

Land-use alters the form of larval density dependence to increase extinction risk in a grassland amphibian

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Abstract

An understanding of interactions between anthropogenic stressors and intrinsic population drivers is needed to fully understand wildlife population declines. Density dependence is a key aspect of population regulation for many species, especially for species that have high reproductive potential, such as amphibians. However, patterns of density dependence have been characterized for only a few species and little work has evaluated how density-dependent interactions may be altered by anthropogenic stressors. We combined the results of a mesocosm experiment with demographic population modeling to investigate how the conversion of native prairie to agricultural grasslands dominated by Tall Fescue grass (*Lolium arundinacea*) affected larval density dependence and adult population size of an imperiled amphibian, *Lithobates areolatus* (Crawfish Frog). Overall, density dependence was overcompensatory, suggesting that *L. areolatus* exhibits 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 project long-term population dynamics. Simulated populations breeding in Fescue-dominated wetlands had a more variable population size and up to 400% higher probability of quasi-extinction within 200 years, compared to populations breeding in ponds with prairie vegetation. Without varying density in experimental treatments and using mathematical models to project emergent population dynamics, our mesocosm experiment results would have suggested a slightly positive effect of Fescue grass on amphibian development and survival. Vegetation changes surrounding breeding wetlands might play an important role in the decline of amphibian populations persisting in low-intensity agricultural areas.

Introduction

To understand animal populations during the Anthropocene, scientists need to consider interactions between 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 (Willson *et al.*, 2012). There is a need to combine experimental results with species demographic and life-history information to better understand the effect of anthropogenic stressors at the population level (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 experience 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; Skelly & Kiesecker, 2001; Vonesh & De la Cruz, 2002). Due to the high fecundity of amphibians, there is often a strong effect of larval density on growth (Skelly & Kiesecker, 2001) and survival (Van Buskirk & Smith, 1991). In some cases, contest competition (i.e., asymmetric effects of competition with a few individuals succeeding at the expense of the remaining larvae) can result in relatively stable recruitment across a wide range of densities. Alternatively, scramble dynamics (i.e., symmetric effects of competition across individuals) can lead to overcompensation, with peak recruitment at intermediate densities and low survival

at high larval densities (Van Buskirk & 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 ephemeral breeding wetlands (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 examined interactions (e.g., Vonesh & 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 the survival of anuran and salamander eggs, this mortality can be compensated for by competitive release (i.e., reduced density) in the larval stage, resulting in increased survival of larvae to metamorphosis (Vonesh & De la Cruz, 2002).

Land-use change and habitat loss are primary drivers of global amphibian population declines (Stuart *et al.*, 2004). 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). 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 (Fish & 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; Kross *et al.*, 2020). 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 & Knopf, 1994). Over 95% of the original tallgrass prairies in the United States have been destroyed (Samson & Knopf, 1994), and many have had their wetlands drained and uplands tilled for agricultural use. As a result, agricultural wetlands are often some of the only suitable amphibian breeding habitats in severely altered landscapes (Knutson *et al.*, 2004). However, spatial subsidies that serve as the resource base for the typically ephemeral wetlands found in grassland habitats have changed because of habitat conversion. Vegetation that dominates seasonally inundated prairie wetlands has been converted 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]). Previous work has shown that Fescue grass can positively affect larval amphibian development under well oxygenated, low-density conditions (Kross *et al.*, 2020). Specifically, nitrogen (N) and phosphorous (P) contents are 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.*, 2020). However, it is unclear how this difference in vegetation could change based on larval density or whether the effect could alter the 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 the 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 moderate densities due to nutrient composition (Kross *et al.*, 2020), leading to stronger (i.e. more overcompensatory) density dependence. Given that strong overcompensatory density dependence can be destabilizing (May, 1975; Wilbur, 1980; Vonesh & De la Cruz, 2002), we ultimately predicted higher variability in population size and greater stochastic extinction risk in Fescue simulations.

