Project T-107-D-1: Demography, community dynamics, and health of reintroduced wood frog populations and resident amphibian communities in restored ephemeral wetlands and oak woodlands in Lake County, IL.

State Wildlife Grant Final Performance Report
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Executive Summary

Several decades of habitat degradation dramatically affected a historic assemblage of amphibians in the Upper Des Plaines region of Illinois (Sacerdote 2009). Forms of degradation included implementation of agricultural drainage tile in amphibian breeding sites, proliferation of invasive shrubs (Sacerdote and King 2014), and a history of fire suppression. These activities altered forest composition which affected pond water chemistry (Sacerdote and King 2009) and resulted in a lack of oak tree recruitment in northern Illinois.

In 1999-2000, the Lake County Forest Preserve District (LCFPD) implemented a major habitat restoration initiative in MacArthur Woods Forest Preserve, focused on restoring the hydrology of the site, rehydrating 100 acres of wetland and 300 acres of hydric soil (Klick 2003). Hydrologic restoration was followed by monitoring of the amphibian community to examine whether three species of extirpated amphibians, wood frogs (*Lithobates sylvaticus*), spotted salamanders (*Ambystoma maculatum*), and spring peepers (*Pseudacris crucifer*) would naturally recolonize the site. When natural recolonization did not occur, Dr. Sacerdote-Velat (project co-PI) and LCFPD carried out a feasibility assessment for egg mass and larval translocation followed by implementation of reintroduction of these three species. After several years of monitoring, successful reproduction of wood frogs was first observed in 2014. Spring peepers successfully reproduced in two ponds in MacArthur Woods following translocation, but densities remained low. Spotted salamanders did not exhibit evidence of persistence following translocation.

In 2015, LCFPD began a restoration effort, the Southern Des Plaines Restoration Project (SDPR) focused on improving oak recruitment in MacArthur Woods, Grainger Woods, Wright Wood, Elm Road Woods, and Ryerson Woods. Restoration management objectives included increasing light transmission to the forest floor through creation of canopy gaps, shelterwood cuts, and removal of largely invasive understory.

During the grant period, LCFPD implemented these oak woodland restoration activities as planned, resulting in a mosaic of canopy cover ranging from 50-80% across a total 910 acres in five study sites (i.e., not including the control site at Old School Preserve). This acreage includes restoration via gap creation and understory thinning in 260 acres of northern flatwoods wetlands and 650 acres of adjacent dry-mesic woodlands.

This study is an effort to examine the effects of this canopy and understory management on the reintroduced and resident amphibian community, demography and health. In this geographic region, natural areas are highly fragmented, presenting obstacles to the resilience of small populations of amphibians and limiting natural dispersal opportunities more generally. As habitat restoration efforts progress within this area, there is great interest in wildlife restoration and translocation to support regional populations of amphibians when fragmentation impedes

natural colonization. However, managers must understand the presence and distribution of *Batrachochytrium dendrobatidis* (*Bd*), the causative fungal pathogen of chytridriomycosis, in the region prior to considering any translocation efforts. Similarly, as it becomes easier for amphibians to move through a landscape with improved habitat quality, the possibility of pathogen movement may also increase.

At the start of the grant period, the PIs began amphibian monitoring efforts in all SDPR sites and in Old School Forest Preserve as a control as it was not part of the SDPR restoration management. The PIs aimed to provide long-term post-translocation monitoring data for the amphibian species reintroduced to MacArthur Woods (i.e., wood frogs and spring peepers), and sought to monitor for any evidence of establishment of spotted salamanders in MacArthur Woods. The wood frog is considered an Illinois Species in Greatest Conservation Need. The spotted salamander is a regionally-rare species in the northeastern morainal division of Illinois, and spring peepers, while considered common at the state level, are a species that may be undergoing local decline in northeastern Illinois (Dan Thompson- Forest Preserve District of DuPage County Pers. Comm. 2014, Bill Graser- Forest Preserve District of Kane County, Pers. Comm., 2015). With local interest in expansion of the wood frog reintroduction effort to other neighboring restoration sites, the PIs included population viability analysis in the study to ensure that the MacArthur Woods wood frog population continues to grow.

As the oak woodland restoration efforts progressed through phased implementation, the PIs also examined changes in community structure through metrics of diversity, richness, evenness, and abundance. The PIs completed analyses of these metrics in resident and reintroduced amphibians across the six sites (i.e., the five study sites plus the control site) to examine changes through time and across management treatments (i.e., gap and understory management versus gap management alone versus a control of unmanaged canopy and understory).

The team also examined changes in health and stress of amphibians as restoration management was implemented for oak woodland restoration efforts. For the examination of amphibian health, the team focused on presence and incidence of *Bd*. In a prior study, Sacerdote-Velat et al. documented the presence of *Bd* in natural areas within Chicago and five surrounding counties, spanning six watersheds, and found that 30% of sites sampled yielded *Bd*-positive samples from bullfrogs, green frogs, or northern leopard frogs (Sacerdote-Velat et al. 2016). Some of these positive samples were found within the Des Plaines watershed, the focus of this study. However, the *Bd* incidence in ephemeral-pool breeding species has not previously been explored in these sites. For this study, the team examined *Bd* incidence across species, sites, years, and restoration management treatments. Paired with *Bd* sampling, the team integrated the use of a novel technique to non-invasively sample amphibian stress by collecting dermal swabs to measure cortisol (Santymire et al. 2018). The examination of amphibian stress via cortisol sampling helped to elucidate changes in amphibian stress response as habitat quality improved. The team also examined species-specific variation in stress with *Bd* infection for those species that were positive for the pathogen.

Highlights of this study's results are detailed below:

- The team observed increasing population size of reintroduced wood frogs in MacArthur Woods and expansion to additional breeding ponds in the site.
- Through the use of call recorders, the team documented in increase in call index of reintroduced spring peepers in MacArthur, reproduction in a third pond within the

site, and the first incident of call documentation for the species in the control site in 2018.

- In sites with gap and understory management prior to the start of sampling (MacArthur, Ryerson, and Elm Road Woods), diversity, and abundance were greatest, and diversity increased as reintroduced wood frogs in MacArthur Woods became more established.
- In the sites undergoing phased implementation of understory removal during the study (Wright Woods and Grainger Woods), abundance and diversity were generally lower. These sites exhibited greater turnover in species composition through the course of the study.
- It became evident that a hydrological issue may persist in the Wright Woods study ponds that is not directly related to the oak management. While site quality improved with removal of invasive shrubs, we observed early pond drying in Wright Woods in each study year, which limited use of the site for recruitment of amphibians.
- The control site, Old School, also exhibited turnover in species composition through the study, with chorus frogs representing main component of the control site community.
- Across species and sites, *Bd* incidence decreased from 17.5% prevalence in 2016 to 8.3% in 2018. Four species exhibited significant decreases in stress as measured by CORT levels in 2018, and all species exhibited a general decrease in CORT in the final season of the study.
- Blue-spotted salamanders and tiger salamanders consistently tested negative for *Bd* despite being captured in ponds with other *Bd*-positive amphibians indicating a lower susceptibility to the pathogen.
- Two of six study sites remained *Bd*-negative throughout the study.
- The team observed a significant effect of oak restoration management on *Bd* incidence with lower *Bd* incidence in both gap only and gap and understory treatment sites as compared to the control.

Study Overview

During this three-year study, the PIs collected demographic, community, and health data for reintroduced and resident amphibian species in MacArthur Wood Forest Preserve, Old School Preserve, Grainger Woods, Wright Woods, Elm Road Woods, and Ryerson Woods (Fig. 1). The PIs completed population estimates and population viability analyses for a reintroduced wood frog population in MacArthur Woods. Additionally, the team completed analyses of community structure and abundance in resident and reintroduced amphibians across six study sites, examining changes through time and across management treatments (i.e., gap and understory management versus gap management alone versus a control of unmanaged canopy and understory). The team also examined changes in health and stress of amphibians as restoration management was implemented for oak woodland restoration efforts. Specifically, the study examined site- and species-specific prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) and stress dynamics in response to oak restoration management treatments.

