 31-Mar-2015 and 22-Mar-2016. We allowed larvae to remain in mesocosms until the appearance of a forelimb (GS 42). We inspected mesocosms daily starting in May. Once at least one forelimb was present, we removed larvae from mesocosms and transported them to the lab, where they stayed until their tail was fully resorbed (GS 46). At GS 46, we measured snout-vent-length (SVL), mass, and time to metamorphosis (TTM). Following emergence of the last metamorph, we calculated biomass and survival for each mesocosm. One control tank failed in 2015 and was excluded from analyses. We compared all development metrics, biomass, and survival data using ANOVA's and type III sums of squares using the Car package (Fox and Weisburg 2019) in R v. 3.6.3 (R Core Team 2020). Since the addition treatment was not replicated across years, we could not include year as a block in our analysis. Thus, to examine the importance of year, we ran an ANOVA that excluded the addition treatment, but included year as a block to determine if there was an effect of year on developmental metrics and survival between the Reduced Biomass and Control

treatments. Following each test, we examined residuals using ggfortify within R (Tang et al. 2016) to check for assumption violations. We arcsine transformed survival data and log transformed SVL, Mass, and Tadpole Biomass to meet the assumptions of normality for both sets of ANOVA's.

<div align="center">**Results**</div>

Average survival was lower in Vegetation Reduction treatments (29%) compared to the Addition (42%) and Control (46%) treatments, but this difference was not statistically significant ($F_{2,32}=2.282$, p=0.12) due to the presence of one mesocosm in the Reduction treatment with very high survival (Figure 1). Exclusion of this outlier (+2.4 SD) resulted in a significant effect of treatment on survival ($F_{2,31}=4.583$, p=0.018). With the outlier excluded, survival was significantly lower in the Reduced Biomass treatment compared to the Control treatment (p=0.014). Survival in the Addition treatment did not significantly differ from the Vegetation Reduction (p=0.09) and Control treatments (p=0.82). Other developmental metrics did not differ among treatments: SVL ($F_{2,32}=0.603$, p=0.553; Figure 2); Mass ($F_{2,32}=0.918$, p=0.409; Figure 3); TTM ($F_{2,32}=1.634$, p=0.211; Figure 4). There was a significant effect of treatment on total biomass of successful metamorphs ($F_{2,32}=3.398$, p=0.046; Figure 5); Tadpole biomass was 50% higher in the Control treatment (31.7 g) compared to the Vegetation Reduction treatment (20.3 g). Larval biomass was significantly lower in the Reduction treatment compared to the Control treatment (Tukey's Test p=0.036). Total biomass in the Addition treatment did not differ significantly from either the Reduction (Tukey's Test p=0.396) or Control treatments (Tukey's Test p=0.526).

Omitting the Addition treatment and including year as a block to compare metrics between the Control and Reduction treatments showed there was an effect of year on TTM

($F_{1,23}=11.75$, $p=0.002$), but not survival ($F_{1,23}=1.716$, $p=0.203$), average SVL ($F_{1,23}=1.90$, $p=0.181$), average mass ($F_{1,23}=1.011$, $p=0.325$), or total tadpole biomass ($F_{1,23}=4.02$, $p=0.06$). There was a significant treatment effect on survival ($F_{1,23}=5.82$, $p=0.024$) and total tadpole biomass ($F_{1,23}=9.28$, $p=0.006$); both were significantly lower in the Reduction treatment. For SVL ($F_{1,23}=0.695$, $p=0.413$), mass ($F_{1,23}=0.543$, $p=0.469$), and TTM ($F_{1,23}=0.316$, $p=0.579$), there was no significant treatment effect. Thus, although we were not able to account for year in our full model, it appears that year primarily affected developmental timing, and not survival or growth.

## Discussion

We found no effects of ash addition, suggesting that deposition from fire in the surrounding terrestrial habitat has minimal positive or negative effects on larvae. Conversely, lower biomass and survival in the Vegetation Reduction treatments suggests that removal of vegetation biomass by fire may reduce amphibian recruitment. Mechanistically, we suspect there was increased competition for forage due to lower periphyton growth during the early stages of the experiment resulting in decreased survival. Following the early die-off, surviving individuals from the Vegetation Reduction treatment might have experienced less competition and were able to consume enough food to reach metamorphosis at a similar time and size as our Control and Addition treatments.