Materials and methods

Study species

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 & Redmer, 2005). Declines in *L. areolatus* are largely associated with the loss of suitable terrestrial and aquatic habitat (Parris & Redmer, 2005). The IUCN (Hammerson & 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 the greatest conservation need in Alabama, Arkansas, Kansas, Kentucky, Louisiana, Mississippi, Missouri, Oklahoma, Tennessee, and Texas. *Lithobates areolatus* is an explosive breeder, with a breeding season that typically lasts 1–4 weeks (Heemeyer & Lannoo, 2012). Adults spend the non-breeding season in terrestrial burrows relatively far from breeding wetlands, thus the density of adults in the terrestrial landscape is presumably low and populations are thought to be regulated primarily through density dependence at the larval state, as well as by stochastic environmental drivers (Stiles *et al.*, 2016, 2020). They are known to breed in 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 the species population dynamics could provide important implications for amphibian conservation and management.

Mesocosm Experiment

We conducted a mesocosm experiment to evaluate the interactive effects of vegetation type 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.* (2020). In

2018, we constructed an array of 50, 379 L cattle tanks, each with a surface area of 0.42 m², and randomly assigned each tank to one of 10 treatments ($N = 5$ tanks per treatment): five densities (5, 15, 45, 135, and 405) and two vegetation types (Prairie and Fescue). These densities represent larval densities produced by 1 to 20 *L. areolatus* females ovipositing (mean clutch size = 5,000 [Parris & Redmer, 2005]) within a single 100 m² wetland, which are typical densities for our region (Kross, unpublished 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 agricultural 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 asteroides*, *B. diffusa*, and *Persicaria* spp.). We cut and raked vegetation from the vicinity of known *L. areolatus* breeding wetlands in February 2018 and the vegetation air dried in the sun for 2 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 (1 g/L) of dry vegetation to tanks on 2 March 2018 after allowing chlorine to dissipate from tanks. We inoculated each tank with 70 mL of a zooplankton/phytoplankton slurry collected from a wetland within WWPS. 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, USA).

In April 2018, we collected three *L. areolatus* egg masses at wetlands in prairie remnants in Northwest Arkansas. All eggs were collected at the same time and had been deposited within hours of each other. We maintained egg masses in aerated pond water in the laboratory until they hatched (~7 days). 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 2018. 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 on 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 42 by the final draw-down were considered mortalities. We analyzed the effects

of density and vegetation on *L. areolatus* larvae survival using a Two-Factor ANOVA with main and interactive effects of Vegetation Type (Fescue vs. Prairie) and Density (categorical) using the Car (Fox & 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 logit transformed survival probabilities (Warton & Hui, 2011), 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; Willson & 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, with static adult survival and no reproductive failure, 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 & De La Cruz (2002). Briefly, this pre-breeding matrix model included two stages: Juvenile (J) and Adult (A). Adults 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 & 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 reaches maturity at 2 years (Jensen & 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 σ_j . Adults have an annual survival rate of σ_a and reproduce annually following the formula $F[A]$. The model can be expressed in matrix form as:

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

The function $F[A]$ represents the production of juveniles per adult at each yearly time step (Vonesh & De la Cruz, 2002). The $F[A]$ function is the product of adult sex ratio (ρ), annual per capita egg production (ϕ), egg survival (σ_e), density-dependent larval survival at that time step (σ_l), and metamorph survival for the remainder of their first year (σ_m) (Vonesh & De la Cruz, 2002):

$$F[A] = \rho\phi\sigma_e\sigma_i\sigma_m \quad (1)$$

Within the $F[A]$ function, density-dependent larval survival (σ_i) is calculated for each time step using a density-dependent function based on the Beverton-Holt fisheries recruitment model (Beverton & Holt, 1957):

$$\sigma_i = \sigma_{max}/(1 + dT)^\gamma \quad (2)$$

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

$$T = \rho\phi\sigma_e A \quad (3)$$

The density-dependent exponent (γ) in equation 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. In these models, carrying capacity is emergent and varies based on model parameters, rather than being a fixed parameter itself.

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 $\rho = 0.5$. Lannoo & Stiles, (2020) documented adult survival rates of 0.35, 0.48, and 0.81 across 3 years and an average adult survival of 0.70 across other years based on a multi-year drift fence study. We set adult survival at 0.75 in our deterministic model and varied annual adult survival probability uniformly between 0.35 and 0.85 in stochastic simulations. We set egg survival to 0.6 based on hatching success of larval leopard frogs in different vegetation types (Adams & Saenz, 2012; Kross *et al.*, 2020). Larval survival was based on the γ 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 σ_m and σ_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) and used equation 2 to determine the γ and d value that best fit our experimental data for each vegetation treatment. To fit γ and d , we used equation 2 to calculate the density of surviving larvae for a range of γ (1000 increments from 0 to 100) and d (1000 increments from 0 to 0.1) combinations, holding σ_{max} constant. For each γ and d combination, we summed the absolute value of the difference between each observed survival