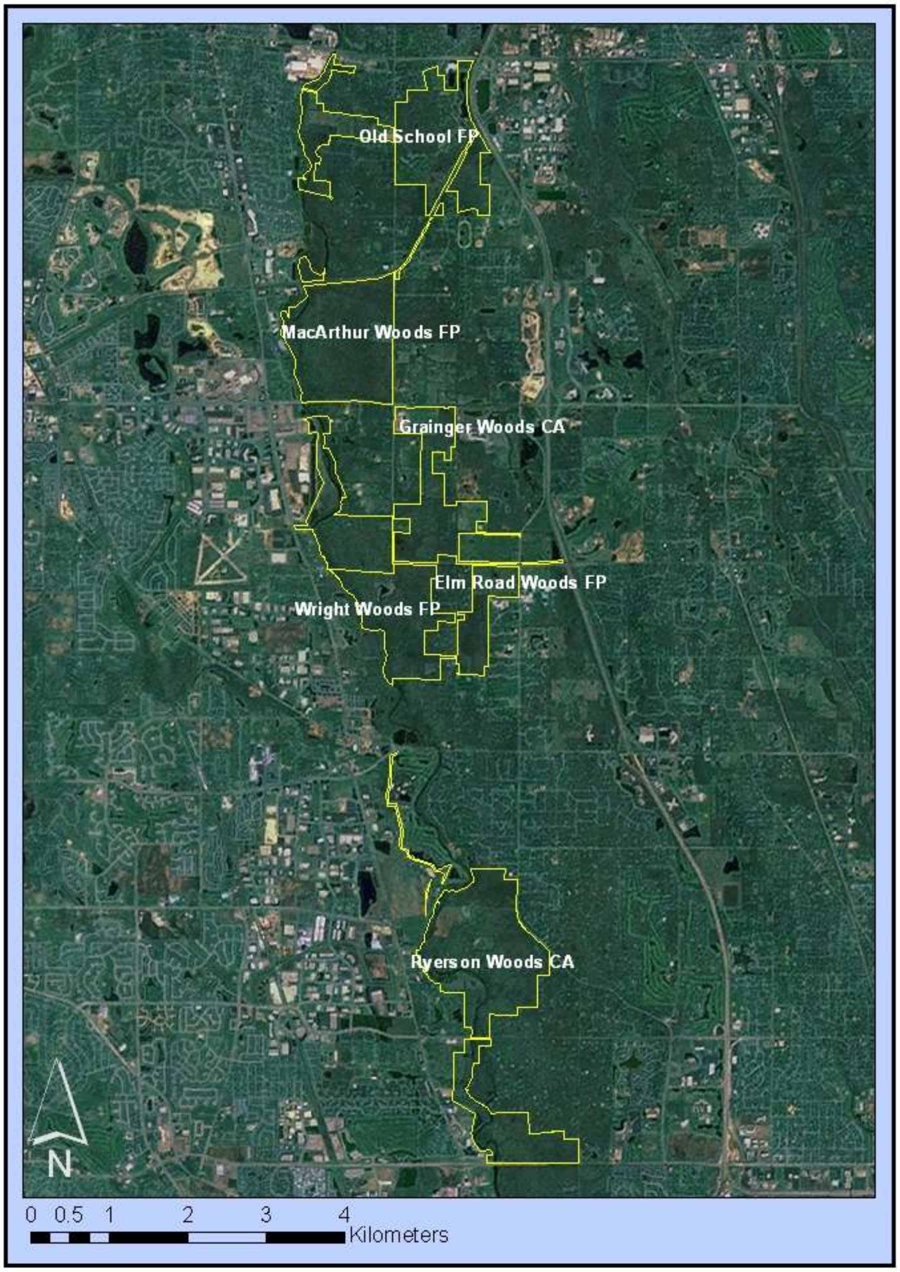

Figure 1. Map of the study sites including the control, Old School Forest Preserve to the north, and the Southern Des Plaines Restoration Project sites: MacArthur Woods, Grainger Woods, Wright Woods, Elm Road Woods, and Ryerson Woods.

Objectives

(1) Collect demographic information for the reintroduced wood frog and spring peeper populations in MacArthur Woods, monitor for evidence of survival and recruitment for translocated spotted salamanders in MacArthur Woods, and examine potential colonization of reintroduced wood frogs and spring peepers into neighboring LCFPD sites in the Des Plaines River Corridor as Southern Des Plaines Restoration efforts progress.

(2) Collect demographic information for the resident amphibian communities (blue-spotted salamanders, western chorus frogs, American toads, tiger salamanders, green frogs, northern leopard frogs, and bullfrogs) in MacArthur Woods and surrounding LCFPD preserves undergoing oak woodland restoration.

(3) Assess health and stress, including presence of the chytrid fungus, (*Batrachochytrium dendrobatidis*; *Bd*), in reintroduced and resident amphibian communities in MacArthur Woods and neighboring LCFPD sites in the Des Plaines River Corridor as habitat restoration efforts progress.

(4) Continue to implement critical measures in LCFPD's Southern Des Plaines Restoration Project benefitting pond-breeding amphibians, including oak woodland restoration, flatwoods wetland restoration, and buckthorn control around breeding pools in LCFPD Des Plaines River Corridor sites: MacArthur Woods, Grainger Woods, Wright Woods/Half Day, Elm Road Woods, and Ryerson Woods Preserves.

Methods

Objectives 1 and 2: Demographic and Community Response of Reintroduced and Resident Amphibians to Oak Woodland restoration management

The team installed 12 three-arm terrestrial drift fence arrays with four pitfall traps each and six funnel traps each in MacArthur Woods, Old School, Grainger Woods, Wright Woods/Half Day, Elm Rood Woods, and Ryerson Woods Preserve. Five terrestrial drift fence arrays were installed in MacArthur Woods: one in Old School, one in Grainger Woods, two in Wright Woods, and three in Ryerson Woods. Additionally, the team installed moveable aquatic drift fences in every study pond to increase captures of breeding adult amphibians and larval amphibians.

For reintroduced amphibians (wood frogs, and spring peepers) and resident amphibians (blue-spotted salamanders, tiger salamanders, American toads, northern leopard frogs, boreal chorus frogs, American bullfrogs, and green frogs), the PIs used photo-mark-recapture to collect species-specific abundance data. The team used the photo-recognition software, Wild-ID, to compute matching scores for cropped images of wood frogs, blue-spotted salamanders, spring peepers, chorus frogs, American toads and northern leopard frogs. The program provides a goodness of fit score, but visual confirmation is used to confirm each match. The program output provides an index of matched images constituting a recapture event, allowing construction of encounter histories for use in Program Mark (White and Burnham 1999).

The team used the robust design model (Pollock 1982) to permit site-by-site estimation of survival and abundance within each season using k primary sampling periods (k= 7 months: late February/early March, late March/early April, late April/early May, late May/early June, late June/early July, late July/early August , late August/early September) and l secondary sampling periods (l= 10 days within each month) with 2 weeks between each primary sampling period. For wood frogs, abundance and survival estimates were incorporated into a stage-structured matrix model to examine growth rate, generation time, reproductive value, sensitivity and elasticity of demographic parameters, and to assess and likelihood of persistence over time (Caswell 2001). To explore the general trend in wood frog breeding effort since translocation and since implementation of oak woodland restoration, annual egg mass counts were regressed across time since 2010 using simple linear regression.

As an additional metric of amphibian richness and breeding effort in each study site, SongMeters (SM2) were deployed at each study pond to continuously record for calling activity of reintroduced wood frogs and spring peepers, and resident frog species (American toad, American bullfrog, western chorus frogs, northern leopard frogs, green frogs) from mid-February through August of each year. Calls were identified to species for each site, and were classified as either index 1, 2, or 3. Call index 1 is defined as when individual frogs can be counted and there is space between calls. Index 2 is defined as when calls of individuals may be distinguished but there is some overlap in the calls. Index 3 is defined as when full choruses occur, calls are constant, continuous, and overlapping. Index 3 is recorded during peak breeding effort at active oviposition sites (Sargent 2000). Call data were used to identify any incidence of dispersal to nearby sites by wood frog, spread of wood frogs and spring peepers to additional ponds within MacArthur Woods, and generally used to supplement trapping efforts in case captures were missed during the gap weeks in sampling due to weather conditions.

To examine changes in community structure across oak restoration treatment sites and the control site, adult and juvenile capture data from drift fences were used to calculate catch per unit effort (CPUE), the number of captures divided by the number of trap nights for each site. Other community structure metrics include the Shannon-Weiner diversity index (H'), $H = -\sum(p_i)x \ln(p_i)$, where p_i is the proportion of the total sample represented by a particular species, evenness (Eh), $Eh = \frac{H}{\ln(S)}$ where S is amphibian richness the number of total species represented in the community. The team used repeated measures analysis of variance (ANOVA) to examine changes in these community metrics across the phased restoration management treatments through time since the number of sites with particular management treatments changed each season as the phased restoration management progressed. The PIs used multivariate analysis of variance (MANOVA) to explore variation in abundance and community metrics across sites and years.

Objective 3: Assessment of Amphibian Health and Stress

The team aimed to collect dorsal swabs of $\leq$ 680 amphibians per season using sterile cotton swabs (3x per sample) for the stress hormone cortisol (CORT). Swabs were placed in individual cryovials with 1 ml of 70% ethanol. The Lincoln Park Zoo Endocrinology Laboratory analyzed the CORT swabs using enzyme immunoassay (EIA) to detect differences in intraspecific stress across study sites. Paired *Bd* swabs were collected by ventrally swabbing amphibians (25 x per sample) with a sterile cotton swab to collect any *Bd* zoospores. Swabs were placed in individual cryovials with 1 ml of 70% ethanol. Standard chytrid disinfection protocol was used on all equipment between sites. *Bd* samples were analyzed by Pisces Molecular Laboratory using qPCR to quantify zoospores. The PIs used ANOVA to examine intraspecific variation in CORT across sites and years. Repeated measures ANOVA was used to examine variation in overall *Bd* prevalence across management treatments. The PIs used MANOVA to explore differences in *Bd* incidence across sites, year, and species.

Objective 4: Implementation of Oak Woodland Restoration Management in the SDPR Sites

LCFPD staff and consultants completed the implementation habitat restoration management objectives for the Southern Des Plaines Restoration Project, including oak woodland and ephemeral wetlands restoration management efforts in preserves along the Upper Des Plaines River Corridor Conservation Opportunity Area. Canopy gaps were created through removal of large overstory competitors of oaks using shelterwood cuts. Phased removal of invasive species and understory thinning in flatwoods, mesic woods, and dry mesic woodlands was carried out between 2015-2018. At the start of the amphibian study in 2016, canopy gap and understory management had been implemented in MacArthur Woods, Ryerson Woods, and Elm Road Woods. Canopy gap management had been implemented in Wright Woods and Grainger Woods. Prior to the start of amphibian surveys in 2017, understory thinning was implemented in Wright Woods. Grainger Woods understory removal was implemented prior to commencement of the 2018 amphibian monitoring.