Ash deposition had a minimal effect on Crawfish Frog tadpole development: the extent to which similar dynamics would hold in natural ponds remains unclear. The larvae in the Addition treatment were similar in size, time to metamorphosis, and had similar survival compared to our Control treatment. In contrast, McDonald et al. (2018) found that Gray Treefrog (*Hyla chrysoscelis*) tadpoles were smaller in ash addition treatments compared to treatments without

ash, which the authors speculate might be related to trace elements associated with fire or changes in vegetation nutrient composition. In the same study, periphyton growth was higher, albeit not significantly so, in ash addition treatments, but larval Gray Treefrog development was slower; zero individuals from the ash treatments had hind limbs after a 3-week period, in contrast over 30% of individuals from the control treatment had hind limbs (McDonald et al. 2018). In a field experiment, Noss and Rothermel (2015) found that larval Oak Toads (*Anaxyrus quercicus*) had higher survival in wetlands that had experienced recent fire, potentially due to their slightly higher pH compared to wetlands that had not been burned within three or more years. These contrasting results highlight the need for understanding species-specific responses to prescribed fire (Pilliod et al. 2003). In particular, the effect of ash deposition on wetland water chemistry still needs to be evaluated. A two-year study on depressional wetlands attributed differences in water chemistry to fire intensity (Battle and Golliday 2003). Understanding the indirect effects of prescribed fire on larval amphibians requires studying interactions between prescribed fire, vegetation, and water chemistry.

The biomass of vegetation that forms the nutrient base for food webs that fuel development of larval amphibians was apparently the strongest driver of total biomass of metamorphs produced. Reduced total biomass production in Vegetation Reduction treatments suggests that removal of standing vegetation by fire prior to wetland filling may reduce amphibian recruitment. This seemed to act primarily through a survival mechanism, indicating contest competition for resources. We suspect lower total biomass and survival in the Reduction treatments were the product of reduced primary production, which might have limited periphyton growth during the early stages of the experiment and resulted in stronger competition for resources within our experimental mesocosms. Although we did not collect periphyton data,

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other studies have shown that periphyton availability is extremely variable (Noss and Rothermel 2015) or can even be higher in fire treatments (McDonald et al. 2018). However, no information is available on within-season changes in periphyton biomass, which might be important for early and explosively breeding amphibians.

Although our experiment found a possible negative effect of recent fire on amphibian development, our experiment did not consider some of the longer-term consequences of fire suppression that have been shown to affect larval development and survival. For example, in the longer term, fire is associated with reduced canopy cover, which increases water temperature and primary production, and can decrease time to metamorphosis and increase larval survival (Harkey and Semlitsch 1988, Anderson et al. 2001). In areas where fire has been suppressed, hardwoods are able to become established in wetlands, increasing canopy cover and decreasing hydroperiod via evapotranspiration, which can negatively affect primary production and amphibian development, and alter amphibian community structure (Halverson et al. 2003, Thurgate and Pechmann 2007, Skelly et al. 2014). Additionally, reduced hydroperiod can increase the risk of catastrophic reproductive failure, which can increase the probability of local extinction (Chandler et al. 2016). While we have examined one set of mechanisms that link fire to amphibian population dynamics, our results need to be interpreted within the broader suite of links among fire, habitat quality, and amphibian populations.

The timing of prescribed fire might have important implications for long-term amphibian reproductive success. Increased time to metamorphosis can negatively affect larval survival by increasing the chance of predation (Anderson et al. 2001) or the risk of desiccation (Skelly 2004) should the wetland dry before larvae have completed metamorphosis. On the one hand, our results suggest that ash deposition has negligible effects on development and thus fire likely

generally provides a net benefit, provided some vegetation remains within wetlands when they fill. However, if fire occurs prior to wetlands filling and vegetation is unable to regrow, spring breeding amphibians might experience lower reproductive success. Growing season burns, which more closely reflect historical burn seasonality (Knapp et al. 2009), might be more beneficial for amphibians and the aquatic habitats they use.