density and estimated survival density for each combination of γ and d (i.e., residuals). We then used the γ 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 adult survival and the frequency of reproductive failure annually in models parameterized to represent *L. areolatus* populations breeding in a wetland dominated by Fescue or Prairie vegetation. Specifically, we varied adult survival by drawing annually from a uniform distribution between 0.35 and 0.85. 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. Frequency of catastrophic reproductive failure is not known for *L. areolatus*, so we set recruitment ($F[A]$) to zero for a range of potential frequencies: 5%, 10%, 15%, and 20% of years. 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 1000 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). The average life span of a Crawfish Frog is 5 years (Parris & Redmer, 2005), and some individuals have been documented to live up to 10 years in Indiana (Lannoo & Stiles, 2020). Generation time has been suggested as a better scale for estimating extinction risk as even strongly declining species can be viable over short time periods (O’Grady *et al.*, 2008; Reed & McCoy, 2014). Thus, we used a 200-year time series, representing 40 generations, to provide a robust estimate of extinction risk over a biologically relevant time scale (Reed & McCoy, 2014). 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 adults/ m^2 . If a population dropped below this value, the population was considered permanently extinct.

Results

Mesocosm experiment

Density ($F_{4,40} = 138.5$, $P < 0.001$) and Litter type ($F_{1,40} = 4.51$, $P = 0.04$) significantly affected larval survival,

and their interaction approached statistical significance ($F_{4,40} = 2.11$, $P = 0.10$; Figure 1). Survival was lowest in the highest density treatments and, generally, survival was higher in Fescue treatments (Figure 1). Average larval survival was high (>50%) in low to moderate density treatments (5, 15, and 45), ranging from 57 to 88% in Fescue treatments and from 53 to 72% in Prairie treatments. The largest difference in average survival between litter type was observed in the moderately-high density (145) with 4% surviving in Prairie treatments compared to 31% 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 overcompensatory dynamics more strongly than larvae in the Prairie treatment.

Population simulations

In deterministic simulations that did not include stochastic reproductive failure, average adult population density in Prairie wetland simulations initially overshoot carrying capacity, but rapidly returned to a stable equilibrium density of 1.67 per m² 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 m² of wetland) (Figure 3a). In stochastic simulations, average adult density was lower in the Fescue wetland population and was much more variable than that of the Prairie wetland population (Figure 3b). Average adult population density was similar across reproductive failure rates for both Fescue (0.92–1.04 per m² of wetland) and Prairie (1.06–1.17 per m² of wetland) simulations, but variance increased as failure rates increased from 0.53 to 0.78 SD in Fescue simulations, and from 0.29 to 0.34 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 up to four times more likely to go extinct, compared to populations in Prairie (Figure 4). At the lowest reproductive failure frequency quasi-extinction probability was 21% for Fescue and 5% for Prairie population simulations. At the 10% and 15% reproductive failure frequencies, quasi-extinction probability was 42% and 52% for Fescue simulations, and 23% and 34% for Prairie population simulations, respectively. At the highest frequency of reproductive failure (20%), quasi-extinction probability was 69% and 54% 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) 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 compared to Prairie treatments. The steep 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. Thus, the apparently positive effect of Fescue vegetation on recruitment (i.e. higher survival at higher densities) 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

TABLE 1 Parameter values used to model *Lithobates areolatus* (Crawfish Frog) population dynamics under varying frequencies of stochastic reproductive failure

| Parameter | Fixed value | Source |
|--|--|--|
| Clutch size (ϕ) | 5000 | Parris and Redmer, 20055 |
| Egg survival (σ_e) | 0.60 | Kross <i>et al.</i> , 2020 (see methods description) |
| Metamorph survival (σ_m) | 0.20 | Kinney, 2011; Lannoo <i>et al.</i> , 2017 (see methods description) |
| Juvenile survival (σ_j) | 0.20 | Lannoo <i>et al.</i> , 2017 (see methods description) |
| Adult survival (σ_a) | 0.75 (0.35–0.85 in stochastic simulations) | Lannoo & Stiles, 2020 (see methods description) |
| Maximum larval Survival (σ_{tmax}) | 1.00 | This Study |
| Prairie density-dependent coefficient (d) | 0.005 | This Study |
| Prairie density-dependent exponent (γ) | 2.102 | This Study |
| Fescue density-dependent coefficient (d) | 0.0001 | This Study |
| Fescue density-dependent exponent (γ) | 37.73 | This Study |