Results

Demographic parameters of reintroduced wood frogs

The reintroduced wood frog population has increased in abundance since 2014, prior to the implementation of oak woodland restoration. As abundance has increased following oak woodland restoration measures, the number of breeding ponds within MacArthur Woods has also expanded from two ponds to four. In 2014, the number of breeding females was estimated at 62 and in 2015, at 65 based on egg mass counts. In 2014 and 2015, wood frogs were largely reproducing in Pond 1 and a few were breeding in Pond 3. With the onset of canopy and understory management, wood frogs expanded breeding into Ponds 2 and 4. In 2016, female breeding wood frog population size was estimated at 96, in 2017 at 308, and in 2018 at 138 (Fig. 2). The apparent decrease in abundance in 2018 was likely related to the prolonged winter weather, with freezing temperatures continuing through most of April, following initial bouts of reproduction on April 11th. Following peak breeding on April 11th and 12th, 2018, the temperature dropped below freezing for eight days, and following the thaw, there was a second bout of breeding activity with full choruses of males, but only 9 additional egg masses were found. In 2017, peak breeding for wood frogs occurred during the last week of March, and wood frogs had left the pond by April. The prolonged freezing conditions in March and April limited trap nights, and may explain the lower abundance estimates, combined with the delayed breeding phenology. The PIs observed a slight decrease across all species in 2018, which may relate to the challenging weather conditions during the typical breeding window. Despite this decrease in egg masses in 2018, wood frogs exhibit a significantly increasing trend in breeding output with time ($r^2 = 0.54$, $p = 0.04$) since the final translocation in 2010 and since the oak woodland canopy management and understory removal was implemented (Fig. 3).

Through the team's drift fence trapping efforts, the sex ratio was determined over the three year study. In 2016, the population was slightly female-biased, but became male-biased in 2017 and 2018 (Fig. 4), Based on photo-mark recapture analysis, the male population size was estimated to be 77 in 2016, 370 in 2017, and 237 in 2018 (Fig. 2). These sex-specific estimates provide a total breeding population size of 173 in 2016, 678 in 2017, and 375 in 2018 (Fig. 2). In addition to trap night limitations and delayed breeding phenology in 2018, the decrease from 2017 to 2018 may be density-related, which is often observed in long-term population studies of wood frogs (Berven 2009, Sacerdote 2009).

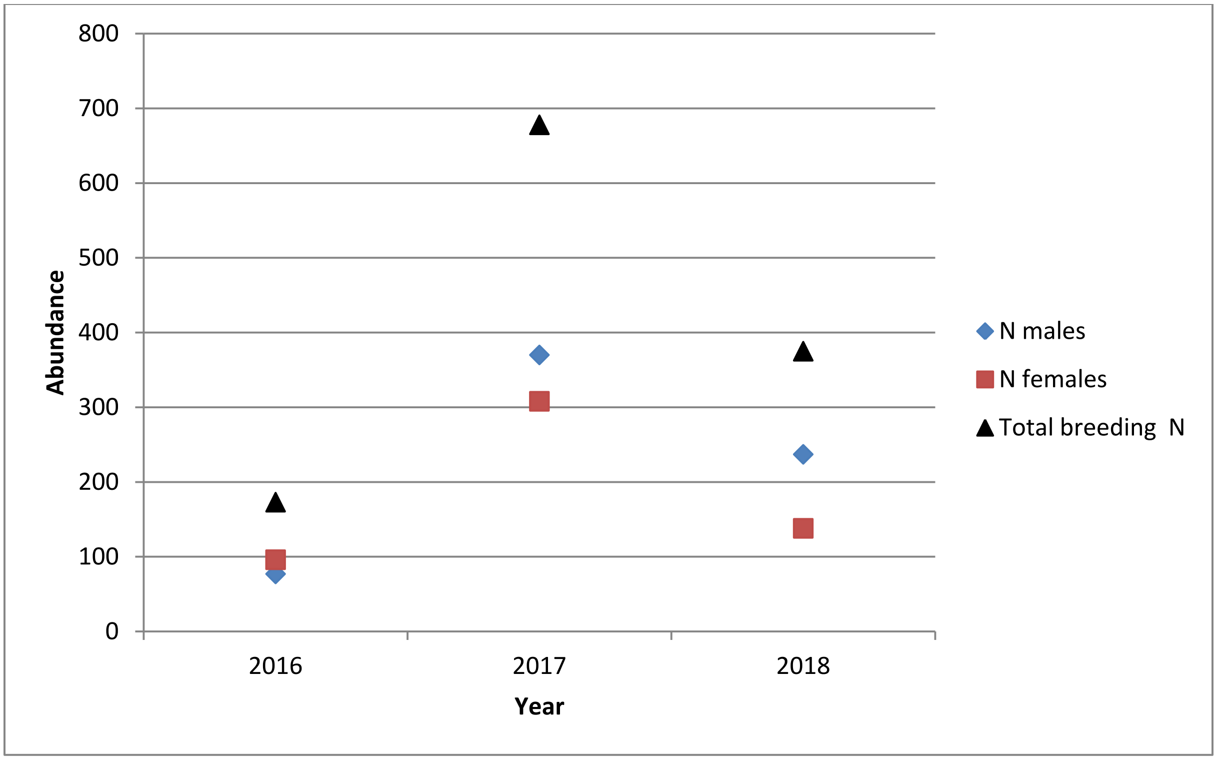

Figure 2. Abundance of breeding males, females, and total wood frog breeding population size, estimated from drift fence captures, photo-mark-recapture, and egg mass counts between 2016-2018.

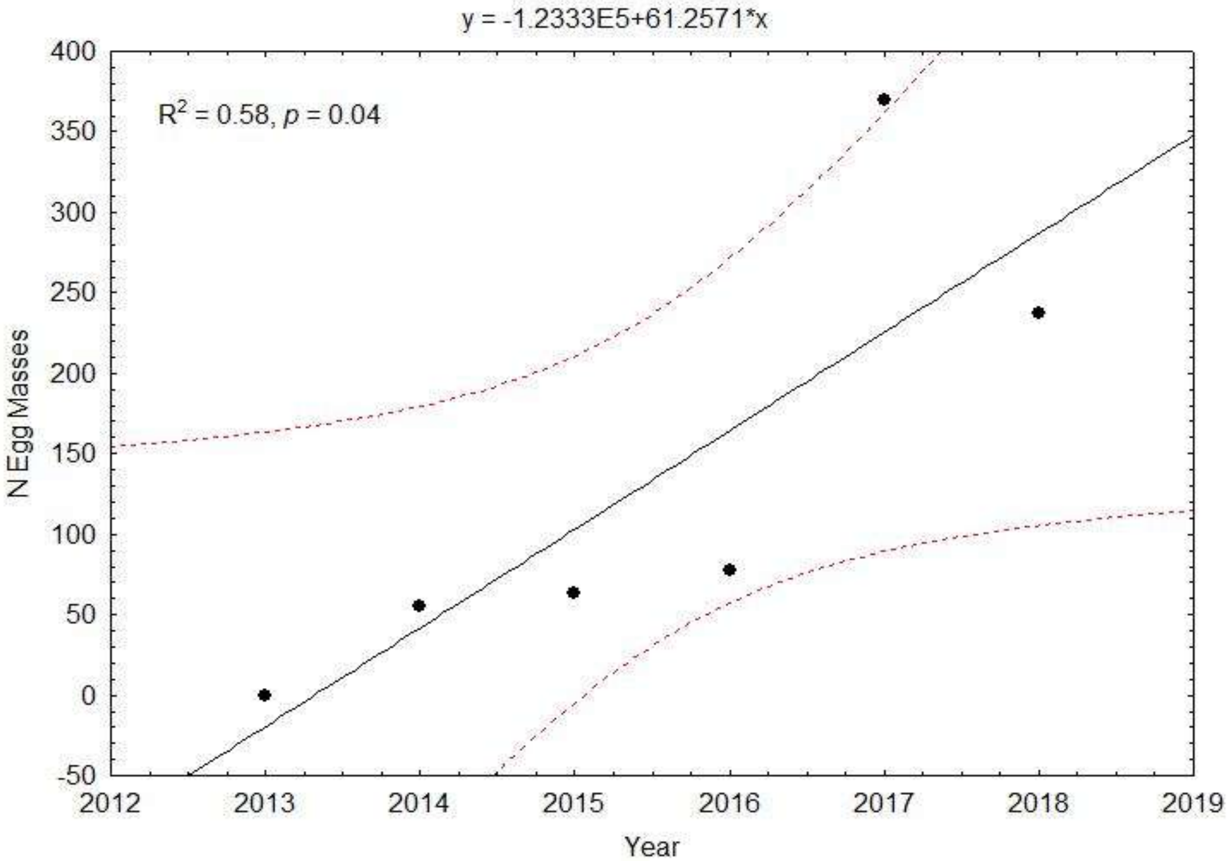

Figure 3. Wood frog egg mass abundance has increased with time since initial egg mass translocation in 2010, and increased additionally since implementation of canopy gap and understory management.

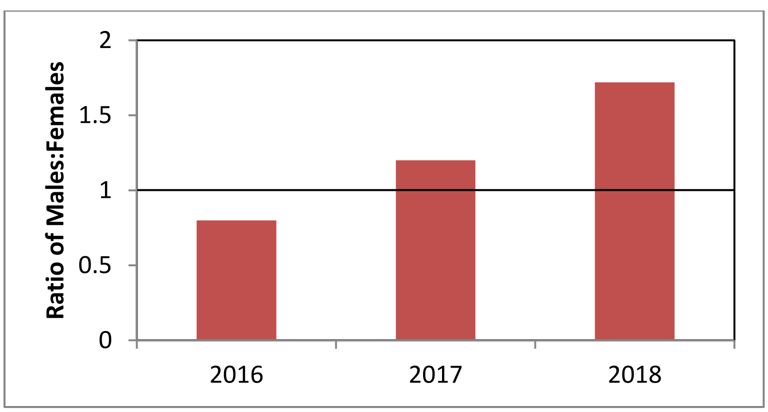

Figure 4. Dynamic sex ratio of breeding adult wood frogs through time in a reintroduced population. The reference line indicates a 1:1 sex ratio. Values below the reference line indicate a female-biased population, and values above the reference line indicate a male-biased population.

