AMPHIBIAN USE OF RESTORED WETLANDS OF DIFFERENT AGES

by

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To Amanda, the love of my life

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ABSTRACT

Wetland-dwelling amphibians are of conservation interest for numerous reasons. They serve as biological indicators of water quality during their fully aquatic larval phase, and as carnivores that prey extensively on both terrestrial and aquatic invertebrates. These amphibians are an important link between terrestrial and aquatic food webs, and their wellbeing is an important factor when considering ecosystem health. Amphibians are facing global declines as their wetland habitats are being lost or degraded by human actions. There are efforts to restore wetland habitats, but it is far from certain which practices encourage amphibian occupancy.

I investigated which factors are important to the persistence of amphibians in restored and naturally formed wetlands to see if restored wetlands can accommodate similar species assemblages. Amphibians were surveyed over two years in a collection of 18 wetlands in Steuben and DeKalb counties, IN owned by The Nature Conservancy. Ambystomatid salamanders were surveyed using plastic minnow traps in springtime, and frogs were surveyed using call surveys in spring and summer. I used linear models to compare wetland plant dominance, wetland hydroperiod, restoration status, distance to nearest mature forest, adjacent forest age and basal area, and inter-wetland distance to amphibian species richness.

The species richness of *Ambystoma* salamanders was positively associated with larger wetlands, higher forest basal area, and central mudminnow presence; and negatively associated with older forests, distance to mature forests, and the presence of sunfishes. *Ambystoma* salamanders besides *A. tigrinum* were associated with ephemeral hydrology, naturally-formed wetlands, and a greater number of wetlands within one km; and negatively correlated with older forests.

Frog species richness was positively associated with larger wetland size, and negatively associated with seasonal wetlands, naturally-formed wetlands, distance to nearest mature forests, naturally formed wetlands, treatment for invasive plants, and number of other wetlands within 500m. Total amphibian species richness models did not perform well, but showed a preference for semi-permanent wetlands, smaller distance to mature forests, greater forest basal area, and greater distance between wetlands; and a preference against Scrub Shrub/Forest wetlands. Hylid frogs

were negatively correlated with naturally formed wetlands. Ranid frogs were associated semi-permanent wetlands and negatively correlated with the number of other wetlands within 500 m.

Ambystomatid salamanders were found in restored wetlands, semi-permanent wetlands, and in wetlands containing central mudminnows. Frogs may dislike the disturbance from removing invasive grasses. Managers should factor the disparate habitat requirements of amphibian taxa into their plans for creating and managing restoration projects. Different amphibian groups appear to differ greatly in their habitat requirements, and diverse wetlands may enhance the species richness of an area. Skillfully restored wetlands appear to serve similar functions to original, naturally formed ones.

CHAPTER 1. LITERATURE REVIEW

North American wetlands have been drained extensively, mainly for conversion to agriculture (Gallant et al., 2007). This reduction in wetland size and number may prove to be harmful to populations of wetland-dwelling species. Amphibian populations in North America trended downward during the latter half of the 20th century (Houlahan et al., 2000) and continue to decrease into the 21st century (Grant et al., 2016). Shrinking populations potentially place some species at risk for local extirpation, or total extinction. This decline is linked, in part, to habitat loss across their species ranges, which is extensive. By 1991, the year of the most recent analysis of Indiana wetlands by the Department of Natural Resources (Indiana DNR, 2021), the state of Indiana had lost 85-87% of its original pre-European settlement wetlands, and many midwestern states have lost comparable levels (Dahl and Johnson, 1991). Research shows that wetlands losses have slowed since the 1980s (Davidson 2014), but there remains a deficit.

Wetland restoration efforts re-create destroyed wetlands, and create new ones to compensate for wetland losses elsewhere. After the wetlands are repaired, the results must be evaluated to determine if they are functioning properly. A succinct and complete definition of wetland function is elusive, so we must use indicators as proxies for ecosystem function. Amphibians are outstanding candidates for indicators, given their central status in wetland food webs (Hocking and Babbitt 2014). The large-scale, government-sponsored movement to preserve and restore wetlands in the United States is less than 40 years old (Beck 1994), and there remain gaps in our understanding of how amphibians recolonize constructed habitats.

Amphibians are essential parts of wetland ecosystems, which themselves are essential parts of the landscape. Amphibians are known to participate extensively in the nutrient flux between terrestrial and aquatic ecosystems, collecting nutrients like nitrogen and phosphorus as aquatic larvae, exporting them to terrestrial habitats upon metamorphosis, and re-depositing some as eggs later (Capps et al., 2015). Some amphibians physically modify the landscape by burrowing (Semlitch, 1983) and may alter soil hydrology (Capps et al., 2015). Amphibians are tied to the wetlands they inhabit, not merely signs of, but enablers of healthy wetlands. In addition to their less-visible benefits to ecosystem health, amphibians provide a wealth of services that directly benefit humans as well, including mosquito control (DuRant & Hopkins 2008, Brodman & Dorton 2006).

Ambystomatid salamanders are common inhabitants of American wetlands and are endemic to North America. These salamanders generally breed in fish-free seasonal wetlands, where in the absence of fish, their larvae are frequently among the top predators (Petranka 1998). As tiny, but important predators, these young salamanders may be responsible for or associated with higher species richness in their natal pools (Sergio et al., 2008), possibly by feeding on a variety of arthropods and preventing any one species from dominating (Freda 1983). These associations render them useful as proxies of overall wetland biodiversity, and of the health of a wetland ecosystem.

Besides salamanders, anurans are potentially useful proxies for biodiversity. The presence of Spring peepers (*Pseudacris crucifer*) have been associated with overall higher amphibian biodiversity in a wetland community (Brodman, 2009), which can be considered as a component of total wetland community health. Anuran species richness has also been previously associated with microhabitat variables, including the amount of vegetation and woody debris along the edge of a pool (Lichtenberg et al., 2006). Adult anurans are carnivorous, so their presence also indicates a productive ecosystem that can sustain the invertebrates that they feed upon.

For amphibian metapopulations to remain robust, amphibians must be able to reproduce and disperse to an extent. The elimination and reduction of wetlands reduces the ability of wetland-dwelling animals to disperse effectively by increasing the distance between wetlands. The increasingly patchy distribution of wetlands and moist forests is of concern because a small, isolated habitat may decrease the ability of an amphibian metapopulation to sustain itself, and significantly increase the probability of extirpation (Sjögren, 1991). Experimental knowledge suggests that amphibians rely on numerous small, interconnected wetlands to maintain robust populations (Zamberletti et al., 2018), but also benefit from some degree of wetland isolation (Heard et al., 2015). These interconnected wetlands may serve as breeding grounds, nurseries, or adult habitats, depending on the species. If numerous small wetlands in a region are destroyed, even if the absolute area of wetlands decreases by only 20%, the mean distance between wetlands can increase by up to 67%, leading to habitat fragmentation for wetland-dwelling animals (Gibbs, 1993). This drastic increase in distance between suitable habitats has been shown to negatively impact migrating wetland animals (Roe et al., 2004).

Amphibians, as a group, tend to be limited in their capacity for natural dispersal. Their vulnerability to desiccation serves as an inherent limit on the movements of many species. In a meta-analysis of amphibian movement studies, Smith and Green (2005) found that most salamanders tend to stray little from their native wetlands, with anurans being capable of longer distance migration. The studies compiled suggest that most Ambystomatid salamander species do not roam much more than 1 km from the wetlands in which they are captured (Funk and Dunlap 1999; Gamble et al., 2007) This is problematic when habitats are more than 1km apart, because it may greatly reduce the likelihood of the salamanders reaching other wetlands, making gene flow unlikely. A lack of gene flow likely reduces the viability of the metapopulation. Anurans have previously found to be more variable in their dispersal capabilities. Some frogs have been recorded travelling several kilometers from one water body to another (Smith and Green 2005), so wetland fragmentation may affect them differently.

Overall amphibian species richness has been shown to be negatively correlated with wetland isolation (Lehtinen et al., 1999). One of the most significant dispersal barriers is thought to be the mean distance between wetlands that exceed the animals' dispersal capabilities (McCauley & Jenkins 2005). Roads have also been found to present immediate barriers to amphibian dispersal (Gibbs, 1998; Marsh, 2005). Loss of habitat often manifests as habitat fragmentation, especially in the American Midwest. Drainage for agriculture and road constructions leaves patchy wetlands and forest habitats surrounded by farmlands and roads (Kolozsvary and Swihart 1999).