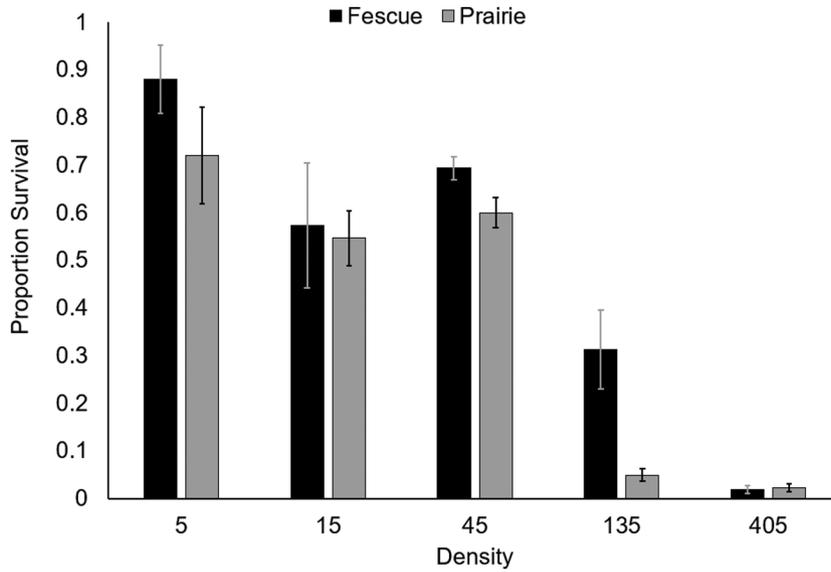


FIGURE 1 Average larval survival (\pm SE) of *Lithobates areolatus* (Crawfish Frog) at each of five stocking densities in Fescue and Prairie vegetation treatments.

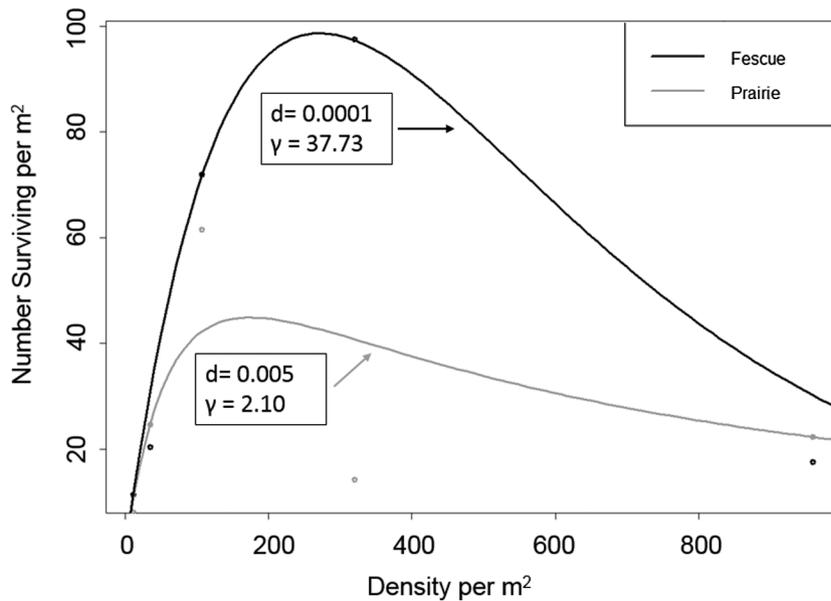


FIGURE 2 Best fit density-dependent survival curves for *Lithobates areolatus* (Crawfish Frog) larvae from Fescue and Prairie mesocosms. Gamma (γ) and (d) represent the density-dependent exponent and coefficient, respectively, from equation 2 (see methods). Densities were transformed to represent density per m^2 .

density-dependent larval survival, to infer population-level effects.

Larval survival in our mesocosm experiment was similar at the lowest and highest densities between vegetation treatments, but overall survival was higher in Fescue treatments at moderate and moderately-high (45 and 135) 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.*, 2020). As a result, larvae can experience enhanced growth (Kross *et al.*, 2020) and survival, under well-oxygenated conditions.