Using the robust design model in Program Mark, and through field data collection, demographic parameters of the wood frog population were prepared in advance of population viability analysis (Table 1). The PIs derived survival estimates for breeding adults from the robust design model (Pollack 1982) in Program Mark (White and Burnham 1999). Mean clutch size was estimated from egg counts from four egg masses per breeding pond per year. Mean hatch rates and tadpole survival rates were estimated using eight replicate pond enclosures (two enclosures/breeding pond) each season. The product of the hatch rate and tadpole survival rate provides a combine aquatic stage survival rate to metamorphosis. Because juvenile captures were too limited to estimate survival, the PIs used the juvenile survival rate provided by a 21-year study of Michigan wood frogs (Berven 2009) to provide the parameter for the population projection and the population viability analysis.

Table 1. Model parameters for population viability analysis of the reintroduced wood frog population. Parameter estimates will be used in a stage-structured matrix model.

Model Parameter	Estimate	Source
Mean clutch size	288.25	Field counts (this study)
SD clutch size	65.29	Field counts (this study)
Mean hatch rate	0.51	Field counts (this study)
SD hatch rate	0.17	Field counts (this study)
Mean tadpole survival	0.15	Field counts (this study)
SD tadpole survival	0.02	Field counts (this study)
Combined aquatic survival	0.07	Field counts (this study)
SD combined aquatic survival	0.004	Field counts (this study)
Adult survival 2016-2017	0.07	Photo Mark Recapture (this study)
Adult survival 2017-2018	0.30	Photo Mark Recapture (this study)
Mean adult survival	0.19	Photo Mark Recapture (this study)
SD adult survival	0.16	Photo Mark Recapture (this study)
Mean juvenile survival	0.11	Mark-Recapture (Berven 2009)
SD juvenile survival	0.08	Mark-Recapture (Berven 2009)

Using these demographic parameters, the PIs have generated a stage-structured matrix model for the reintroduced wood frog population:

$$A = \left\{ \begin{array}{ccc} 0 & 0 & 288.25 \\ 0.07 & 0 & 0 \\ 0 & 0.11 & 0.19 \end{array} \right\}$$

Each column in the matrix represents a life-stage of metamorph, juvenile, and adult, from left to right. The top row of the matrix represents stage-specific mean fecundity (clutch size). The values on the sub-diagonal represent survival rates or transition probabilities from one life-stage to the next. The transition probabilities include metamorph survival to juvenile, juvenile survival and transition to adult stage, and adult survival. Only adults reproduce, so the mean clutch size from the field counts of eggs (Table 1) was used to represent average female adult fecundity.

For the population viability analysis, the team used the stage-structured matrix generated from the demographic parameters (Table 1) to estimate the finite rate of growth, λ, the intrinsic rate of growth, *r*, generation time, mean breeding age, and reproductive value of each life stage (Table 2).

Table 2. Population Viability Parameters for the Reintroduced Wood Frog Population.

Population Parameter	**Estimate**
Finite Rate of Population Growth (λ)	1.30
Intrinsic Rate of Growth (*r*)	0.27
Generation Time (T)	3.30
Mean Breeding Age of Parents of a Cohort	3.35
Reproductive Value of Metamorphs	0.004
Reproductive Value of Juveniles	0.083
Reproductive Value of Adults	0.912

The positive intrinsic rate of growth $r = 0.27$ and the finite rate of growth, λ of 1.3 indicate increasing population growth trends. The generation time estimate and mean breeding age of parents of a cohort of ~3 years is consistent with long-term studies of wild populations (Berven 2009). The age distribution of the population was 93% metamorphs, 5.4% juveniles, and 0.53% adults, consistent with most pond-breeding frog populations. Matrix models are typically females-only for incorporation of fecundity rates (Caswell 2001), so female population size was projected over the next 15 years using the matrix model and an initial stage vector of 3,039 metamorphs, 769 juveniles, and 138 adult females (Fig. 5). The number of breeding adults fluctuates slightly, but continues to grow, reaching 3,000 individuals within 15 years. This projection does not account for extreme catastrophic events such as severe multi-year droughts. However, the trend and growth rates indicate a persistent population with density-dependent limitations (Berven 2009).

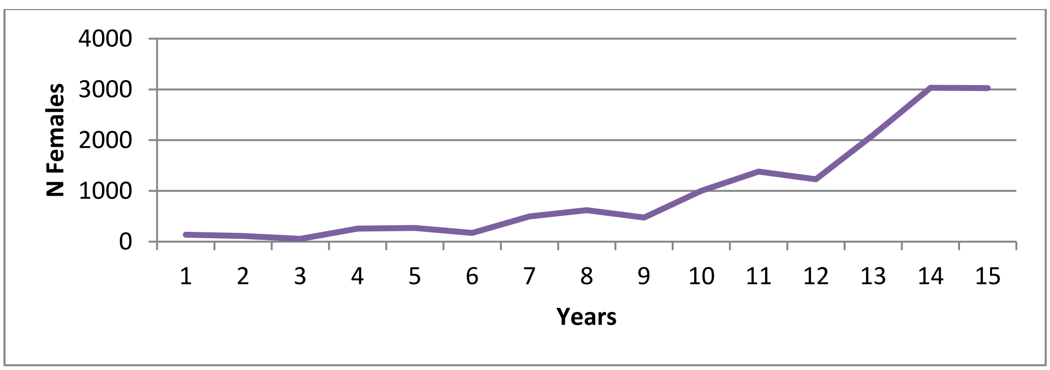

Figure 5. Projected population growth of the reintroduced wood frog population (adult females only) over 15 years.

Natural Expansion of Wood Frogs to Additional Sites

While the team observed the expansion of wood frogs to two additional breeding ponds within MacArthur Woods following oak restoration, wood frogs did not naturally colonize any neighboring preserves near the reintroduction sites. No sites other than MacArthur Woods produced captures of adults, juveniles, and larvae, and egg masses were not observed in any other sites. From the call recordings, wood frog calls were only detected at index 1, 2, and 3 in MacArthur Woods. Despite lack of expansion beyond the site, movements within the site to occupy new breeding ponds entailed movements of 400-500 meters beyond the initial two breeding ponds. Successful colonization of these additional ponds following understory removal indicates that terrestrial habitat quality can support metapopulation movements (local colonizations of multiple ponds and turnover in use of breeding ponds).

Spring Peeper Persistence in the Reintroduction Site

While spring peepers have persisted in MacArthur Woods following translocation in 2008-2010, and following oak woodland restoration, the captures of adult spring peepers within MacArthur Woods were limited to fewer than 10 individuals in each season. Eggs were observed in two breeding ponds but because spring peepers lay individual eggs rather than discrete egg masses, the eggs cannot be used to estimate breeding female abundance. Larval counts from dip net surveys were 382 in 2016, 497 in 2017, and 603 in 2018. In 2016 and 2017, spring peeper calls were detected by the team during sampling at index 2 (individuals distinguishable, with overlapping calls) in three of the study ponds. Previously, only two ponds produced index 2 calls. However, in 2018, call recorders at four study ponds in MacArthur Woods produced sustained index 3 calls in mid-April. The recordings were from one of the two-week gaps in sampling, when traps were not open. Additionally, index 3 calls were recorded in the control site, Old School for the first time during the same week in 2018. Dr. Sacerdote-Velat plans to continue monitoring spring peeper activity and demography in future years to assess whether the apparent expansion and increase in abundance as indicated from the recordings will persist.

Spotted Salamanders, Blue-Spotted Salamanders, and Tiger Salamanders

No evidence of spotted salamander persistence was documented in MacArthur Woods between 2016-2018. While conditions appear suitable and dissolved oxygen was generally in the 50-74% saturation range in MacArthur Woods ponds during the breeding window, there were occasional bouts of hypoxia where dissolved oxygen dropped below the 50% saturation necessary for spotted salamander hatchling success (Sacerdote and King 2009). The other factor which may have prevented establishment of spotted salamanders is competition with blue-spotted salamanders which represent between 25-40% of the captures in MacArthur Woods in all years, and potential predation by tiger salamanders. Tiger salamanders represent $\leq$ 5% of the captures in MacArthur Woods in 2016-2018. However, over 60 egg masses of tiger salamanders were found in two breeding ponds in MacArthur Woods, so they may become more established in future years. From our photo mark-recapture of MacArthur Woods blue-spotted salamanders, breeding adult abundances were estimated at 447 in 2016, 553 in 2017, and 571 in 2018. The abundance of blue-spotted salamanders remained stable following oak woodland restoration measures, with a slight (non-significant) increase with time. Since eggs are deposited singly or in small clumps, breeding output cannot be used as a direct correlate of female breeding population size for blue-spotted salamanders.

Community Structure and Abundance

The PIs used a multivariate analysis of variance (MANOVA) to examine variation in amphibian community metrics including Shannon-Weiner diversity index, evenness, species richness, and catch per unit effort (CPUE) with differences among sites and years. The MANOVA detected significant differences in Shannon-Weiner diversity (H), evenness (Eh), and richness (S) among sites ($F_{3, 15}$=22.4, p = 0.016) (Fig. 6), and significant variation in CPUE among study years ($F_{3, 6}$=16.0, p = 0.009) (Fig. 7). A *post hoc* Tukey's test was used to identify significant differences in diversity, evenness, and species richness among specific sites. MacArthur Woods and Wright Woods significantly differed in H' diversity and evenness (p = 0.003), while other sites did not significantly differ from each other. Species richness differed significantly among MacArthur Woods and all other study sites (p = 0.002). This greater richness was expected since MacArthur Woods is the only study site with wood frogs. The persistence of the reintroduced wood frog population maintained greater species richness in MacArthur Woods, and their increased representation in the amphibian community (Fig. 8) produced a greater diversity index through increased evenness and abundance (Fig. 8). Wright Woods had significantly lower species richness than both MacArthur and Elm Road Woods (p=0.032). The low diversity, richness and CPUE of Wright Woods are attributable to early pond drying in all three years of the study resulting in limited captures. The CPUE of all sites differed significantly by year, with 2016 having lower capture rates than either 2017 or 2018 (Fig. 7). The repeated measures ANOVA detected a significant effect of restoration management on the amphibian community metrics ($F_{6, 26}$=3.78, p = 0.008). Sites with both gap management and understory removal had greater diversity, richness (Fig. 6) and CPUE (Fig. 9) than the gap-only sites prior to implementation of understory removal and compared to the control site.