Wetland habitat heterogeneity, in the form of multiple adjacent pools possessing disparate hydroperiods, is considered to promote higher amphibian abundance, occupancy, and diversity (Brodman 2009). Efforts are being made to increase the size of remnant wetlands and restore some that were eliminated. These efforts may improve the quantity and quality of wetlands, using metrics such as mean inter-wetland distance, favorable habitat structure, and heterogeneity of wetland hydroperiods.

Increasing isolation of wetlands makes recolonization of sites by *Ambystoma* salamanders after local extinction events difficult, as their colonization efforts are likely constrained by proximity to source populations. Frogs are likely more capable of long-range dispersal. It is, however, unknown how these dispersal constraints apply across amphibian taxa.

In this study, I hope to establish whether there is an association between wetland age and distance to older habitat and amphibian biodiversity. I hypothesized that salamander diversity will be higher in the older pools and those closer to potential dispersal sources. Anurans are more mobile than salamanders, so I anticipated a weaker association between wetland age, distance from the nearest other habitats, and anuran diversity. In order to evaluate the restoration efforts, I compared wetlands that have been recently enhanced with older ones. Direct comparisons between mature and restored wetlands are uncommon in publications, so further research comparing them will further understanding of wetland ecosystem progression.

CHAPTER 2. METHODS

2.1 Study Site

The study was conducted at Douglas Woods, a Nature Preserve in Northeastern Indiana owned and managed by The Nature Conservancy (TNC). Douglas Woods, with its diverse wetlands, provides a natural laboratory for investigating wetland restoration. The preserve was previously a mixture of agricultural land, wet forest, and vernal pools that was purchased in parcels between 1993 and 2014. It is the subject of ongoing restoration efforts by TNC. The wetlands within the preserve were restored following the land acquisitions. The preserve wetlands range in age from much greater than 27 years to less than 10 years, and those that were restored have documentation of when they were created. The range in age of wetlands combined with a known timeline of their creation enables the study of temporal and spatial factors of recolonization by wetland species.

The studied wetlands are spread across 6 sites and vary in age. The sites are the units that TNC used for restorations, and all restored wetlands in a site were restored at the same time. Most of the unforested land was previously used for row-crop agriculture. During restoration, drainage tiles were broken and basins were excavated if suitable depressions were not already present. Native wetland vegetation was planted in and around the wetlands, and native trees were planted in the remainder. Maps of the preserve can be found in Figures 1 and 2.

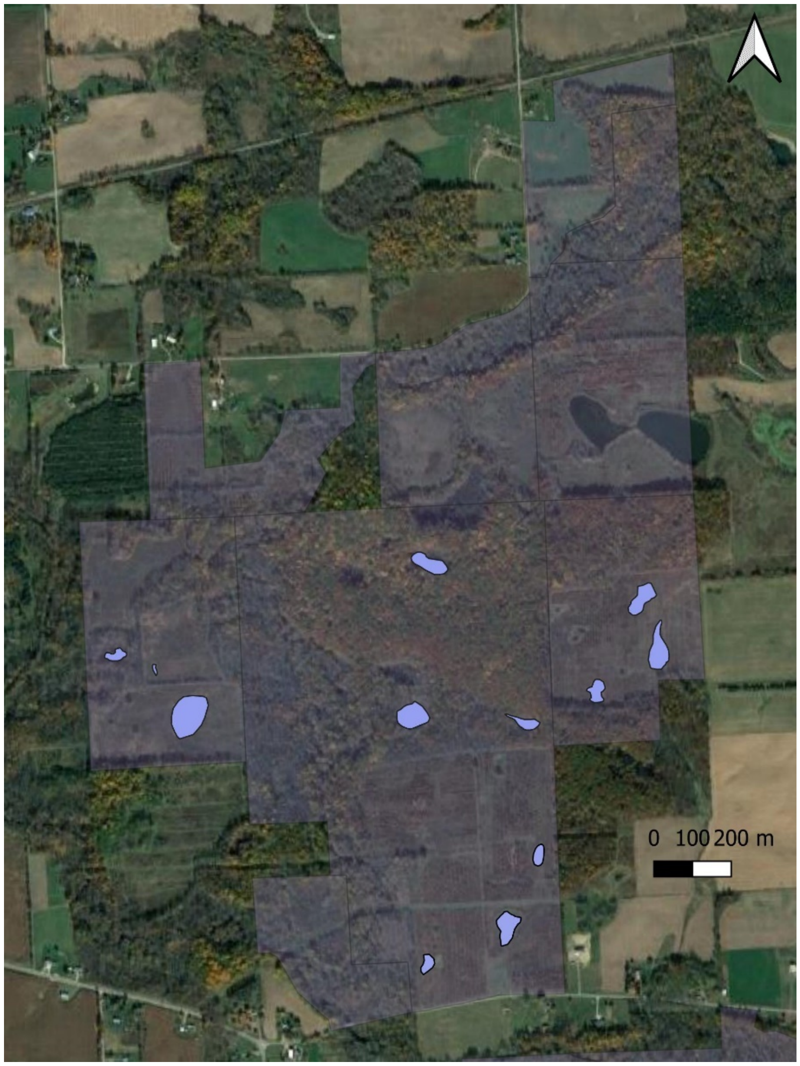

Figure 1. A map of the bulk of the wetlands in Douglas Woods. Studied wetlands are shaded blue. Preserve land is shaded transparent purple.

Figure 2. A map of the 3 northeastern sites. Sites are separated by roads. Studied wetlands are shaded blue. Preserve land is shaded transparent purple.

2.2 Wetland Characterization

Each wetland was characterized based on its hydrology, restoration status, dominant vegetation growth forms, size at high water, the age and basal area of the forest surrounding it, recent management efforts, its distance to the nearest mature forest, and its distance from other wetlands. These variables were evaluated as continuous or categorical, and used for statistical analysis.

The hydroperiod was assessed as a binary based on observed drying. If a wetland was observed to have no visible standing water at any point during Spring or Summer 2018, 2019, or 2021, it was classified as 'ephemeral'. If the wetlands were not observed to have dried completely, they were classified as 'semi-permanent'. These classifications were confirmed by TNC staff working on the property.

To test whether amphibians were willing to utilize restored wetlands, the restoration status was also assessed as a binary. If a wetland had been created since 1994, it was counted as restored. If a wetland appeared on a satellite image taken in 1994, it was counted as naturally-formed. The 1994 image was the best quality available, and it is assumed to represent the preserve immediately before restoration work began. The restoration efforts began in the late 1990s, and it is unlikely that any restorations were performed before TNC acquired the property. Assessments were corroborated by TNC staff.

Dominant vegetation of each wetland was assessed visually according to broad structure and growth forms (Cowardin et al., 1979). I was able to divide wetlands into three broad categories for analysis: Emergent, Scrub Shrub/Forest, and Scrub Shrub. Emergent wetlands have a periphery dominated by soft-stemmed semiaquatic plants including graminoids (rushes, sedges, true grasses) Juncaceae, Poaceae, and Cyperaceae, and forbs along their periphery with submergent cores. A Scrub Shrub wetland is dominated by woody vegetation less than 6m tall without detectable shading from adjacent trees. A Scrub Shrub/Forest wetland is dominated by woody vegetation less than 6m tall, with the presence of multiple adjacent trees of height greater than 6m that form a canopy over at least some of the wetland.

Wetland size at high water was assessed using satellite imagery and GIS mapping. The satellite imagery used was taken October 25, 2015 and was obtained from Google Earth®. Using the NAD83 spatial reference, an outline of each wetland was drawn atop its satellite image. The images were drawn based on visible vegetation shifts between frequently flooded areas and areas that remain dry. If a wetland had multiple basins, only the area of the basin in which traps were placed was counted to control for variable degrees of connectivity. Basins were considered separate if there was an observed break in the vegetation forms between them visible from satellite imagery, and if on-site observation confirmed separation by very shallow or dry areas. The computed areas of these polygons were used as an estimate of the wetland surface area. The distance from the nearest mature, closed-canopy forest was estimated by drawing lines between the polygons and the nearest canopy visible from satellite imagery.