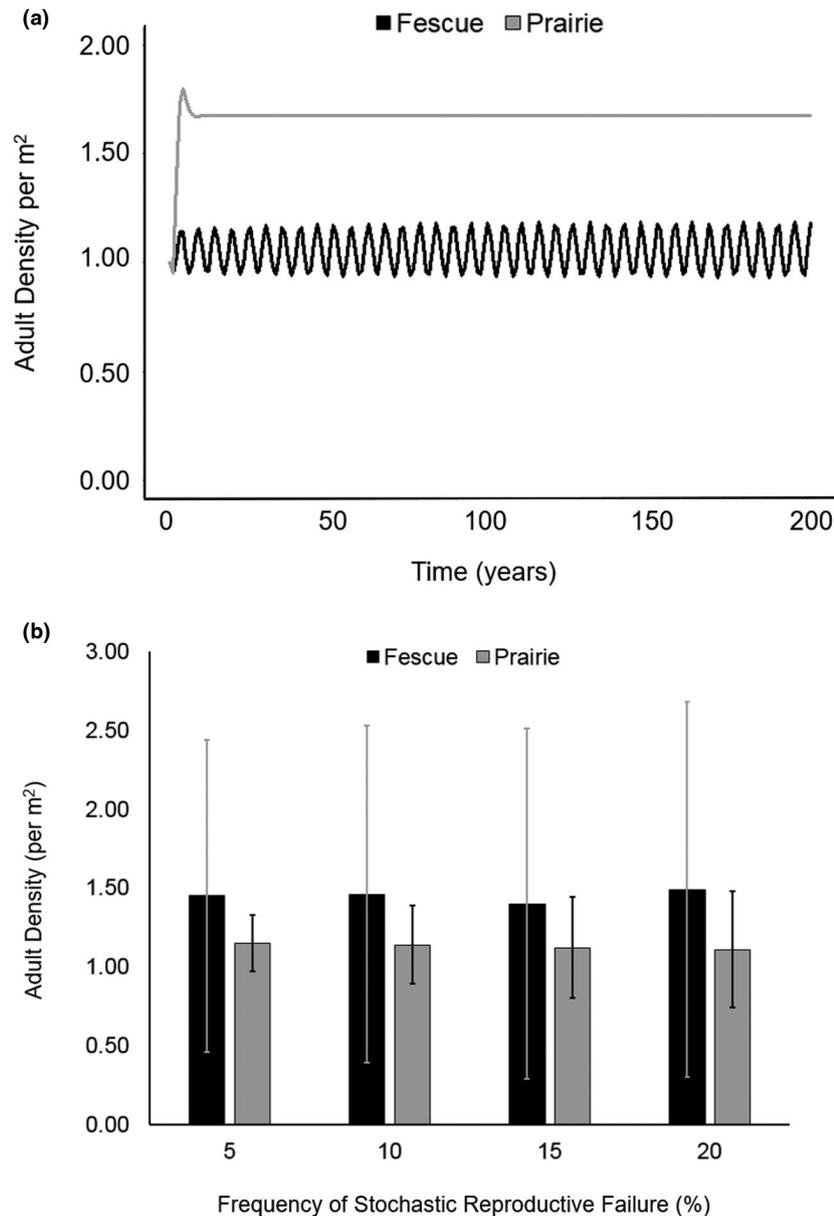


FIGURE 3 (A) Density of adult *Lithobates areolatus* (Crawfish Frog) in a Fescue and Prairie wetland, predicted from a single 200-year deterministic model simulation. (B) Average density (\pm SD) of adult *L. areolatus* in a Fescue and Prairie wetland across 1000 stochastic model simulations under four frequencies of stochastic reproductive failure (5%, 10%, 15%, and 20%).

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 (i.e., a more strongly hump-shaped curve; Figure 2), which led to stable limit cycles (Figure 3a) in deterministic simulations. Stable limit population cycles are an indication of unstable dynamics, driven by high intrinsic rates of population increase (Hassell *et al.*, 1976; May & Oster, 1976). Stochastic demographic

and environmental forcing (i.e. reproductive failure; May, 1975; Melbourne & 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 with favorable conditions where hundreds or thousands of juveniles are recruited into the population (Pechmann *et al.*, 1991). 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.