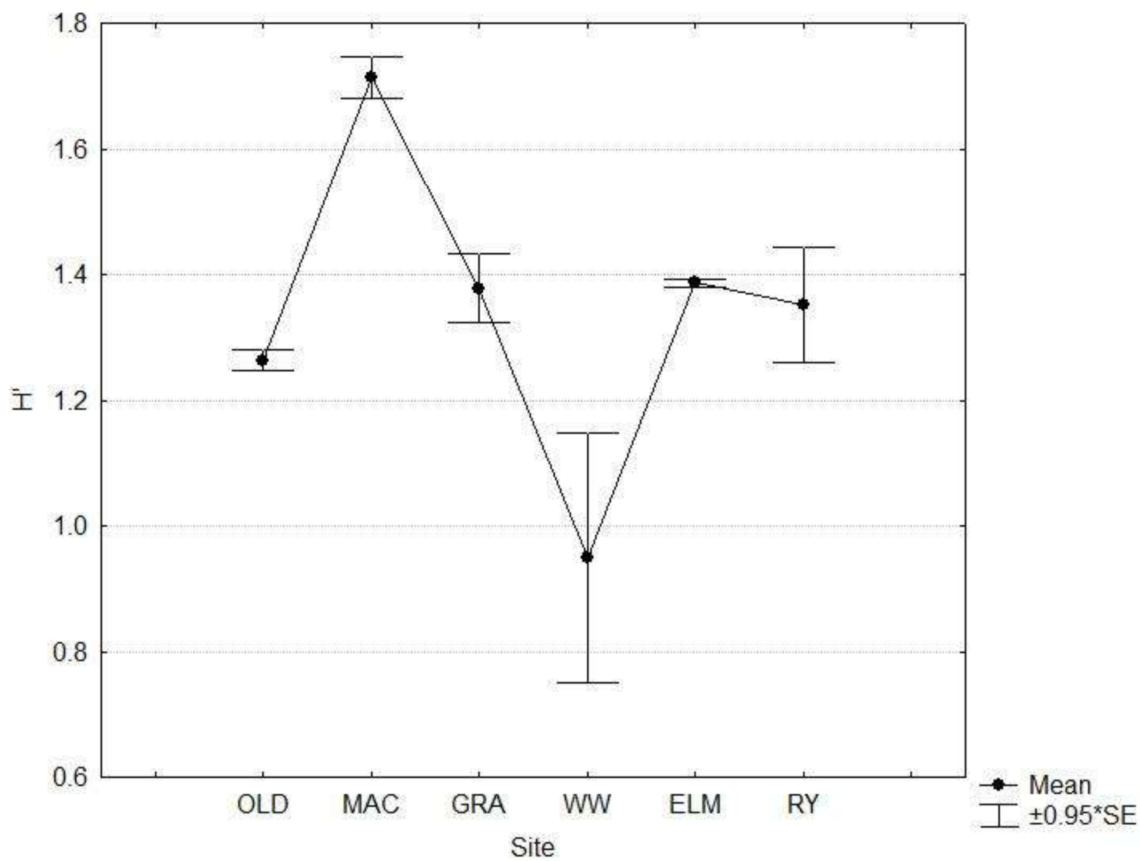

Figure 6. Variation in H' (Shannon-Weiner diversity index) of the amphibian communities across the study sites, pooled among years. MacArthur Woods had the greatest amphibian diversity, while Wright Woods had the lowest amphibian diversity.

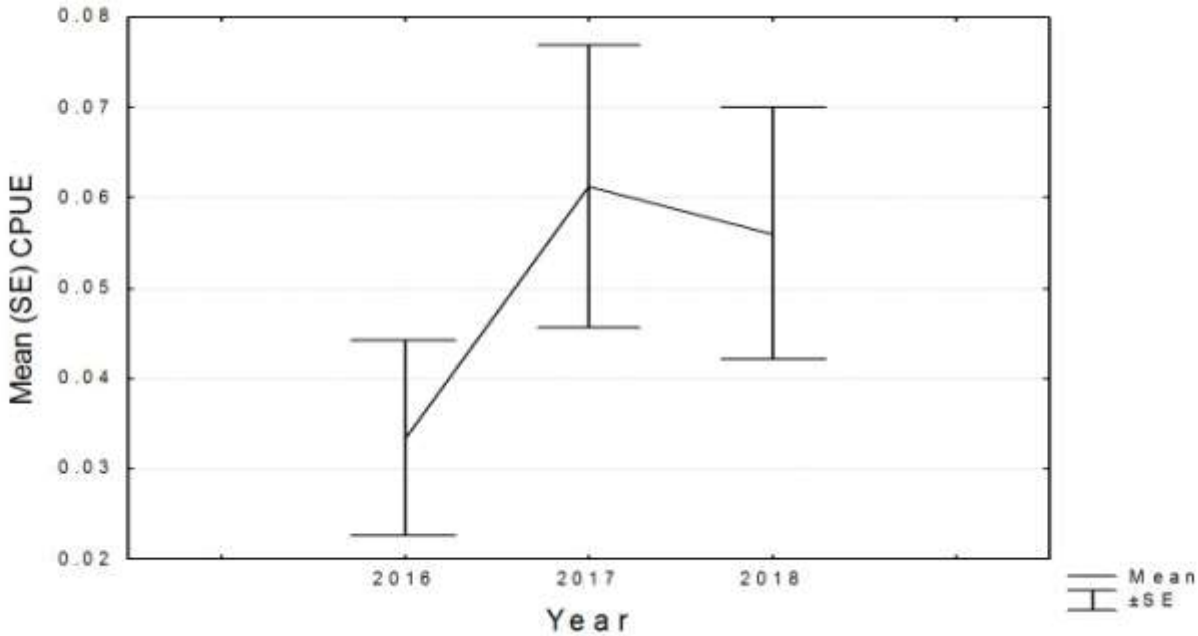

Figure 7. Variation in catch per unit effort (CPUE), a metric of abundance, across study years. CPUE was significantly lower in 2016, at the start of restoration implementation, than in 2017 and 2018.

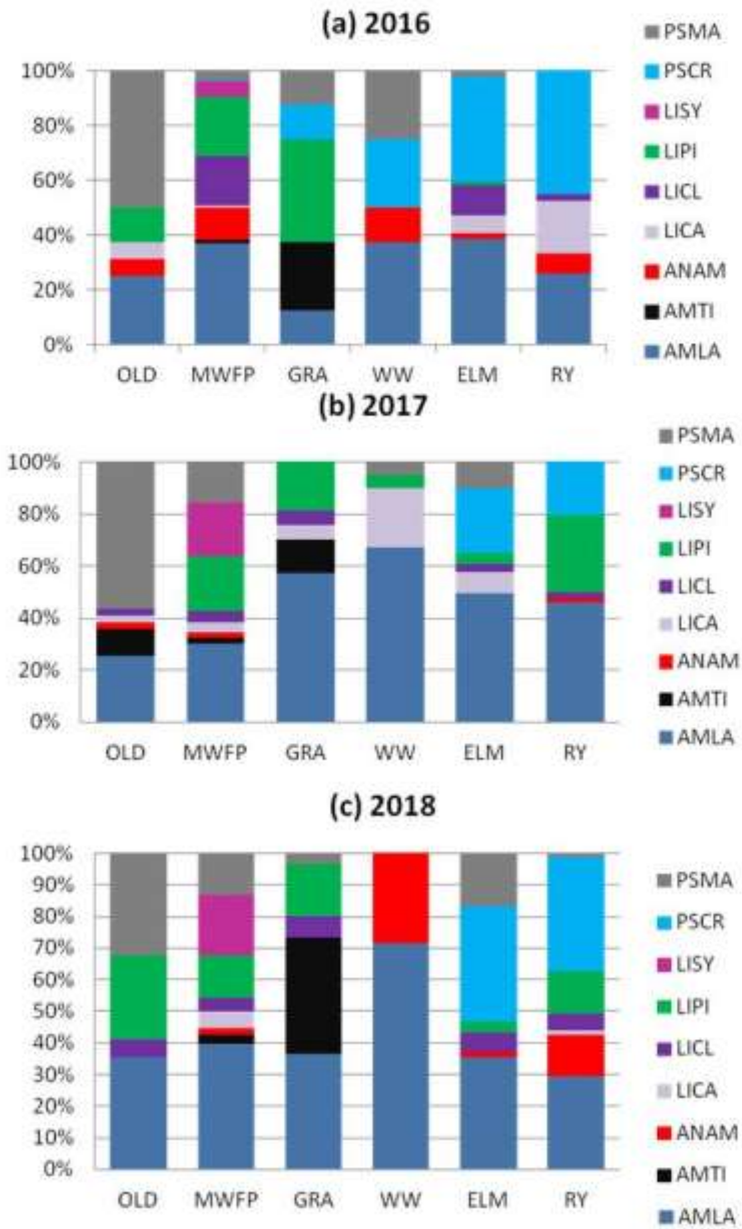

Figure 8. Relative abundance of amphibian species captured in drift fences during the (a) 2016, (b) 2017, and (c) 2018 sampling seasons. Species abbreviations are as follows: Blue spotted salamander (AMLA), tiger salamander (AMTI), American toad (ANAM), bullfrog (LICA), green frog (LICL), northern leopard frog (LIPI), wood frog (LISY), spring peeper (PSCR), and chorus frog (PSMA).