The ages of the forests surrounding the wetlands were determined using TNC documents and historical satellite imagery. Forests planted 2009 and later were classified as "recent". Forests planted from 1994-2008 were classified as "intermediate". Forests predating 1994 were classified as "old". These classifications were coded into the models categorically.

The basal area of the forest was assessed using an angle gauge and a plotless sampling method. The angle gauge is tethered to the face while the assessor peers through it. The assessor pivots on one foot and notes every tree with a trunk that appears wider than the inside of the angle gauge at 1.2m high. Trees that are wider are counted as a hit, and trees that appear approximately equal in width are counted as a half hit. The forest around each wetland was measured four times with an angle gauge and averaged to determine the basal area of the forest (Shanks 1954). The measurements were all taken within 10 m of the wetland edge, once in each cardinal direction from the wetland. The basal area was then computed by: $Area = (Hits \times 10) \div n$, where n is the number of measurements taken, and Area is in ft^2/acre, which was converted to m^2/hectare.

To assess whether disturbance from recent restoration efforts affected amphibian use of them, the management status of each wetland was assessed as a binary. Wetlands classified as under management had significant restoration efforts made since 2017. These efforts include burning, tree girdling, and herbicide applications to remove invasive or problematic plant species.

Wetlands were analyzed for nearness to each other using GIS mapping software. A wetland location layer was obtained from the national wetland inventory. A point was manually placed in the centroid of each wetland in the area, and 500-meter and 1000-meter buffers were drawn around the edges of the 18 wetlands studied. The number of other wetlands within each buffer was determined by adding up the number of centroid points contained within each buffer. To estimate the minimum distance between wetlands, the Euclidean distance between the centroid of each studied wetland and that of its nearest neighbor was calculated.

2.3 Amphibian Surveying Approach

Ambystomatid salamanders were surveyed in 2019 and 2021. Surveys were conducted when nightly low temperatures exceeded 5° C and rain was predicted. Salamanders were collected using un-baited plastic minnow traps, with two traps placed in each wetland. If a wetland was totally dry at the time of trapping, no traps were set and a capture count of zero was recorded. Traps were set in water during the day at a depth that allowed access to air for trapped animals. Traps were tethered to an anchor object, left overnight, and opened the following day. When the traps were opened, salamanders were identified counted, and released. Other salamanders and fishes were also counted. All animals were released alive. If Ambystomatid egg masses were observed while laying traps but no salamanders captured, the wetland was counted to have an Ambystomatid species richness of 1. Traps were checked in sites 1,2,3, and 4 on March 25, 29, and 30 of 2019. Sites 5 and 6 were checked March 21, 29, and 31 of 2019. Traps in all wetlands were checked on March 26, 2021 and April 25, 2021. Each wetland was thus sampled 5 times total.

Frogs were sampled using call surveys. Each wetland was surveyed twice per year for two years, once in Spring and once in the Summer. Spring recordings were taken between April 28 & May 18 of 2018, and April 12 & May 6 of 2019. Summer Recordings were taken between June 24 & June 28 of 2018, and July 22-28 of 2019. Wetland 3b was only call-surveyed in 2019. Surveys were conducted a minimum of 30 minutes after sunset and lasted 5 minutes each. Lights were extinguished and silence maintained for 1 minute before a 5-minute recording was taken. Preliminary call identifications were made, then validated by listening to each recording. Only calls appearing to come from the direction of the wetland in question were counted. Distant calls or ones that appeared to come from another direction were excluded.

2.4 Statistical Analysis

Despite the small sample size of wetlands (n = 18), the data were analyzed using linear models. Recent research has questioned the conventional threshold of n=30 for linear models to be effective

(Carter and Wojton 2018). Linear models were chosen for their abilities to compare multiple effect variables to a response variable. Linear models are still quite capable of detecting strong effects even at such sample sizes. The response variables were analyzed using a Shapiro-Wilk test for normality. If they were normally distributed, a parametric linear fixed effects model was used. If they were not normally distributed, non-parametric generalized linear models were used instead. Analysis was performed using the "lm" and "glm" functions found in the basic R software.

Models were created with all measured variables and assessed using the Akaike Information Criterion (AIC) for fit. Variables with highest p values were subtracted and the models were re-run. If the AIC increased, the removed variable was re-added, and the variable with the next largest p-value was subtracted. The models were pruned of variables until subtracting any variable increased the AIC. Eight models were created (Table 1). Three models were made with Ambystoma richness as response variables, with habitat and co-occurring species as the predictor variables; and four with frog richness as response variables, with habitat and and co-occurring species as the predictor variables. One model was made with total amphibian species richness as a response variable and habitat variables as independent variables. Multiple models were created to avoid creating large models with unnecessarily large prediction error (Breiman and Freedman 1984) Final models were selected based upon their AIC values.

Due to the difficulty in identifying them using morphology, *Ambystoma laterale* and the unisexual *Ambystoma* were grouped together as one species for analysis. Unisexual females strongly resemble female *A. laterale*, and often share their characteristic blue spots. One male *A. laterale* was captured and is of interest for discussion but is grouped in with the unisexual species for analysis due to inconsistent morphology among the species complex. The male was only identified by its very swollen cloaca.

Table 1. A summary of the model types and variables used to predict amphibian species richness.

Response Variable	**Model Name and Type**	**Predictor Variables Used**
Ambystoma Richness	Ambystoma – Habitat; Generalized Linear Model	Habitat: Distance to nearest mature forest, surrounding forest age, wetland hydrology, dominant vegetation forms, forest basal area, wetland size, wetland management status, wetland restoration status, wetlands within 500 m, wetlands within 1 km, and distance to nearest other wetland.
Ambystoma Richness	Ambystoma – Other Animal Species; Generalized Linear Model	Other amphibian & fish species: Frog Species Richness, presence of *N. viridiscens*, presence of *Lepomis* spp.
Ambystoma Richness minus *A. tigrinum*	Ambystoma minus *A. tigrinum* – Habitat; Generalized Linear Model	Habitat: Distance to nearest mature forest, surrounding forest age, wetland hydrology, dominant vegetation forms, forest basal area, wetland size, wetland management status, wetland restoration status, wetlands within 500 m, wetlands within 1 km, and distance to nearest other wetland.
Total Frog Species Richness	Total Frogs – Habitat; Fixed Effects Linear Model	Habitat: Distance to nearest mature forest, surrounding forest age, wetland hydrology, dominant vegetation forms, forest basal area, wetland size, wetland management status, wetland restoration status, wetlands within 500 m, wetlands within 1 km, and distance to nearest other wetland.
Total Frog Species Richness	Total Frogs – Other Animal Species; Fixed Effects Linear Model	Other amphibian & fish species: *Ambystoma* Species Richness, presence of *N. viridiscens*, presence of *Lepomis* spp.
Ranid Frog Species Richness	Ranid Frogs – Habitat; Fixed Effects Linear Model	Habitat: Distance to nearest mature forest, surrounding forest age, wetland hydrology, dominant vegetation forms, forest basal area, wetland size, wetland management status, wetland restoration status, wetlands within 500 m, wetlands within 1 km, and distance to nearest other wetland.
Hylid Frog Species Richness	Hylid Frogs – Habitat; Fixed Effects Linear Model	Habitat: Distance to nearest mature forest, surrounding forest age, wetland hydrology, dominant vegetation forms, forest basal area, wetland size, wetland management status, wetland restoration status, wetlands within 500 m, wetlands within 1 km, and distance to nearest other wetland.
Total Amphibian Species Richness	Total Amphibians – Habitat; Generalized Linear Model	Habitat: Distance to nearest mature forest, surrounding forest age, wetland hydrology, dominant vegetation forms, forest basal area, wetland size, wetland management status, wetland restoration status, wetlands within 500 m, wetlands within 1 km, and distance to nearest other wetland.

The response variables for the models were the number of *Ambystoma* and frog species detected at each wetland. The initial independent variables for the habitat models were age, hydrology, dominant vegetation, forest basal area, wetland size, distance from mature forest, management status, restoration status, distance to nearest other wetland, number of wetlands within 500m, and number of wetlands within 1000m. The response variables for co-occurring species were the presence of sunfishes, mudminnows, red-spotted newts, and the number of frog or *Ambystoma* species, respectively.