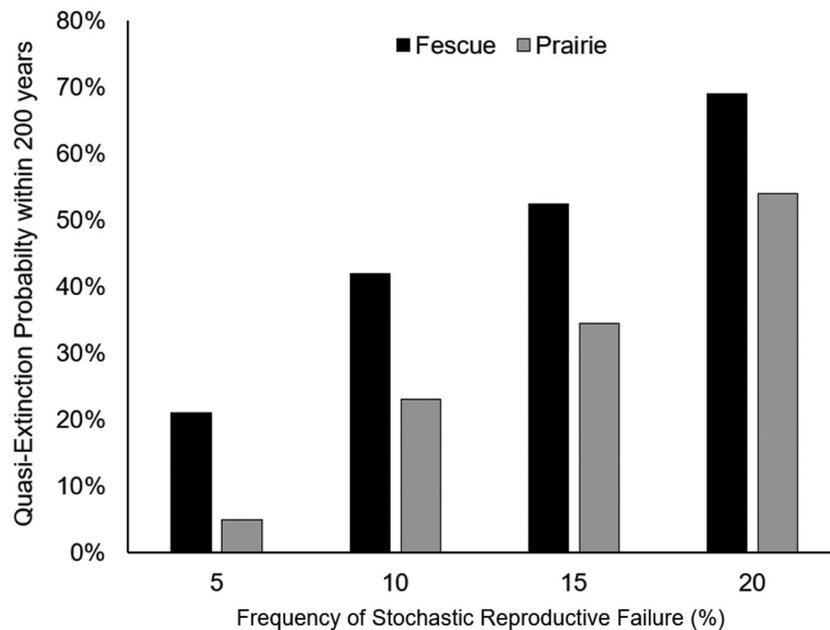


FIGURE 4 Quasi-extinction ($N < 0.05$ females per m^2) probability within 200 years under four frequencies of stochastic reproductive failure (5%, 10%, 15%, and 20%) for a simulated *Lithobates areolatus* (Crawfish Frog) population breeding within a Fescue-dominated wetland and a wetland dominated by mixed Prairie vegetation.

Populations in Fescue simulations were more likely to go extinct due to stronger overcompensation compared to the Prairie populations and extinction risk increased as frequency of stochastic reproductive failure increased. While not incorporated in our model, stochastic variation may also be higher in agricultural wetlands. Amphibians breeding in Fescue-dominated 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.*, 2020). If this crash coincides with oviposition, complete mortality of eggs or larvae can occur (Kross *et al.*, 2020). Additionally, many agricultural wetlands have been modified to maintain longer hydroperiods, which can facilitate colonization by fish, making the wetlands temporarily or permanently unsuitable for amphibian reproduction (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). Any or all of these factors could lead to increased frequency of reproductive failure in populations breeding in agricultural wetlands, thus increasing extinction probability relative to those breeding in 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. While some studies do manipulate density, they are usually not comprehensive enough to model emergent effects on population dynamics (e.g., Williams *et al.*, 2008; Cotton *et al.*, 2012). 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 the results of experimental studies focusing on amphibians (Vonesh & De la Cruz, 2002; Willson *et al.*, 2012). Models from Willson *et al.* (2012) revealed that some detrimental effects measured in the lab had minor population-level consequences, while others were strong drivers of population 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 (Pittman *et al.*, 2014) and when reproductive success is high, hundreds or thousands of juveniles can disperse to the surrounding landscape, as well as to 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 (Willson & Hopkins, 2013). More complex modeling efforts (e.g., Willson & 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 across taxonomic groups (Gaston & Fuller, 2008). In our system, replacing the vegetation forming the nutrient base of breeding wetlands increases the risk of reproductive failure (Kross *et al.*, 2020) 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 persisting in low-intensity agricultural areas. Further, there is a need for understanding the effects of vegetation changes in grasslands on adults in terrestrial habitats. Direct mortality as a result of human or agricultural activities (e.g., mowing, trampling, soil compaction, etc) or indirect effects on growth, survival, reproduction, or dispersal resulting from changes in the food base, predator community, vegetation structure, or abiotic environment (e.g. thermal or hydric conditions, burrow availability, etc.) could all present additional threats to amphibian populations.

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.*, 2013). 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 a 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). 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.

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Author contributions

CSK and JDW conceived the study. CSK collected data and completed analysis with input from JDW. CSK led writing with contributions from JDW.

Data availability statement

Data are available via reasonable request to the corresponding author.

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