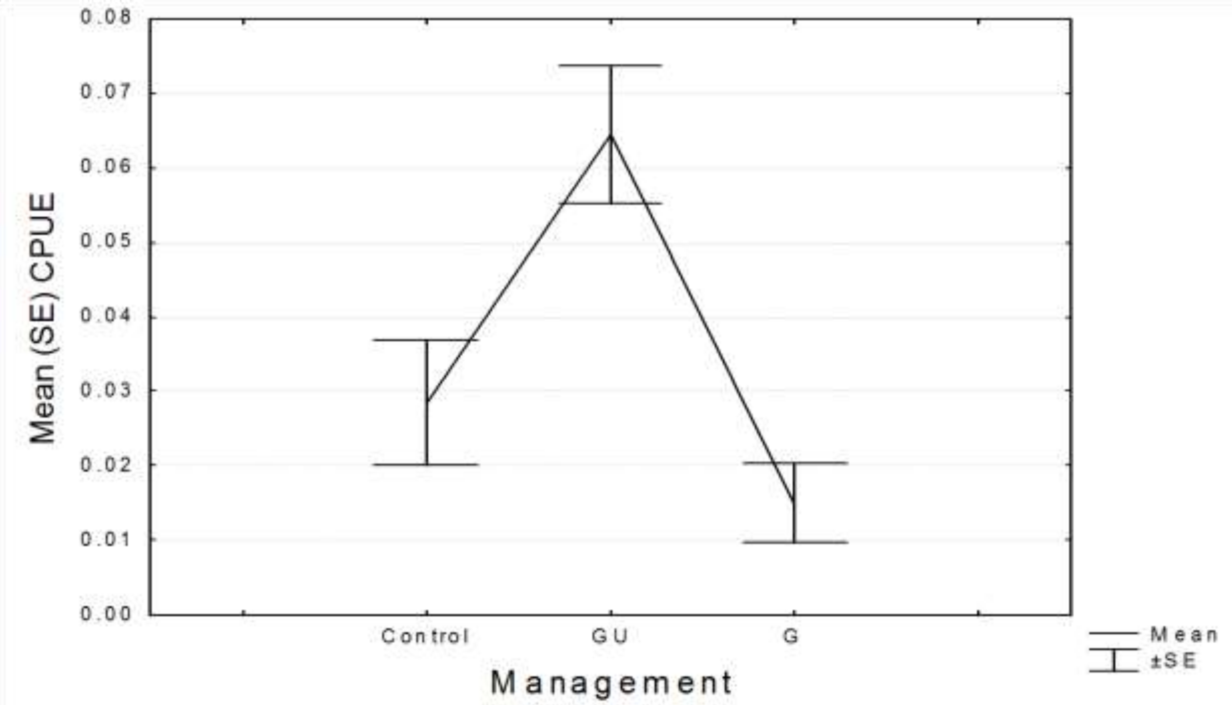

Figure 9. Repeated measures ANOVA variation in mean species richness with restoration management. Treatments include the control site, gap and understory management (GU) and gap-only management (G).

The team used repeated measures ANOVA to examine effects of management treatments on the relative abundance of the ephemeral pond-breeding amphibians including blue-spotted salamanders, chorus frogs, and spring peepers. Tiger salamanders were only repeatedly captured in two sites, MacArthur Woods, and Grainger Woods, with one isolated capture in Old School, so they were not included in the analysis. There was a significant management effect on relative abundance of these species, ($F_{12, 20}$= 3.58, p = 0.005). However, the trends differed for the species, with greater relative abundance of blue-spotted salamanders in the gap and understory sites than gap only or control sites (Fig. 10), greater relative abundance of spring peepers in both gap and understory and gap only sites than in the control, and greater relative abundance of chorus frogs in the control site than in the canopy treatment sites. These trends may be explained by the dominant effect of terrestrial habitat quality on the presence of blue-spotted salamanders, such that they should be most abundant in sites that have had invasive understory shrubs removed. The spring peepers have an apparent threshold of necessary canopy openness for their oviposition sites (Lawrence 2018). Accordingly, they will be more abundant in sites with gap management as these sites have greater emergent vegetation in the breeding ponds. This pattern may be less influenced by the understory management surrounding the ponds given that they are more arboreal than chorus frogs. Chorus frogs as canopy generalists were one of few species regularly captured in the control site.

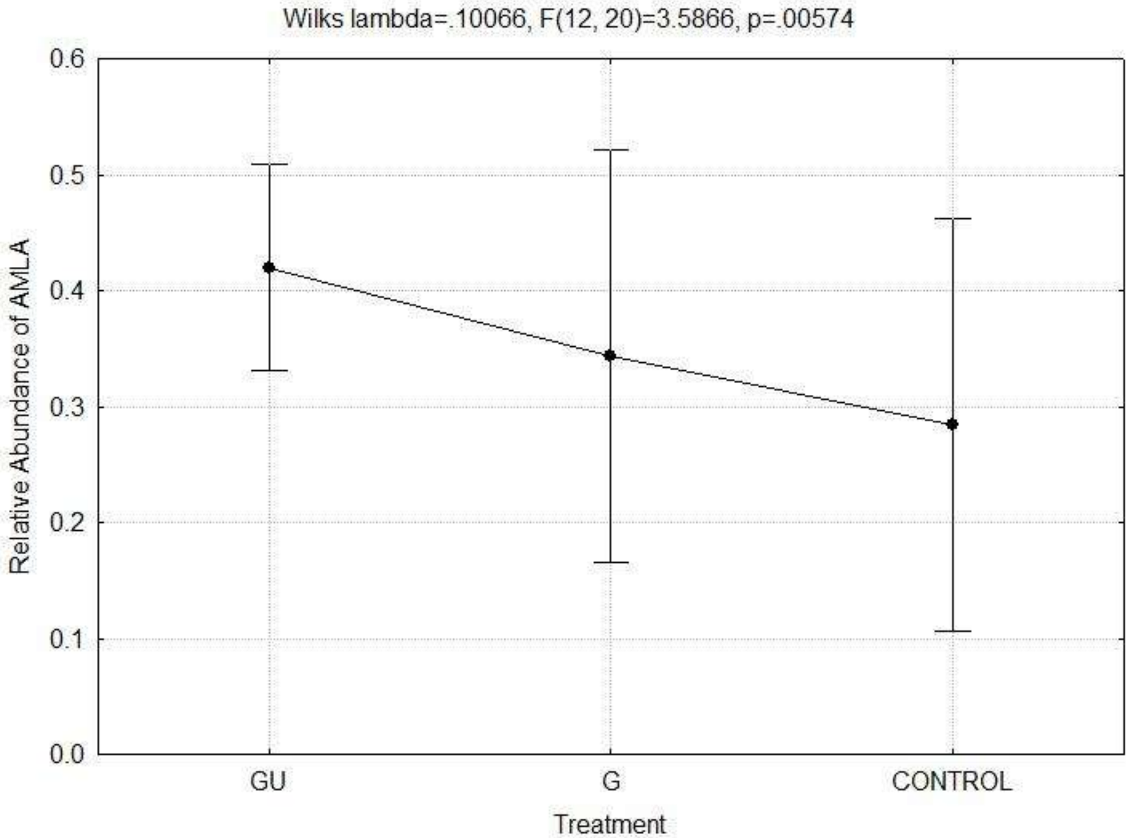

Figure 10. Mean (SE) relative abundance of blue-spotted salamanders across oak restoration management treatments from 2016-2018. The blue-spotted salamanders constituted approximately 45 % of the captures in gap and understory sites, 35% in gap only sites, and 28% in the control site.

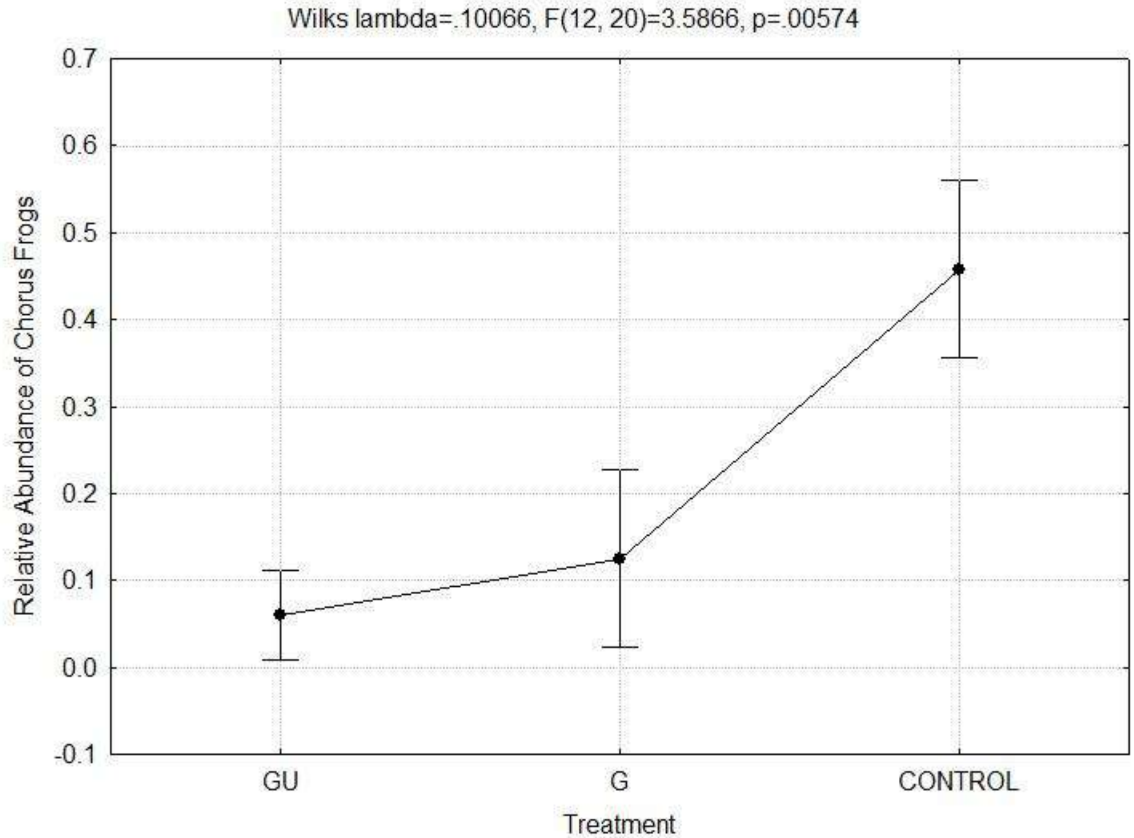

Figure 11. Mean (SE) relative abundance of chorus frogs across oak restoration management treatments from 2016-2018. The chorus frogs constituted approximately 8% of the captures in gap and understory sites, 11% in gap only sites, and 48% in the control site.