CHAPTER 3. RESULTS

3.1 Amphibian Survey Findings

Four species of *Ambystoma* salamanders were found to use the wetlands within Douglas Woods: *Ambystoma texanum, A. maculatum, A. tigrinum,* and *A laterale* plus the putative unisexual species. One wetland contained *Ambystoma* egg masses but no adults were captured. The verifiable *A. laterale* was represented by a single male found in a restored wetland. Adult *Ambystoma* salamanders were captured in 9 out of the 18 wetlands. Of the wetlands in which they were detected, three were restored and 6 were naturally formed. 120 *Ambystoma* salamanders were captured in original wetlands, and 12 were captured in restored wetlands. The newt *Notophthalamus viridiscens* was captured in 8 out of 18 wetlands, four of which were original and four were restored.

Sunfishes *Lepomis spp.* were captured in 3 wetlands, all of which were restored. The central mudminnow, *Umbra limi*, was found in 3 wetlands, 2 original and 1 restored. A total of 8 species of anurans were found in Douglas Woods. *Lithobates clamitans, Lithobates pipiens, Lithobates catesbeianus, Lithobates sylvaticus, Pseudacris crucifer, Pseudacris triseriata, Dryophytes versicolor,* and *Acris crepitans*. All but *L. sylvaticus* were found in both restored and original wetlands, with the former only found in original wetlands. A total of 13 amphibian species were detected in the preserve.

Table 2. Descriptions of each of the 6 sites studied in Douglas Woods. Original wetlands are defined as being present before 1994, restored wetlands are defined as created after 1994.

Site Name	Wetlands Studied	Restoration Timeline
Site 1	3 – all restored	Wetlands: 2014 Forest: 2011
Site 2	1 – restored	Wetland: 2009-2010 Trees: 2010
Site 3	2 – all original	Wetland: 2009-2010 Trees: 2010
Site 4	3 – two original, one restored	Wetlands: 2009 Trees: 2009
Site 5	6 – three original, three restored	Wetlands: 2003-2004 Trees: 2003-2004
Site 6	3 – all restored	Wetlands: 2004 Trees: 2004

3.2 Statistical Analysis

The species richness among Ambystomatid salamanders was found to not be normally distributed, so generalized additive models were used. Among habitat variables, Ambystomatid salamander species richness was found to be negatively correlated with wetland distance to mature forest and intermediate and old adjacent forest age. Ambystomatid species richness was weakly positively correlated with a higher basal area of the surrounding forest. Wetland original status and size did not contribute significantly (Table 4).

Among species variables, Ambystomatid species richness was significantly positively associated with presence of the central mudminnow, *Umbra limi.* Ambystomatid species richness was also significantly negatively associated with the presence of sunfishes, *Lepomis spp.* Frog species richness appeared to have no effect on the salamander species richness (Table 6).

When *Ambystoma tigrinum* was removed from analysis, Ambystomatid species richness was positively correlated with ephemeral wetlands, naturally formed wetlands, and a higher number of wetlands within 1 km. Species richness was still negatively correlated with intermediate and old adjacent forest age. Forest basal area and wetland size at high water have no significant effects (Table 8).

Table 3. The initial results of the unpruned generalized linear model with Ambystoma species richness as the response variable and habitat variables as effects. 95% CI. Nagelkerke $R^2 = 0.925$. AIC = 34.589. Rows shaded in gold represent habitat variables that were removed during model fitting.

Fixed Effect on *Ambystoma* Species Richness	**Regression Coefficient**	**CI**	**p-value**
Distance to Mature Forest	-0.82	-1.41 – -0.24	**0.032**
Adjacent Forest Age (Intermediate)	-2.58	-4.15 – -1.01	**0.018**
Adjacent Forest Age (Old)	-4.99	-8.33 – -1.64	**0.026**
Hydrology (Ephemeral)	0.84	-0.08 – 1.76	0.122
Wetlands within 1 km	0.02	-0.04 – 0.08	0.541
Basal Area of Surrounding Forest	0.09	-0.02 – 0.19	0.145
Wetland Size at High Water	0.66	0.07 – 1.25	0.069
Under_Management (Yes)	0.64	-0.78 – 2.06	0.410
Restoration Status (Naturally Formed)	0.95	0.03 – 1.88	0.090
Wetlands within 500 m	-0.08	-0.19 – 0.04	0.228
Distance to Nearest other Wetland	0.15	-0.32 – 0.63	0.555

Table 4. A Summary of the fitted generalized linear model with Ambystoma species richness as the response variable and habitat variables as effects. 95% CI. Nagelkerke R2 = 0.908. AIC = 32.076. Bold p-values are significant.

Fixed Effect on *Ambystoma* Species Richness	**Regression Coefficient**	**CI**	**p-value**
Distance to Mature Forest	-0.78	-1.25 – -0.31	**0.010**
Adjacent Forest Age (Intermediate)	-1.89	-2.78 – -1.01	**0.002**
Adjacent Forest Age (Old)	-4.96	-7.25 – -2.67	**0.002**
Hydrology (Ephemeral)	0.56	-0.08 – 1.19	0.119
Basal Area of Surrounding Forest	0.11	0.05 – 0.17	**0.004**
Wetland Size at High Water	0.47	0.07 – 0.87	**0.048**
Under Management (Yes)	0.69	0.03 – 1.35	0.071
Restoration Status (Naturally Formed)	-0.06	-0.12 – 0.00	0.083

Table 5. The initial results of the unpruned generalized linear model with Ambystoma species richness as the response variable and other species variables as effects. 95% CI. Nagelkerke R2 = 0.629. AIC = 46.479. n = 18. Bold p-values are significant. Rows shaded in gold represent species variables that were removed during model fitting.

Fixed Effect on *Ambystoma* Species Richness	**Regression Coefficient**	**CI**	**p-value**
Mudminnow Presence	1.30	0.35 – 2.25	0.0019
Sunfish Presence	-1.09	-2.08 – -0.11	0.049
Red Spotted Newt Presence	0	-0.77 – 0.77	0.993
Frog Species Richness	-.021	-0.46 – 0.04	0.130

Table 6. A Summary of the fitted generalized linear model with Ambystoma species richness as the response variable and other species presence variables as effects. 95% CI. Nagelkerke R2 = 0.629. AIC = 44.479. n = 18. Bold p-values are significant.

Fixed Effect on *Ambystoma* Species Richness	**Regression Coefficient**	**CI**	**p-value**
Mudminnow Presence	1.30	0.35 – 2.25	**0.0019**
Sunfish Presence	-1.09	-2.08 – -0.11	**0.049**
Frog Species Richness	-.021	-0.46 – 0.04	0.130

Table 7. A Summary of the unpruned generalized linear model with *Ambystoma* species richness minus *A. tigrinum* as the response variable and habitat variables as effects. 95% CI. Nagelkerke R2 = 0.948. AIC = 24.602. n = 18. Bold p-values are significant. Rows shaded in gold represent species variables that were removed during model fitting.

Fixed Effect on *Ambystoma* Species Richness minus *A. tigrinum*	**Regression Coefficient**	**CI**	**p-value**
Distance to Mature Forest	-0.15	-0.52 – 0.22	0.461
Adjacent Forest Age (Intermediate)	-1.85	-2.92 – -0.79	**0.011**
Adjacent Forest Age (Old)	-2.36	-4.19 – -0.52	**0.040**
Hydrology (Ephemeral)	1.11	0.43 – 1.78	**0.015**
Wetlands within 1 km	0.05	0.01 – 0.09	0.056
Basal Area of Surrounding Forest	0.03	-0.03 – 0.09	0.406
Wetland Size at High Water	0.38	-0.06 – 0.81	0.134
Under Management (Yes)	0.44	-0.46 – 1.34	0.373
Restoration Status (Naturally Formed)	0.79	0.13 – 1.44	0.050
Wetlands within 500 m	0.18	-0.15 – 0.51	0.319

Table 8. A Summary of the fitted generalized linear model with Ambystoma species richness minus *A. tigrinum* as the response variable and habitat variables as effects. 95% CI. Nagelkerke R2 = 0.936. AIC = 22.240. n = 18. Bold p-values are significant.