Subsidies (i.e., vegetation from the terrestrial environment) form the energy base of temporary wetlands. Subsidies have been shown to have important effects on primary production, microbial activity, and aquatic consumer (e.g. amphibians) development (Stephens et al. 2013, Cohen et al. 2012, Kross et al. Accepted). Prescribed fire directly affects subsidy composition and biomass, which are the most important drivers of water chemistry and nutrient availability in wetland habitats (Maerz et al. 2010, Kross et al. Accepted). Here, we showed that ash deposition has little effect on amphibian development and survival, however, timing of fire may negatively affect larval survival by reducing vegetation biomass. An understanding of how timing interacts with subsidy composition and biomass to affect juvenile recruitment could provide important insights for managing and conserving amphibian populations within grasslands and other fire-maintained ecosystems.

## Acknowledgements

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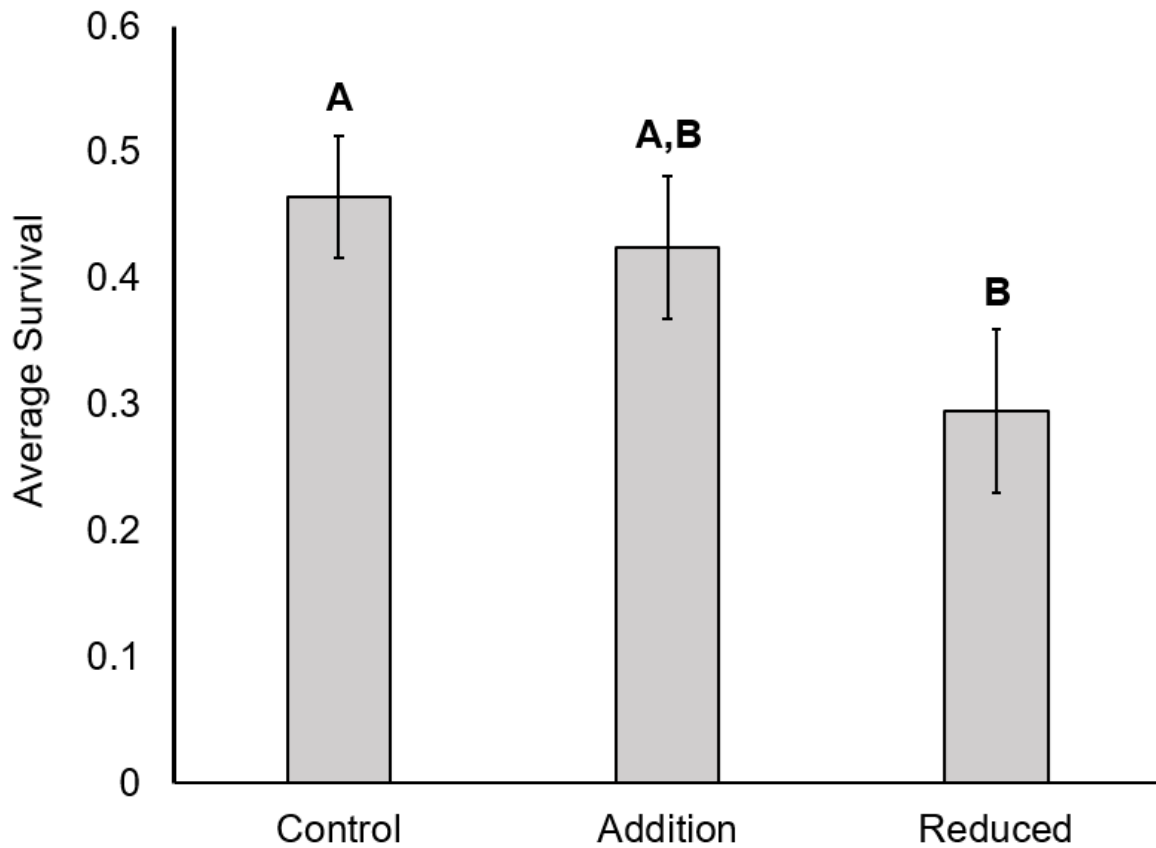
**Figure 1.** Average survival to metamorphosis (± 1 SE) of Crawfish Frog (*Lithobates areolatus*) larvae introduced to control, ash addition, and vegetation reduction with ash mesocosms. Letters indicate significant differences based on an ANOVA and Tukey's HSD. An outlier was removed from the reduced treatment (See Results).
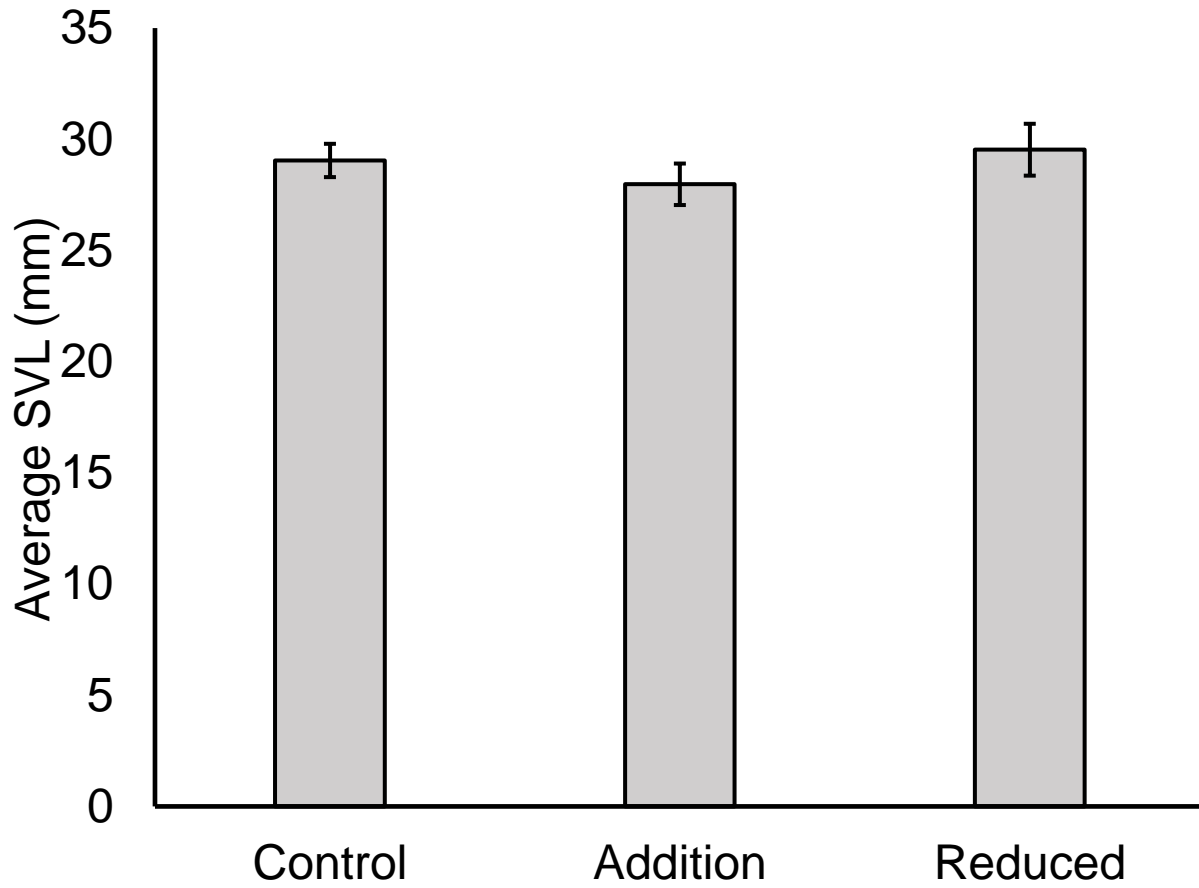