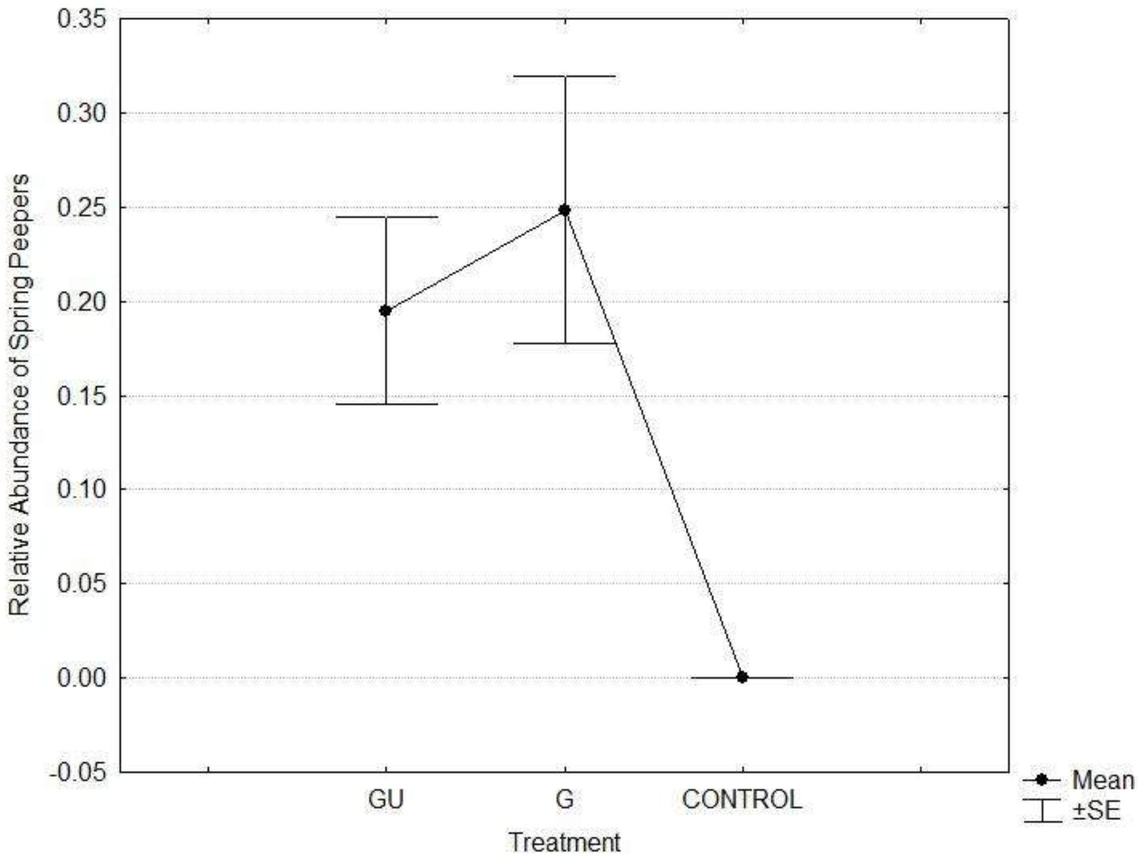

Figure 12. Mean (SE) relative abundance of spring peepers across oak restoration management treatments from 2016-2018. The chorus frogs constituted approximately 20% of the captures in gap and understory sites, 25% in gap only sites, and less than 1% in the control site.

Health and Stress of Amphibians

Analysis of all cortisol swabs was completed at the Lincoln Park Zoo Endocrinology Laboratory. The PIs received the results of all *Bd* swabs from Pisces Molecular laboratory. Within-species analyses of CORT by *Bd* status, year, and management were completed for all species with the exception of blue-spotted salamanders and tiger salamanders, as these two species were consistently *Bd*-negative throughout the three-year study.

Variation in Bd

In 2016, 2017, and 2018, the team collected 194, 307, and 308 *Bd* swabs respectively, across the six study sites. Sample prevalence decreased across years from 17.5 % in 2016, to 14.3% in 2017, and to 8.7% in 2018. Individual sites varied in overall *Bd* incidence with Grainger Woods and Ryerson Woods producing consistent negative swabs across years and species. MacArthur, Elm Road, and Old School produced positive samples in all years, and Wright Woods produced positive samples in 2016 and 2017, but not in 2018. However, in the case of Wright Woods, sample size was limited by early pond drying in 2017 and 2018. Across species, blue-spotted salamanders and tiger salamanders were consistently *Bd*-negative in all years. Wood frogs were negative in 2016 and 2017, but 4% were positive in 2018 (Fig. 13). All other positive species except American toads underwent a decrease in *Bd* prevalence from 2017 to 2018. Spring peepers tested negative for the first time in 2018 (Fig. 13).

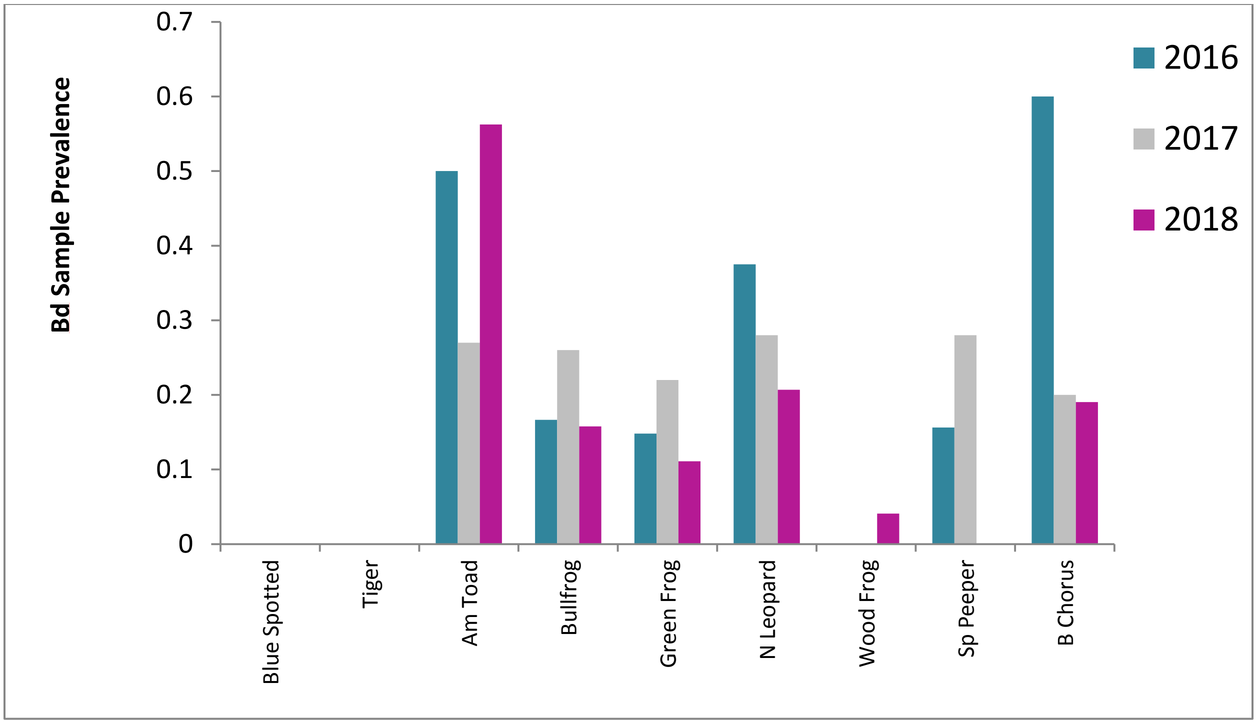

Figure 13. *Bd* prevalence across species, and years, pooled across study sites in 2016-2018.

The team observed a significant effect ($F_{2,13}$ = 13.25, p = 0.0007) of restoration management treatment on *Bd* incidence pooled across species and sites (Fig. 14). Gap and understory treatment sites and gap-only sites had a significantly lower mean sample prevalence of approximately 5% positives for both treatments, while the control had a mean sample prevalence of 37%. This effect may be related to water temperature, as *Bd* is often considered a cold-associated fungus and zoospore growth is expected to increase in cooler water, resulting in higher incidence earlier in the season, and potentially in more shaded ponds. Mean water temperatures recording weekly during peak breeding averaged 7.8° C for the gap and gap plus understory ponds and averaged 3.9°C for the control site. Because the canopy gaps above the ponds in the treatment sites permitted greater solar insolation, the water temperatures are warmer than in the control site.