Fixed Effect on *Ambystoma* Species Richness minus *A. tigrinum*	**Regression Coefficient**	**CI**	**p-value**
Adjacent Forest Age (Intermediate)	-1.39	-2.09 – -0.69	**0.003**
Adjacent Forest Age (Old)	-2.54	-4.04 – -1.03	**0.008**
Hydrology (Ephemeral)	0.85	0.36 – 1.33	**0.006**
Wetlands within 1 km	0.04	0.01 – 0.08	**0.030**
Basal Area of Surrounding Forest	0.05	0.00 – 0.09	0.056
Wetland Size at High Water	0.15	-0.07 – 0.37	0.211
Restoration Status (Naturally Formed)	0.67	0.16 – 1.17	**0.027**

Frog species richness was found to be normally distributed, so linear fixed effects models were used. Among habitat variables, frog species richness was found to be negatively correlated with distance to mature forest, temporary wetland status, recent management, original wetland status, distance to other wetlands, and number of other wetlands within 500 m. Species Richness was positively correlated with larger wetland size. No combination of species variables produced a statistically significant model for predicting frog richness, so the species effects model was not included.

When only Hylid frog species were analyzed (Table 12), only wetland original status was found to have a significant effect on their richness. Hylid frogs were significantly more speciose in restored wetlands than in original ones. When only Ranid frog species were analyzed (Table 14), species richness was negatively correlated with ephemeral hydrology and a higher number of other wetlands within 500 m.

Table 9. A summary of the unpruned fixed effects model with total frog species richness as the response variable and habitat variables as fixed effects. 95% CI. R2/R2 adjusted = 0.923/0.781. AIC = 44.898. n = 18 Bold p-values are significant. Rows shaded in gold represent species variables that were removed during model fitting.

Fixed Effect on Total Frog Species Richness	Regression Coefficient	CI	p-value
Distance to Mature Forest	-1.13	-2.10 – -0.16	**0.029**
Hydrology (Ephemeral)	-2.47	-3.99 – -0.94	**0.007**
Wetland Size at High Water	0.49	-0.48 – 1.46	0.265
Under Management (Yes)	-1.45	-3.82 – 0.91	0.183
Restoration Status (Naturally Formed)	-1.39	-2.92 – 0.15	0.070
Number of Wetlands within 500 m	-0.20	-0.39 – -0.01	**0.040**
Distance to Nearest other Wetland	-0.84	-1.63 – -0.05	**0.041**
Wetlands within 1 km	-0.04	-0.14 – 0.06	0.346
Basal Area of Surrounding Forest	0.12	-0.05 – 0.29	0.137
age (Intermediate)	0.02	-2.59 – 2.63	0.986
age (Old)	-3.52	-9.08 – 2.04	0.172

Table 10. A Summary of the fitted fixed effects model with total frog species richness as the response variable and habitat variables as fixed effects. 95% CI. R^2/R^2 adjusted = 0.883/0.801. AIC = 44.383. n = 18. Bold p-values are significant.

Fixed Effect on Total Frog Species Richness	Regression Coefficient	CI	p-value
Distance to Mature Forest	-0.91	-1.51 – -0.31	**0.032**
Hydrology (Ephemeral)	-1.94	-2.85 – -1.02	**0.003**
Wetland Size at High Water	0.71	0.17 – 1.25	**0.016**
Under Management (Yes)	-1.40	-2.64 – -0.17	**0.030**
Restoration Status (Naturally Formed)	-1.56	-2.61 – -0.51	**0.008**
Number of Wetlands within 500 m	-0.12	-0.23 – -0.02	**0.028**
Distance to Nearest other Wetland	-0.44	-0.87 – -0.00	**0.049**

Table 11. A Summary of the unpruned generalized linear model with Hylid frog species richness as the response variable and habitat variables as effects. 95% CI. Nagelkerke R2 = 0.841. AIC = 46.1825. n = 18. Bold p-values are significant. Rows shaded in gold represent species variables that were removed during model fitting.

Fixed Effect on Hylid Frog Species Richness	Regression Coefficient	CI	p-value
Distance to Mature Forest	-0.58	-1.38 – 0.22	0.207
Adjacent Forest Age (Intermediate)	-0.03	-2.20 – 2.14	0.980
Adjacent Forest Age (Old)	-2.51	-7.12 – 2.10	0.327
Hydrology (Ephemeral)	-0.85	-2.11 – 0.42	0.237
Wetlands within 1 km	-0.05	-0.14 – 0.03	0.250
Basal Area of Surrounding Forest	0.08	-0.07 – 0.22	0.342
Wetland Size at High Water	0.17	-0.63 – 0.98	0.688
Under Management (Yes)	-0.93	-2.90 – 1.03	0.387
Restoration Status (Naturally Formed)	-0.94	-2.22 – 0.34	0.198
Number of Wetlands within 500 m	-0.09	-0.24 – 0.06	0.296
Distance to Nearest other Wetland	-0.48	-1.14 – 0.18	0.202

Table 12. A Summary of the fitted fixed effects model with Hylid frog species richness as the response variable and habitat variables as effects. 95% CI. R^2/R^2 adjusted = 0.883/0.801. AIC = 44.383. n = 18. Bold p-values are significant.

Fixed Effect on Hylid Frog Species Richness	Regression Coefficient	CI	p-value
Distance to Mature Forest	-0.40	-0.97 – 0.16	0.185
Hydrology (Ephemeral)	-0.46	-1.25 – 0.33	0.276
Wetlands within 1 km	-0.03	-0.08 – 0.02	0.204
Wetland Size at High Water	0.38	-0.08 – 0.84	0.134
Under Management (Yes)	-0.87	-1.81 – 0.08	0.100
Restoration Status (Naturally Formed)	-1.13	-2.05 – -0.22	**0.034**

Table 13. A Summary of the unpruned generalized linear model with Ranid frog species richness as the response variable and habitat variables as fixed effects. 95% CI. Nagelkerke R2 = 0.900. AIC = 39.099. Bold p-values are significant. Rows shaded in gold represent species variables that were removed during model fitting.

Fixed Effect on Ranid Frog Species Richness	**Regression Coefficient**	**CI**	**p-value**
Distance to Mature Forest	-0.58	-1.24 – 0.08	0.135
Adjacent Forest Age (Intermediate)	-0.54	-2.32 – 1.24	0.572
Adjacent Forest Age (Old)	-1.79	-5.57 – 2.00	0.392
Hydrology (Ephemeral)	-1.26	-2.30 – -0.22	0.055
Wetlands within 1 km	0.01	-0.06 – 0.07	0.850
Basal Area of Surrounding Forest	0.06	-0.06 – 0.18	0.347
Wetland Size at High Water	0.38	-0.29 – 1.04	0.309
Under Management (Yes)	-0.21	-1.82 – 1.40	0.805
Restoration Status (Naturally Formed)	-0.16	-1.21 – 0.89	0.779
Number of Wetlands within 500 m	-0.18	-0.31 – -0.05	**0.031**
Distance to Nearest other Wetland	-0.31	-0.85 – 0.23	0.303

Table 14. A Summary of the fitted generalized linear model with Ranid frog species richness as the response variable and habitat variables as fixed effects. 95% CI. Nagelkerke R2 = 0.866. AIC = 34.098. Bold p-values are significant.

Fixed Effect on Hylid Frog Species Richness	**Regression Coefficient**	**CI**	**p-value**
Distance to Mature Forest	-0.38	-0.77 – 0.01	0.081
Hydrology (Ephemeral)	-1.20	-1.85 – -0.56	**0.004**
Basal Area of Surrounding Forest	0.02	0.00 – 0.05	0.068
Wetland Size at High Water	0.29	-0.06 – 0.65	0.135
Number of Wetlands within 500 m	-0.16	-0.22 – -0.09	**0.001**
Distance to Nearest other Wetland	-0.20	-0.48 – 0.09	0.199

The total amphibian species richness was found to not be normally distributed, so a generalized linear model was created (table 16). The model deleted four observations to run, due to colinearity, which rendered it less predictive than the others. When Ambystomatid salamander richness, frog richness, and the red-spotted newt presence were combined, the total amphibian richness was positively correlated with higher forest basal area, and negatively correlated with distance to mature forest, ephemeral hydrology, scrub shrub/forest vegetation, management, number of other wetlands within 500 m and 1 km, and distance to the nearest other wetland.