**Figure 2.** Average snout-vent-length (SVL) at metamorphosis (± 1 SE) of Crawfish Frog (*Lithobates areolatus*) larvae reared in control, ash addition, and vegetation reduction with ash treatments.
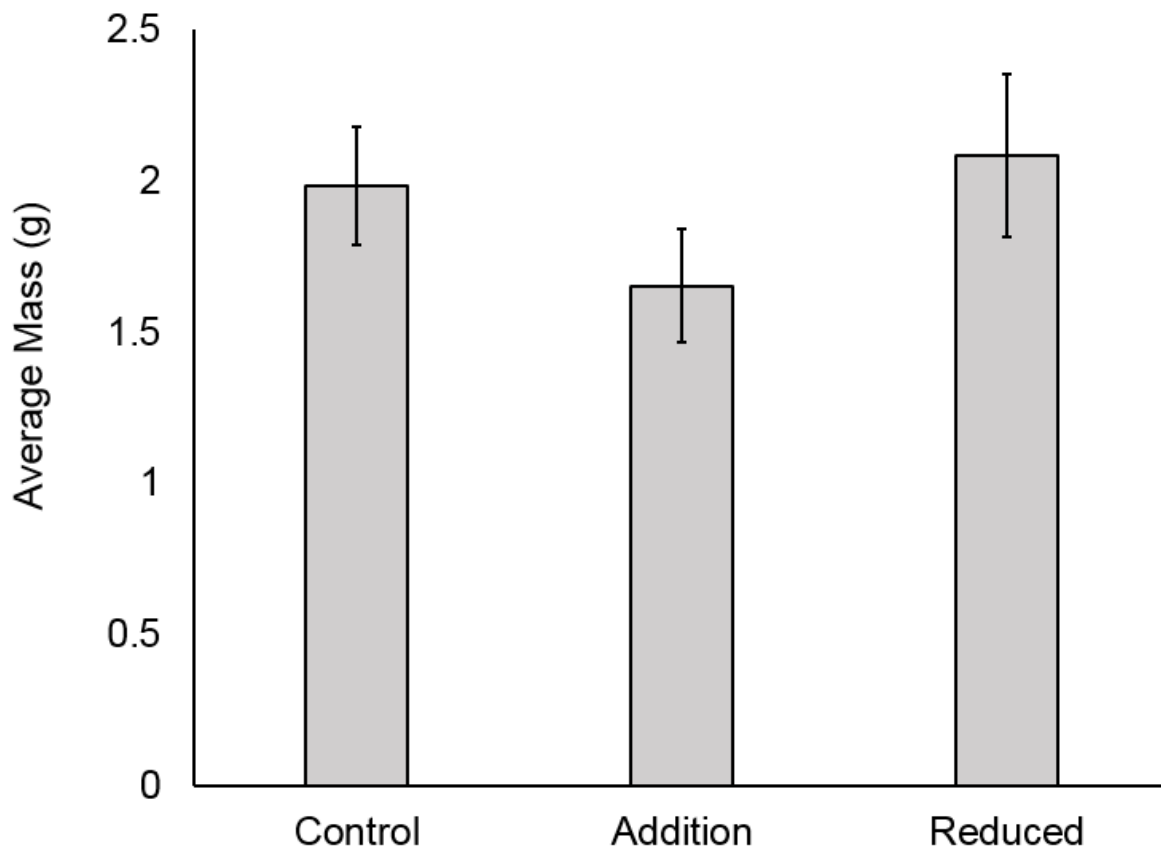
**Figure 3.** Average mass at metamorphosis (± 1 SE) of Crawfish Frog (*Lithobates areolatus*) larvae introduced to control, ash addition, and vegetation reduction with ash treatments.
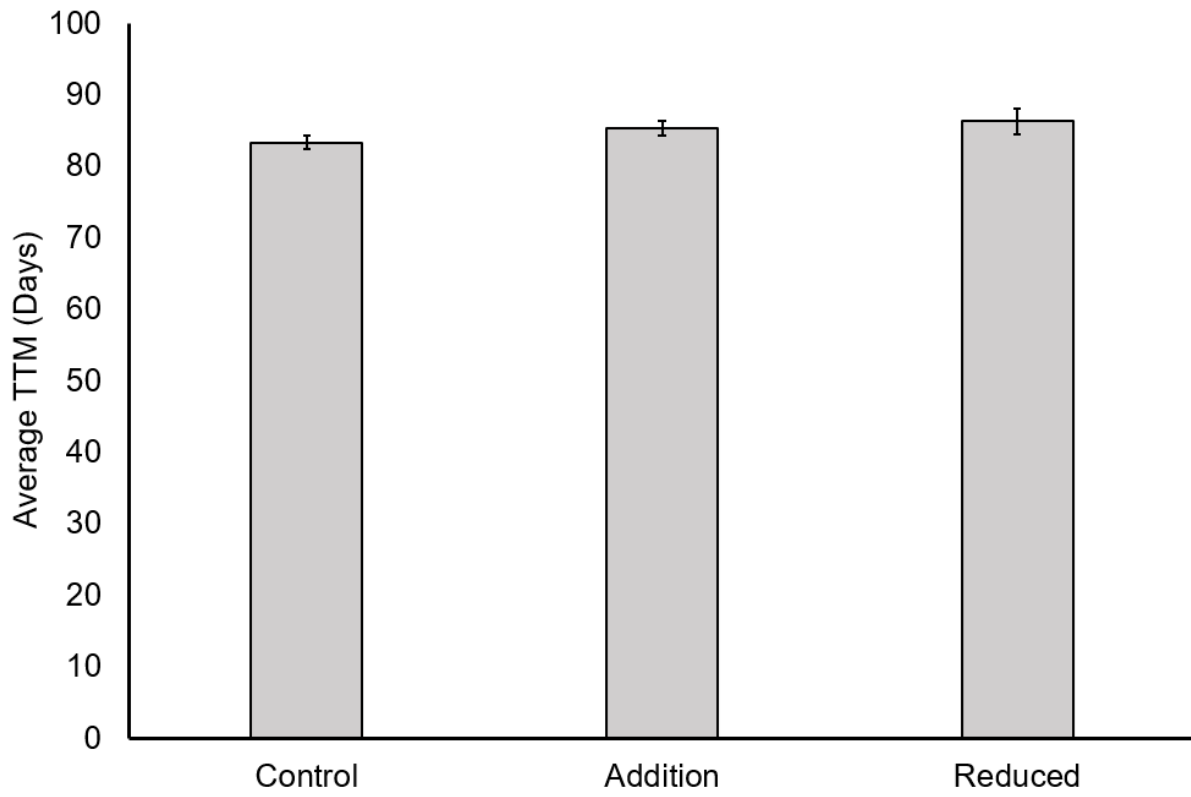
**Figure 4.** Average time to metamorphosis (± 1 SE; TTM) of Crawfish Frog (*Lithobates areolatus*) larvae introduced to control, ash addition, and vegetation reduction with ash treatments.