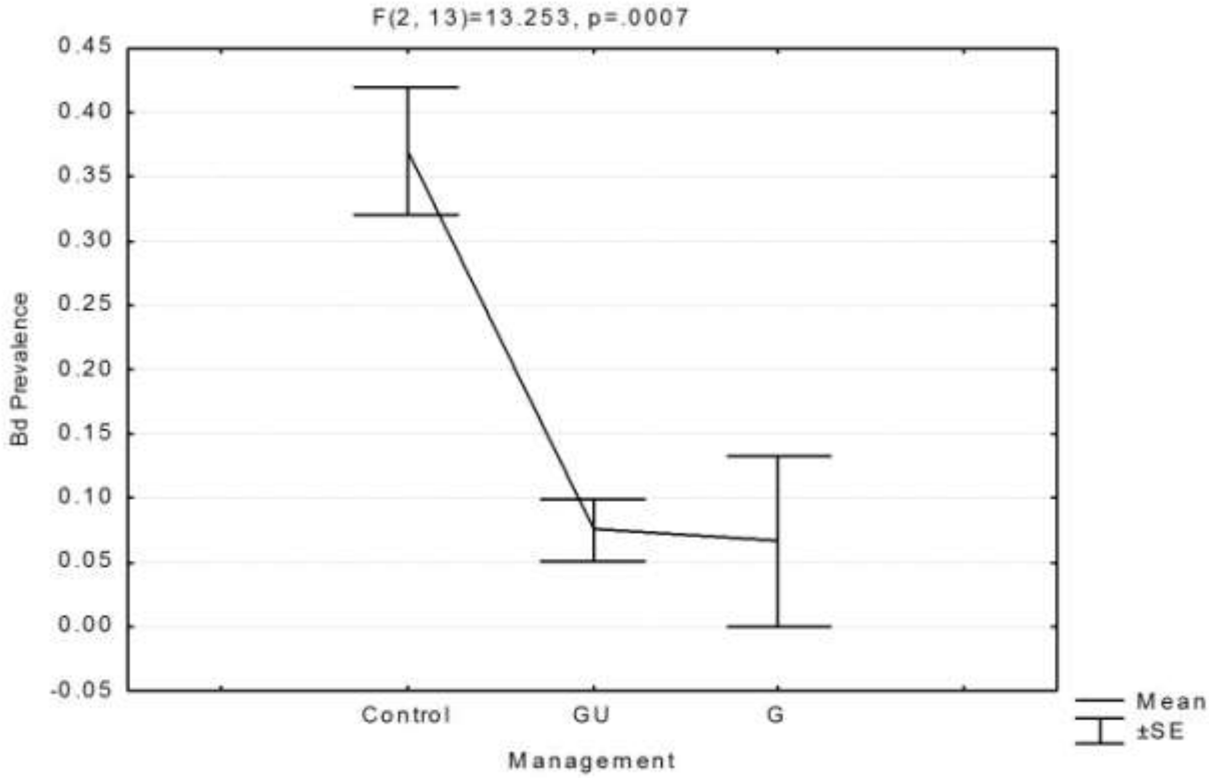

Figure 14. Variation in mean (SE) *Bd* prevalence across restoration management treatments.

Variation in CORT within species by year, Bd status and management treatment

CORT varied significantly by year for blue-spotted salamanders ($F_{(2, 206)}$=13.89, p<0.0001), wood frogs ($F_{(2, 130)}$= 6.03, p=.003), American toads ($F_{2, 49}$=3.21, p=.04868), and bullfrogs ($F_{2, 45}$=6.8, p=.002). CORT was significantly lower for these four species in 2018 (Fig. 15). Similarly, there was nearly significant variation in CORT by year for chorus frogs and spring peepers with a general decrease in mean CORT in 2018 (Fig. 15).

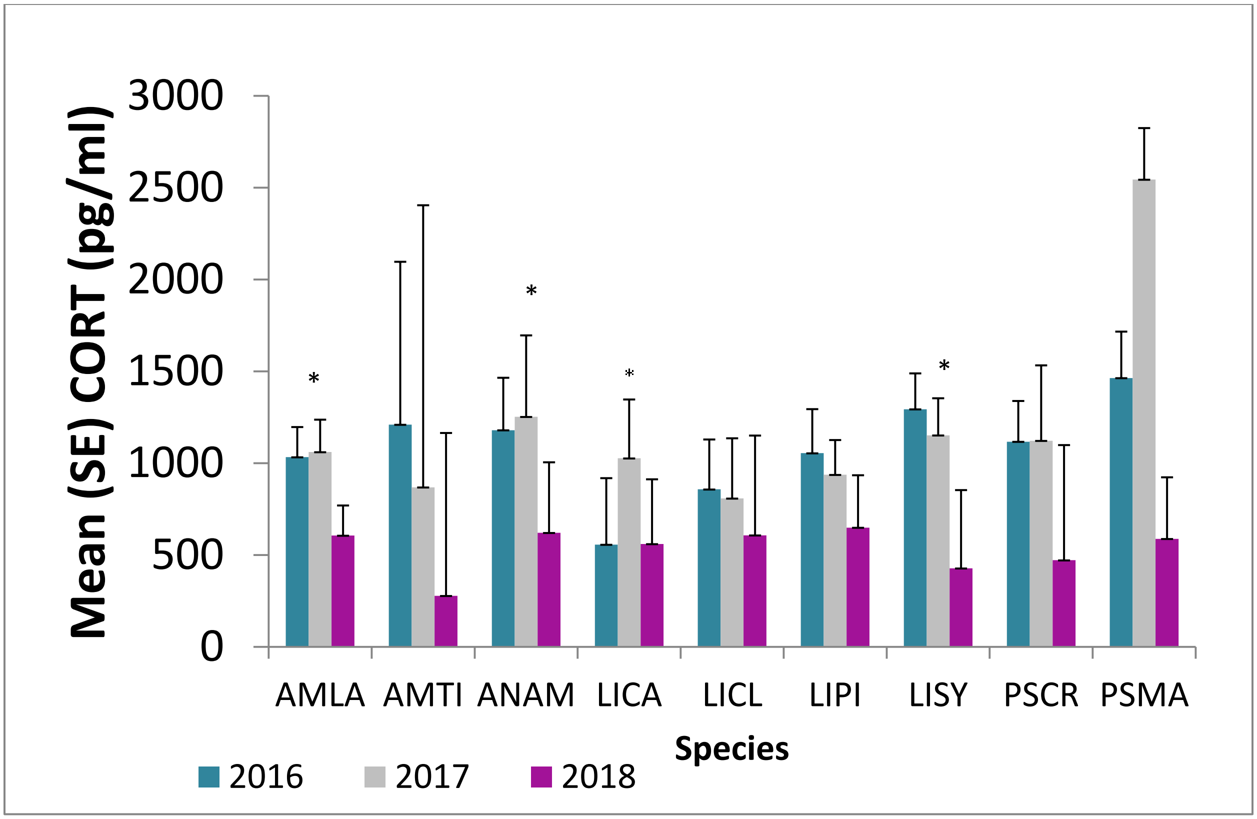

Figure 15. Species-specific variation in mean CORT (pg/ml) across years. All species exhibited lower CORT levels in 2018. Species abbreviations are as follows: blue-spotted salamander (AMLA), tiger salamander (AMTI), American toad (ANAM), bullfrog (LICA), green frog (LICL), northern leopard frog (LIPI), wood frog (LISY), spring peeper (PSCR), and chorus frog (PSMA).

For species with sufficient numbers of positive *Bd* samples, the PIs examined variation in CORT with *Bd* status. The PIs also examine intraspecific variation in CORT with management treatment. Spring peeper CORT varied significantly with management treatment ($F_{1, 52} = 5.14$, $p = 0.028$), but not with *Bd* status ($F_{1, 52}= 0.386$, $p = 0.53$). Spring peepers had significantly greater CORT levels in gap-only restoration sites than in gap and understory sites (Fig. 16). Only a single spring peeper was captured in the control site during the three-year study, in 2018, although call recordings did produce evidence of use of the control site by spring peepers. This pattern of occurrence indicates a positive association with greater canopy openness generally as observed in the patterns of relative abundance (Fig. 12), but the presence of invasive understory may represent a stressor to the species. In contrast to spring peepers, chorus frogs, a canopy generalist, did not exhibit any significant trends in CORT with management treatment ($F_{2, 51} = 0.474$, $p = 0.625$), *Bd* status ($F_{1, 51} = 0.002$, $p = 0.96$. American toad CORT did not vary significantly with *Bd* status ($F_{1,49}$=.032, p=.85714) or management ($F_{2, 49}$=1.41, p=.25). Northern leopard frog CORT did not vary significantly with *Bd* status ($_{F1, 111}$=.86, p=.35), or management treatment ($F_{1, 111}$=1.91 p=.16). Green frog CORT did not vary with *Bd* status ($F_{1, 53}$=.47, p=.49) or management treatment ($_{F1, 53}$=.13, p=.71). In the case of northern leopard frogs and green frogs, captures were limited in the gap-only treatment. Tiger salamander sample size was too limited for a multivariate analysis of CORT and all captures were all *Bd* negative.

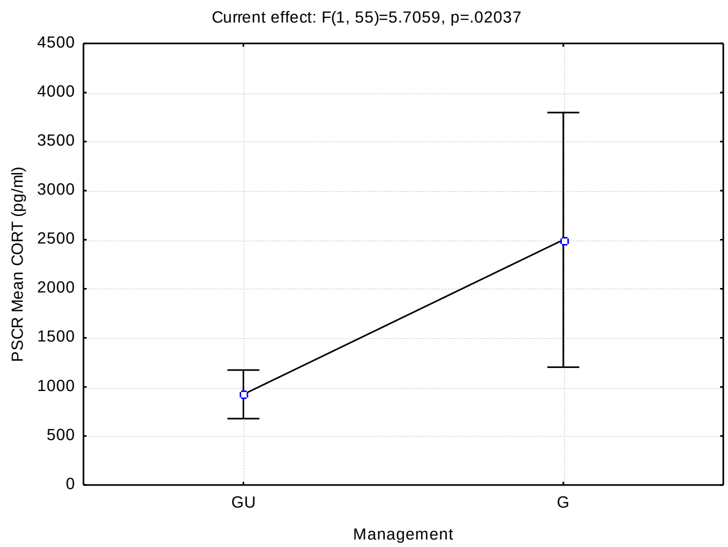

Figure 4. Variation in mean (SD) CORT across management types for spring peepers pooled across years.

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