Table 15. A Summary of the unpruned generalized linear model with total amphibian species richness as the response variable and habitat variables as effects. 95% CI. Nagelkerke $R^2 = 0.985$. AIC = 29.249. n = 14. Bold p-values are significant. Rows shaded in gold represent species variables that were removed during model fitting.

Fixed Effect on Total Amphibian Richness	Regression Coefficient	CI	p-value
Distance to Mature Forest	-2.03	-2.90 – -1.16	**0.044**
age (Intermediate)	-0.71	-5.27 – 3.85	0.789
Hydrology (Ephemeral)	-2.52	-4.82 – -0.21	0.166
Scrub Shrub/Forest Dominant Vegetation	-2.22	-5.76 – 1.31	0.343
Basal Area of Surrounding Forest	0.38	0.05 – 0.71	0.154
Wetland Size	0.34	-1.24 – 1.91	0.717
Management Status (Yes)	-0.89	-3.46 – 1.68	0.569
Restoration Status (Naturally Formed)	-0.15	-1.43 – 1.13	0.838
Wetlands Within 500 m	-0.35	-0.56 – -0.13	0.091
Wetlands Within 1 Km	-0.11	-0.33 – 0.11	0.422
Distance to Nearest other Wetland	-1.01	-1.89 – -0.13	0.153

Table 16. A Summary of the fitted generalized linear model with total amphibian species richness as the response variable and habitat variables as effects. Nagelkerke R2 = 0.983. AIC = 25.014. n = 14. Bold p-values are significant.

Fixed Effect on Total Amphibian Richness	Regression Coefficient	CI	p-value
Distance to Mature Forest	-2.03	-2.60 – -1.45	**0.001**
Hydrology (Ephemeral)	-2.92	-3.76 – -2.09	**0.001**
Scrub Shrub/Forest Dominant Vegetation	-2.78	-4.41 – -1.14	**0.021**
Basal Area of Surrounding Forest	0.42	0.24 – 0.60	**0.006**
Management Status (Yes)	-1.01	-2.24 – 0.23	0.171
Wetlands Within 500 m	-0.38	-0.50 – -0.25	**0.002**
Wetlands Within 1 Km	-0.15	-0.22 – -0.08	**0.009**
Distance to Nearest other Wetland	-1.15	-1.57 – -0.73	**0.003**

CHAPTER 4. DISCUSSION

For habitat restorations to be effective, they must be done purposefully and with particular goals in mind. These goals should be attainable and measurable (SER International 2004). They ought to also include ideas of what the restored habitat should be, and which animals will inhabit it. In analyzing which wetland characteristics are associated with higher numbers of amphibian species, I hope to elucidate some of the markers of a successful restoration. The models shown here are moderately conclusive, but the small sample sizes create some uncertainty. Additionally, the models that pooled all amphibian species together required discarding four of the 18 observations to run, which further reduces statistical power. The conclusions presented here ought to be interpreted cautiously, but they remain useful for assessing potential habitat preferences of amphibians. The different amphibian taxa exhibit markedly different habitat preferences, and some flexibility in their use of restored wetlands. Amphibian use of restored wetlands is a positive sign of their development and is a worthy goal of restoration projects. In designing wildlife sanctuaries and protecting wetlands, amphibian habitat preferences must be considered.

4.1 Distance from Mature Forest and Adjacent Forest Age

Smaller distance from the nearest closed-canopy forest was significantly correlated with both a higher *Ambystoma* species richness and frog species richness. All the Ambystomatid species, besides *A. tigrinum* (Petranka 1998), and Hylid frogs are known to inhabit woodlands, so these findings are unsurprising. *A. maculatum* and *L. sylvaticus* have both been documented to preferentially move toward closed forest canopies post-metamorphosis (DeMaynadier and Hunter 1999). The analysis suggests that older and intermediate forest ages are associated with decreased numbers of Ambystomatid species, which seemingly contradicts these other findings. I discuss this apparent contradiction under its own section, but I believe it to be a statistical anomaly. This negative association remained when *A. tigrinum* was removed, which suggests that its open canopy preferences (Petranka 1998) are not responsible for pulling the model toward younger forests. These data, taken together, might indicate that the age of the forest immediately adjacent to the wetlands is largely not relevant for its use by *Ambystoma* salamanders. The forests that were within 10-15 m of a wetland may not support salamanders, but salamanders may still use a wetland if it is reachable from their forest.

When all amphibian species were pooled, the results indicate that lower distance from mature forest is still preferred. This is intuitive for the species that inhabit woodlands. Curiously, when analyzed alone, Hylid frog species was not significantly associated with the wetland's distance from mature forests. The variable was retained in the fitted model, implying some effect, but it was not significant. Although Hylid frogs are mostly arboreal, they may not be strongly selective about the maturity of the trees they inhabit.

4.2 Basal Area

Likewise, higher basal area had a slight positive correlation with *Ambystoma* salamanders. Some of the forests adjacent to the Douglas Woods preserve contain wetlands that the salamanders probably also use, but they are privately owned and not surveyable.

In the pooled model with all amphibian species, higher forest basal area was also positively correlated with higher species richness. This may be the salamander preferences breaking through, since frogs appear to be indifferent according to the other models.

4.3 Restoration Status

The non-significant effects of wetland original status on overall *Ambystoma* richness suggests that they might prefer original wetlands, but that this preference is not an absolute requirement . The presence of *Ambystoma* salamanders in many restored wetlands indicates that they will indeed attempt to use restored wetlands for breeding. Furthermore, when *Ambystoma tigrinum* was removed from the model, a significant correlation was seen between species richness and naturally formed wetlands. *A. tigrinum* may simply be less selective than the other salamanders, which is supported by Petranka (1998).

Wetland original status being negatively correlated with frog species richness poses interesting questions about frog wetland preferences. They appear to prefer restored wetlands over original ones, and hydroperiod may be a factor. Many of the original wetlands are ephemeral, which would discourage animals with lengthy aquatic larval periods from using them. However, when analyzed alone, Ranid frogs did not display a significant correlation with restoration status. Hylid frogs, however, showed a negative correlation with original wetlands. Lehtinen and Galatowitsch (2001) found that *P. crucifer* was absent from recently restored wetlands, but their wetlands were less than 12 months old. It appears that Hylid frogs indeed colonize restored wetlands, and may even prefer them.

4.4 Management

The negative correlation between frog species and the managed wetlands suggests that clearing wetlands of vegetation, even invasive vegetation, deters their use by frogs. The commotion around the management, the herbicides, or the loss of vegetation around the wetlands may have disturbed the frogs enough to decrease their use of the wetlands. The elimination of invasive plants must be balanced with the negative effects on the wetland amphibians, which appear to be substantial (Wagner 2013). Reed Canarygrass, *Phalaris arundinacea,* one of the invasive plants targeted at Douglas Woods, may even be attractive for some native North American frog species (Holzer and Lawler 2015). Japanese stilt grass, *Microstegium vimineum*, has also been found to provide habitat for native frogs in the midwestern United States (Nagy et al., 2011). These findings raise questions about invasive plants and their roles in the ecosystems they colonize. They may be harmful in some ways, but beneficial in others. The removal of the invasive vegetation might have unforeseen consequences. The managed wetlands still appeared to have less vegetation overall than others nearby, even three years after the invasive plants were removed.

4.5 Wetland Size

Larger wetland size also appears to be positively associated with larger numbers of *Ambystoma* and total frog species. This may simply be a result of larger wetlands having more habitat in absolute terms, with more space for more species. The larger wetlands may contain more diverse habitats that meet the requirements of more species (Lichtenberg et al., 2006). Theories of island biogeography suggest that the larger a habitat is, the more species it can potentially support (Preston 1962). Wetlands and other isolated habitats may be thought of as islands of habitability set within a larger matrix of unsuitable habitat.