**Figure 5.** Average total biomass of successfully metamorphosed (± 1 SE) Crawfish Frogs (*Lithobates areolatus*) produced in control, ash addition, and vegetation reduction with ash treatments. Letters indicate significant differences based on an ANOVA and Tukey's HSD.
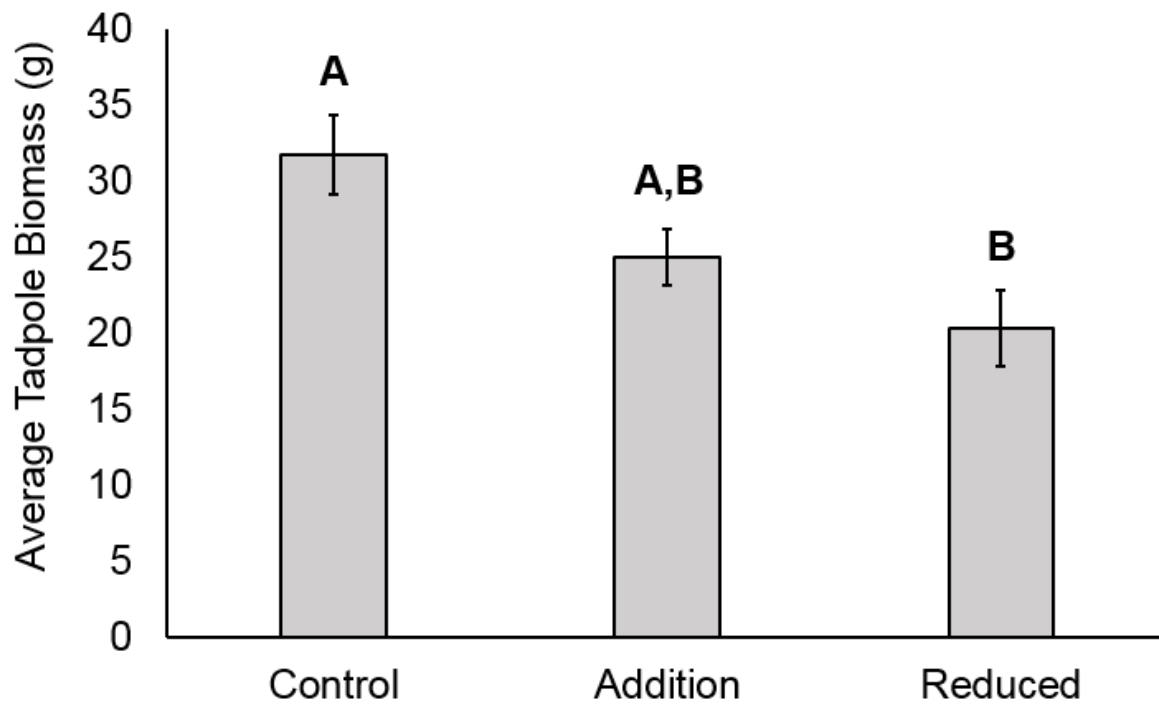
# IACUC Approval Forms

UNIVERSITY OF
ARKANSAS

MEMORANDUM

TO: J.D. Willson

FROM: Craig N. Coon, Chairman

DATE: Feb 6, 2015

SUBJECT: IACUC Approval

Expiration Date: Feb 28, 2018

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your
Protocol: 15033 Effects of litter type on development of larval Crawfish
Frogs (Lithobates areolatus) to begin March 1, 2015

In granting its approval, the IACUC has approved only the information provided. Should
there be any further changes to the protocol during the research, please notify the IACUC
in writing (via the Modification form) prior to initiating the changes. If the study period is
expected to extend beyond Feb 28, 2018 you must submit a **newly drafted protocol**
prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for
more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal
guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

UNIVERSITY OF
ARKANSAS

Office of Research Compliance

To:            John Willson
Fr:            Craig Coon
Date:          May 4th, 2017
Subject:       IACUC Approval
Expiration Date:  May 3rd, 2020

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **17079**: *Effect of non-native vegetation development of amphibian larvae.*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond May 3rd, 2020, you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study:    Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

I, Dr. John D. Willson, certify that Chelsea S. Kross completed the required training as required by the Institutional Animal Care and Use Committee (IACUC) for work completed in Chapter 4.

*J. D. Willson*
J. D. Willson (Jul 16, 2020 20:23 CDT)

7/16/2020

_____

John D. Willson                                    Date
Associate Professor
Department of Biological Sciences

## Conclusions

The threatened status of Crawfish Frogs requires that conservation stakeholders have an understanding of their biology and threats to their persistence across their range. In this dissertation, I have presented a foundation for the continued monitoring of Crawfish Frog populations in Arkansas and elsewhere, threats to their persistence in modified landscapes, and an experimental evaluation of how management of habitat with fire might affect larval development.

Chapter 1 reports results of the most comprehensive landscape-scale occupancy study of *L. areolatus* to date, focusing on an increasingly human-altered region at the core of its historic range: Northwest Arkansas. We had two primary hypothesizes: 1) *L. areolatus* occupancy would be negatively associated with urban land cover and positively associated with historic prairie and prairie mound density, and 2) that many of the historic records near urban centers would have been locally extirpated. We performed 405 surveys at 81 sites across Northwest Arkasnas and found that  the overall naïve occupancy of Crawfish Frogs was 26% indicating that much of the apparently suitable habitat was unoccupied. We did not detect *L. areolatus* at almost 40% (6/16) of historic locations during our study, indicating these populations have likely have likely been extirpated due to increasing urbanization in the region. Crawfish Frog detection probability was high; thus, we expect that had Crawfish Frog been present at the historic sites, we would have detected them. Our results indicate that Crawfish Frog populations are more likely to be found in areas where habitat has remained relatively intact, as indicated by a high density of prairie mounds. Weak associations with soil covariates hinted that soils may have been important in mediating occupancy historically, but these relationships may have been weakened by past and recent extirpations. Our study provides a framework for optimizing Crawfish Frog inventory and

monitoring strategies, including identifying survey conditions that maximize detectability and prairie mounds as an integrative proxy for habitat quality that can be rapidly derived from remote-sensed data. The Crawfish Frog is threatened throughout most of its range due to the loss of habitat and our results suggest that trend is no different in Northwest Arkansas.