4.6 Hydrology

Hydroperiod appears to exert a less than significant effect the *Ambystoma* species richness. However, when *Ambystoma tigrinum* was removed from analysis, the remaining species did display a correlation with ephemeral wetlands. Still, my observations on hydroperiod call into question the emphasis on ephemeral wetlands as their breeding grounds, which is consistent with the observations of De Lisle and Grayson (2011). So long as the factors that stymie recruitment, such as sunfishes, are not present, the salamanders should be able to breed successfully in semi-permanent wetlands. Frog species richness actually appears to decrease in ephemeral wetlands, indicating a preference for semi-permanent ones. Ranid tadpoles often overwinter in their natal wetlands before metamorphosing (Tattersall and Ultsch 2008), which would explain a preference against wetlands that dry frequently. If a wetland dries completely, recruitment of Ranid frogs could be negligible that season. Low recruitment would decrease local sub-populations, and the frogs would eventually no longer inhabit that wetland. With all amphibian species pooled semi-permanent wetlands were preferred, possibly due to the frog species pulling the model that way.

4.7 Forest Age

The data on the wetland habitat's effects on their use by *Ambystoma* salamanders tell a somewhat puzzling story. Old and intermediate ages were significantly negatively correlated with Ambystomatid species richness, but this is likely a statistical artifact caused by the low sample size, or by how the forests were categorized by age. This correlation is seemingly incongruous to the results of Vasconcelos and Calhoun (2004) who observed strong preferences for closed-canopy forest among *Ambystoma maculatum*. Three out of the four wetlands located in the oldest forest each contained 2-3 *Ambystoma* species, with three being the highest number observed in any wetland. Also, as discussed earlier, a smaller distance to mature forest was associated with greater numbers of frog and Ambystomatid salamander species. Homan et al., (2008) also believed that *A. maculatum* carrying capacity increases as young forests mature. Rothermel and Luhring (2005) found that a mature, closed-canopy forest significantly decreased mortality by desiccation in *A. talpoideum*. Unfortunately, there may not be enough statistical power to fully explain this anomaly. These data should not be construed to mean that Ambystomatid salamanders do not thrive in mature forests.

4.8 Vegetation

Only the model analyzing all amphibian species together returned significant correlations with vegetation. Scrub Shrub/Forest wetlands were associated with lower total amphibian species richness. These wetlands had a mean of four frog species versus the overall mean of 4.94. Werner and Glennemeier (1999) demonstrated that some frog species strongly prefer wetlands in open-canopy areas, which would explain why these forested wetlands had fewer species overall. Frogs are also more speciose than salamanders and are therefore better able to influence the model. However, when the frogs were analyzed alone, wetland vegetation did not contribute enough to remain in the model. This discrepancy could be explained by the addition of the red spotted newt and tiger salamander, both of which thrive in open areas (Petranka 1998).

Ambystoma species richness also does not appear to be impacted by dominant vegetation and management status. The lack of significant effects of dominant vegetation types further supports the idea that the vegetation surrounding the wetland is less important than the wetland's proximity to the salamanders' forest habitat. It appears that neither the invasive species nor the management exerted significant effects on *Ambystoma* species richness either.

4.9 Wetland Connectivity

None of the wetland connectivity variables seemed to exert any effect on Ambystomatid species richness when all species were pooled. Distance to the nearest other wetland and the number of other wetlands within 500-1000 m had no significant impact. However, when *Ambystoma tigrinum* was removed, a significant positive correlation with greater numbers of wetlands was found. It appears that *A. tigrinum* may skew the models away from more connected wetlands. This particular salamander is known to have a broad range of suitable habitats (Petranka 1998) and may be less selective with its breeding wetlands. I posit that when analyzing wetland connectivity, as it concerns these salamanders, their terrestrial habitats should be factored in. The shaded, moist upland forests that these salamanders might provide corridors for their dispersal even in the absence of wetlands. Gamble et al (2007) found that *A. opacum* could travel as much as 1350 m from its natal wetland, and posited that forest continuity may have played a role in their capacity to disperse. Coulon et al., (2004) demonstrated that mammal movement is governed by least-cost rules rather than simple Euclidean distance, and it would be plausible that such rules affect amphibians as well. The abundance of northern watersnakes, *Nerodia sipedon sipedon*, have been shown to be influenced by upland as well as wetland structure (Attum et al., 2007). It would be beneficial if future studies examined the upper limit of Ambystomatid dispersal capabilities and how terrestrial habitat affects them.

The frogs' apparent preference for more isolated wetlands, with fewer others within 500 m, is also curious. One might attribute it to their preference for restored wetlands, but both restored and naturally formed wetlands have identical averages of 11.1 other wetlands within 500 m. If they truly do prefer isolated wetlands, it may relate to disease ecology, with more isolated habitats decreasing disease transmission (McCallum and Dobson 2002). With the advent of *Ranavirus* and other amphibian diseases (Lesbarrères et al., 2012), it is not implausible that frogs would prefer more isolated wetlands. Heard et al., (2015) suggest that heterogenous patchy wetlands may serve as disease refugia, but they acknowledge that some degree of interconnectedness is essential to maintain a metapopulation.

4.10 Co-Dwelling Vertebrate Species

The data on wetland co-dwelling animal species and their effects on *Ambystoma* species richness are a bit easier to comprehend. Ambystoma species richness is significantly negatively correlated with the presence of sunfishes, and none of the 3 wetlands found to contain the fish yielded *Ambystoma* salamanders to traps. A related species, *A. barbouri,* has been found to avoid laying eggs in pools containing fish (Kats and Sih 1992). This avoidance is likely due to fish predation on eggs. Contrastingly, presence of the central mudminnow is significantly positively correlated with *Ambystoma* species richness. It is possible that the mudminnow thrives in conditions that the larvae also thrive in. These small fish likely do not pose a large predation risk to salamander eggs and larvae, asthey feed mostly on invertebrates that the larvae also prey upon (Panek and Weis 2013). The presence of red-spotted newts and numbers of frog species does not appear to impact the number of *Ambystoma* species present. The newts feed primarily on invertebrates (Proehl et al., 2017), similar to the *Ambystoma* larvae, and are probably too small to pose a serious predation risk to the eggs or larvae. When all frog species were analyzed, no association was found between their species richness and any of the other wetland-dwelling animals.

4.11 Other Observations

Ambystoma laterale is listed as a special of special concern in Indiana (Indiana DNR 2017), and its range in Indiana is confined to the extreme north. A single male of the species was found in a restored wetland, indicating that they are both present in the area and willing to use restored wetlands. This finding indicates that these salamanders do use restored wetlands, which is an encouraging development in their conservation.

The models that pooled all amphibian species found in a wetland were disappointing. The models were only able to use 14 observations out of 18, so the statistical power is much reduced. I believe that the frogs and salamanders also may be pulling the model in different directions, given the differences between the frog and salamander models.

4.12 Assessment of the Restoration

A wetland that is not suitable for amphibians is performing sub-optimally. Amphibians are sensitive to environmental degradation, and places they avoid are likely of poor quality for other sensitive species. The results of this study serve as an evaluation of the health of the amphibian community of Douglas Woods, and indirectly, as the health of the wetlands it contains. It is assumed that more amphibian species equals a healthier ecosystem, which has been demonstrated in previous studies (Saber et al., 2017; Xu-Dong et al., 2003). The Nature Conservancy has an interest in the health of the amphibian community, for the sake of the amphibians themselves and by virtue of their role as indicators of habitat quality. Health of an amphibian community is difficult to gauge, but the overall number of amphibian species found in an area can serve as a proxy.

Douglas Woods appears to be performing relatively well with 13 wetland-dwelling amphibian species detected there, with less than 25 years since restorations began. For comparison, Werner et al., (2007) found 14 wetland-associated amphibian species in a Michigan preserve of comparable size that had been managed as a restricted access preserve for over 60 years. The restored wetlands in Douglas Woods are used by frogs, newts, and *Ambystoma* salamanders. These results are encouraging for future restorations, and appear to demonstrate that amphibians will rapidly begin to use restored wetlands if the wetlands are suitable. It is likely that conditions for amphibians will improve further as the planted trees near the wetlands mature into a functional forest ecosystem. Once the canopies close adequately and the habitat more resembles a native forest, Ambystomatid salamanders may begin to inhabit the land around the restored wetlands and colonize more of them.

4.13 Conclusions

Wetland restorations are not a one-size-fits-all endeavor. It appears that different amphibian species require different types of wetlands. When attempting a restoration, diverse wetlands should be a goal. Upland areas also likely play a role in amphibian wetland use.

Ideal wetlands for ambystoma salamanders should be created close to any existing mature forest near a proposed restoration site if such a forest is available, and forests planted if not. The wetlands need not be so shallow that they dry regularly and should be fairly large to increase the chances of amphibians finding suitable habitat within them. Ideal wetlands for frogs should also be relatively large, rarely dry, and be near mature forests if possible. Necessary depth will depend on local climate and precipitation patterns, but the deepest wetlands in Douglas Woods seldom dry fully and were approximately 1.5-2 m deep in the center during late Spring.

Management of the wetlands post-creation should be balanced with the risk of disturbing amphibians there. Special caution should be taken to prevent wetlands being colonized by predatory fishes, and consideration should be given to removing existing populations when possible. Once wetland-adjacent planted forests mature further, amphibian relocation may be a viable option to assist their dispersal if there are no source populations nearby. Weyrauch and Amon (2002) found good success in relocating *A. maculatum* to created wetlands near planted forests, and their results could likely be replicated at Douglas Woods and other preserves.

Wetland restorations are costly and time-consuming endeavors, but they are important to safeguard biodiversity. With wetlands continually being destroyed, restorations must happen to counter-balance losses, or risk an enormous loss of biodiversity. With proper design, restored wetlands can function similarly to naturally formed ones.

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APPENDIX A. SURVEY RESULTS

Table 1A. The overall results of wetland surveys. For Hydrology, SP = "semi-permanent", a wetland that typically holds water for the entire year E = "Ephemeral", a wetland that dries completely most years. Basal Area refers to the cross-sectional area of the trees surrounding each wetland. For vegetation, EM means emergent, SS means scrub shrub, and SS/F means scrub shrub/forest. For forest age, Y means young (<12 years), I means intermediate (13-24 years), O means old (>25 years).

Wetland	Frog Species Richness	*Ambystoma* Species Richness	Site	Hydrology	Vegetation	Basal Area (m^2/hectare)	Forest age
1a	5	0	1	SP	EM	1.721763085	Y
1b	6	2	1	SP	EM	1.434802571	Y
1c	6	0	1	SP	EM	1.721763085	Y
2	7	0	2	SP	EM	6.600091827	I
3a	7	1	3	SP	EM	4.017447199	I
3b	2	1	3	E	SS	7.747933884	I
4a	5	1	4	SP	EM	5.739210285	I
4b	5	0	4	E	EM	0.573921028	I
4c	6	0	4	SP	SS/F	20.94811754	O
5a	7	1	5	SP	SS	10.61753903	I
5b	4	0	5	SP	EM	8.608815427	I
5c	4	1	5	E	EM	6.887052342	I
5d	5	2	5	E	SS/F	41.89623508	O
5e	3	3	5	E	SS/F	41.03535354	O
5f	2	2	5	E	SS/F	39.31359045	O
6a	5	0	5	SP	EM	8.034894399	I
6b	5	0	6	SP	EM	3.730486685	I
6c	5	0	6	SP	EM	10.33057851	I

Table 2A. Results of wetland surveying. Size refers to the estimated surface area of the wetland from satellite imagery. "Dis mat forest" is the distance from the nearest edge of the wetland to the nearest closed-canopy forest in meters. Management status refers to whether herbicides or fire have been applied from 2017-2021 to combat invasive plant species. Original refers to whether the wetland formed naturally before 1994 or was created by TNC since then.

Wetland	**Size (m^2)**	**Dis Mat Forest (m)**	**Under Management (<4y)**	**Original**	**Total *Ambystoma* Salamanders Captured**
1a	2786	114.5	No	No	0
1b	2427	51.1	No	No	2
1c	3024	187.2	No	No	0
2	25638	251.2	No	No	0
3a	14692	73.8	No	Yes	1
3b	710	80.2	No	Yes	1
4a	7847	90.4	No	Yes	1
4b	210	26.3	No	No	0
4c	1383	0	No	Yes	0
5a	1180	12	No	No	0
5b	3305	113.8	Yes	No	0
5c	1835	29.1	Yes	No	7
5d	4161	0	No	Yes	17
5e	3097	0	No	Yes	62
5f	1550	0	No	Yes	35
6a	2594	5.1	Yes	No	0
6b	4626	12	Yes	No	0
6c	3182	27.8	Yes	No	0

Table 3A. The presence/absence data of salamander and fish species in Douglas Woods. A "+" means present. A "- " means absent. * means the blue-spotted salamander, *Ambystoma laterale*, and the unisexual Ambystoma species grouped together due to their morphological similarities.

Wetland	***Ambystoma species richness***	***A. tigrinum***	***A. texanum***	***A. maculatum***	***A. jeffersonianum Complex****
1a	0	-	-	-	-
1b	2	+	-	-	+*
1c	0	-	-	-	-
2	0	-	-	-	-
3a	1	+	-	-	-
3b	1	-	+	-	-
4a	1	-	-	+	-
4b	0	-	-	-	-
4c	0	-	-	-	-
5a	1	-	-	-	-
5b	0	-	-	-	-
5c	1	-	-	-	+
5d	2	-	+	-	+
5e	3	-	+	+	+
5f	2	-	+	-	+
6a	0	-	-	-	-
6b	0	-	-	-	-
6c	0	-	-	-	-

Table 4A. The presence/absence data of red-spotted newt, *Notophthalmus viridescens*, sunfishes of genus *Lepomis*, and the central mudminnow, *Umbra limi*, in Douglas Woods. A “+” means present. A “-“ means absent.

Wetland	***N. viridiscens***	***Lepomis spp.***	***U. limi***
1a	-	-	-
1b	-	-	-
1c	-	-	-
2	-	+	+
3a	-	-	-
3b	-	-	-
4a	+	-	-
4b	-	-	-
4c	-	-	-
5a	-	-	-
5b	+	+	-
5c	+	-	-
5d	+	-	-
5e	-	-	+
5f	+	-	+
6a	+	-	-
6b	+	+	-
6c	+	-	-

Table 5A. The presence/absence data of Hylid frog plus toad species found in Douglas Woods. A "+" means present. A "-" means absent. Presence was determined using call surveys.

Wetland	***Pseudacris crucifer***	***Pseudacris triseriata***	***Dryophytes versicolor***	***Acris crepitans***	***Anaxyrus americanus***
1a	+	-	+	+	-
1b	+	+	+	+	-
1c	+	+	+	+	-
2	+	+	+	+	-
3a	+	+	+	+	-
3b	+	+	-	-	-
4a	+	-	+	-	-
4b	+	+	+	-	-
4c	+	+	+	-	-
5a	+	+	+	+	-
5b	+	-	+	-	-
5c	+	+	+	-	-
5d	+	+	-	-	-
5e	+	-	+	-	-
5f	+	-	-	-	-
6a	+	+	+	-	+
6b	+	-	-	+	+
6c	+	+	+	-	+

Table 6A. The presence/absence data of Ranid frog species found in Douglas Woods. A "+" means present. A "-" means absent. Presence was determined using call surveys.

Wetland	***Lithobates clamitans***	***Lithobates catesbeianus***	***Lithobates pipiens***	***Lithobates sylvaticus***
1a	+	-	+	-
1b	+	+	-	-
1c	+	-	+	-
2	+	+	+	-
3a	+	+	+	-
3b	-	-	-	-
4a	+	+	+	-
4b	+	-	+	-
4c	+	+	+	-
5a	+	+	+	-
5b	+	-	+	-
5c	+	-	-	-
5d	+	-	+	+
5e	+	-	-	-
5f	+	-	-	-
6a	+	-	-	-
6b	+	+	-	-
6c	+	-	-	-

APPENDIX B. PHOTOGRAPHS OF SALAMANDERS AND WETLANDS

Figure 1B. A spotted Salamander, *Ambystoma maculatum,* captured in wetland 5e.

Figure 2B. A possible unisexual Ambystoma salamander that resembles *Ambystoma laterale* captured in wetland 5f.

Figure 3B. A Smallmouth Salamander, *Ambystoma texanum,* captured in wetland 5e.

Figure 4B. An image of wetland 1a, classified as emergent.

Figure 5B. An image of wetland 5a, classified as scrub shrub.

Figure 6B. An image of wetland 5f, classified as scrub shrub/forest.