In Chapter 2, we used a mesocosm approach to investigate how litter (Native Prairie or Non-Native Tall-Fescue Grass) surrounding wetlands and timing of oviposition affected larval amphibian development. We hypothesized that non-native Tall- grass Fescue would increase survival and growth of amphibians due to its expected labile (i.e. more readily broken down) properties. Our results suggested that changes in vegetation composition and the alteration of subsidies forming the base of ephemeral aquatic systems can have mixed effects on Crawfish Frog larvae, making it difficult to determine how land-use change and introduction of non-native vegetation might affect anuran populations. Specifically, our experiments demonstrated that non-native Fescue vegetation can have either positive or negative effects on anuran hatching success and larval development, depending on when wetlands are filled and breeding occurs. When eggs and larvae were reared in adequately oxygenated Fescue Treatments, eggs hatched successfully and larvae metamorphosed 7-days earlier and had twice the mass than larvae reared in Prairie Treatments. In contrast, when eggs and larvae were introduced to mesocosms at the same time as litter, dissolved oxygen (DO) concentrations crashed and resulted in fewer or no eggs hatched and larvae did not survive to metamorphosis. Low DO conditions were driven by the higher nutrient concentrations found in Fescue Treatments, which increased microbial activity, leading to DO depletion and subsequent death of eggs and larvae. The pattern of the short-term DO depletion in Fescue was also observed in Fescue-dominated wetlands in the field. Collectively, our data suggest that nonnative Fescue

grass could have positive effects on the development of some amphibians but could cause mass mortality for species that exhibit explosive breeding or early-spring breeding coincident with wetland inundation.

In Chapter 3, we combined the results of a mesocosm experiment and demographic population modeling to demonstrate that larval density and rearing habitat (vegetation type) interact to affect Crawfish Frog population dynamics and extinction risk. Specifically, we compared the effect of larval density and vegetation composition (native prairie or non-native Tall-grass Fescue) on larval Crawfish Frog survival and used computer simulations to extrapolate our emergent effects of experimental results on adult Crawfish Frog population size and extinction risk. Overall, as larval density increased, survival decreased, indicating strong density-dependence in the larval stage. Average survival was similar between vegetation treatments at low and high densities, but survival was much higher in Fescue treatments at moderate and moderately-high densities, indicating more strongly overcompensatory density-dependence in Fescue treatments. Models parameterized using our experimental data revealed that Crawfish Frog population size was more variable in Fescue simulations, leading to increased extinction risk compared to Prairie simulations. Additionally, increasing frequency of reproductive failure greatly increased quasi-extinction probability in Fescue simulations when compared to Prairie simulations, suggesting that an apparently positive effect on recruitment can destabilize population dynamics and increase local extinction risk. The results highlight that while rearing habitat can differentially affect survival, these data need to be considered within the context of natural population drivers, such as density-dependent larval survival, to infer population-level effects.

In Chapter 4, we assessed two indirect effects of fire on larval amphibians. Although mortality of terrestrial amphibian life stages due to prescribed fire has been well-documented, few studies have assessed the effects of fire on larval amphibians. Using a mesocosm approach, we compared the effects of ash addition and reduced vegetation biomass on larval Crawfish Frog development. We found no effects of ash addition on larval development and survival, suggesting that ash deposition from fire in the surrounding terrestrial habitat has minimal positive or negative effects on Crawfish Frog larvae. Conversely, lower biomass and survival in the Vegetation Reduction treatments suggests that removal of vegetation biomass by fire may reduce amphibian recruitment. Mechanistically, we suspect there was increased competition for forage due to lower periphyton growth during the early stages of the experiment, resulting in decreased survival. Following the early die-off, surviving individuals from the Vegetation Reduction treatment might have experienced competitive release and were able to consume enough food to reach metamorphosis at a similar time and size as our Control and Ash Addition treatments. Our results suggest that prescribed fire has negligible effects on the development of aquatic amphibian larvae, but might result in reduced survival and metamorph biomass if burning occurs in the dormant season, prior to wetlands filling.

The combined results of this work indicate that Crawfish Frogs require high quality natural habitat for population persistence. The loss of historical populations in urban areas, increased extinction risk in agricultural habitats, and common occurrence of populations on agricultural land suggests that Crawfish Frog populations within Northwest Arkansas, and likely elsewhere, will continue to decline. Restoration and active management can be successful for Crawfish Frogs (Lannoo et al. 2017, Baecher et al. 2018) and should be used to maintain and perhaps reintroduce viable populations throughout their range.

# Literature Cited

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