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Restoration for The Small and Slimy: How Pond-breeding Amphibians Utilize Natural, Restored, and Created Wetlands

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Restoration for The Small and Slimy: How Pond-breeding Amphibians Utilize Natural, Restored, and Created Wetlands

Miranda Florent

2.12.2024

Abstract

This research project for the Williams Honors College sought to examine how local pond-breeding amphibians utilized natural, restored, and created wetlands. Presence/absence data was gathered from field surveys, historical records, and iNaturalist data for the six study sites. It was predicted that created and restored wetlands may support less sensitive species with lower coefficient of conservatism scores, based on past surveys of a similar nature. No statistically significant results were found in this survey, but that could be due to low sample sizes and limited data sets, among other considerations. Therefore, more in-depth studies should still be undertaken to examine this question in our local ecosystems. Restoration and habitat creation are becoming more common practices as natural spaces are lost and climate change worsens. When restoring and creating wetland habitat, more considerations should be made for sensitive, pond-breeding species, and extra care should be taken to not cause harm via hydrology changes, human disturbance, introduction of predatory species, etc. In Ohio, our wetland protections and restoration practices likely need an overhaul to truly support and protect sensitive, pond-breeding amphibian species.
Introduction

In a time where natural spaces are facing ever-increasing development and disturbance, restoration has become a popular topic in conservation and land management. Protecting wild spaces is no longer just about limiting development or preserving what exists naturally. An increasing number of land managers and conservationists are turning to restoration as a way to take once-wild spaces and make them wild again, if possible. When making changes within an ecosystem, even those meant to be positive, it is impossible to fully understand or predict the outcomes. Additionally, many ecosystems have overlooked inhabitants that are harder to study or less charismatic. Amphibians can be a good example of these inhabitants and their relationship with restoration efforts can be very complex. This project seeks to unwind some of these complexities by examining local restoration efforts and amphibian communities - how they interact and how restoration can better serve these often overlooked but critical species.

Restoration work often poses a complicated and difficult to understand challenge, which can yield surprising results (Holl, 2020). Amphibian conservation can be impacted by this reality, but there has been important work examining the relationship between the two topics. Past research has examined how pond-breeding amphibians may utilize or avoid restored and created wetlands (Denton and Ritcher, 2013). This work found that community composition tended to differ between natural and created sites, with more sensitive species like marbled salamanders (*Ambystoma opacum*) and wood frogs (*Lithobates sylvaticus*) being found almost exclusively at natural, undisturbed wetlands (Denton and Ritcher, 2013). In the case of this study, created wetlands fell into two creation methods; dammed wetlands that retained permanent water and wetlands that were ephemeral, acting as vernal pools (Denton and Ritcher, 2013). The permanent, created wetlands attracted predatory amphibian species like green frogs (*Lithobates clamitans*), bullfrogs (*Lithobates catesbeianus*), and eastern newts (*Notophthalmus viridescens*), all of which prefer to inhabit permanent wetlands due to their need to overwinter in bodies of water (Denton and Ritcher, 2013). Although native to the region, their
presence in this particular system (ridge-top wetlands in eastern Kentucky) is atypical, and due to their predatory nature, their presence can often preclude other amphibians from utilizing wetlands for habitat and breeding (Denton and Ritcher, 2013). These unintended consequences, among others, beg the question - is wetland creation or restoration always a positive, or could it cause more harm than good in some scenarios?

Wetland restoration is a very imperfect process. In states like Ohio, where wetland mitigation systems are in place, the goal of wetland restoration could be the first place where things go awry. In Ohio, the development of wetlands is required to be balanced out via a compensatory wetland mitigation system, overseen by a variety of agencies including the Ohio Environmental Protection Agency and the United States Army Corps of Engineers (Ohio EPA, 2023). This system is designed to maintain the overall percentage of wetlands in our state, preventing a loss in wetland acreage to development. However, in the case of wetlands being restored or created as part of a compensatory credit, there are valid concerns to be raised about the ecological soundness of the process. Although fully dependent on who is managing and overseeing the project, many wetland restorations do not hold the goal of creating habitat for amphibians (and reptiles) or pond-breeding habitat in general (Denton and Ritcher, 2013). This is a crucial point to overlook as wetlands offer critical habitat for many species, especially for breeding and the rearing of larval and aquatic young. Often, wetland restoration is done without a clear ecological goal or adequate management, maintenance, and monitoring (Holl, 2020). Localized, wetland specific restoration can be impactful, but literature surveys have suggested that landscape-wide or watershed-wide restorations can be far more beneficial for local amphibian communities (Barrett and Price, 2014). Additionally, many amphibian species like mole salamanders (Ambystoma sp.) require additional upland habitat to live out a majority of their biphasic life cycle (Pfingsten et. al., 2013). To simply preserve or restore an isolated wetland, while developing or altering the upland habitat around it, would leave the site
unsuitable for many pond-breeding amphibian species. Therefore, wetland restoration efforts for amphibians are further complicated by the need to look outside of the wetland boundaries.

To make matters more complicated, restoration activities can accidentally harm the system or species that conservationists are seeking to protect. As discussed earlier, the creation of permanent wetlands in ridge-top communities has allowed for the invasion of predatory amphibian species, which can push out native species that may otherwise benefit from restored wetlands (Denton and Ritcher, 2013). Additionally, the invasion of these species can bring along an increased risk for chytrid fungus or Ranavirus infections (Denton and Ritcher, 2013).

However, some research has found that removing invasive plants may disturb local amphibian species, perhaps due to changes in microhabitat and anthropogenic disturbance (Lehtinen et. al., 2022). In lab settings, salamanders have been documented to detect and avoid chemical herbicides, which may help account for disturbance during invasive species removal (Gertzog et. al., 2011). In contrast, some research has found no occurrence change in response to the removal of invasive species, so the issue seems unresolved (Smith, 2018). Whether it is due to the method chosen, anthropogenic disturbance, or other factors, restoration activities may negatively impact amphibians, at least in the short term.

Despite the complexities, restoration is still an important and powerful tool in modern-day land management and herpetological conservation. Although the removal of invasive species can be tricky, leaving species like Amur honeysuckle (*Lonicera maackii*) to grow and expand in ecosystems can allow them to act negatively as ecosystem engineers (Watling et. al., 2011). In lab settings, amphibian larvae (*Anaxyrus americanus*, the American toad) have been shown to accelerate development when exposed to extracts from Amur honeysuckle (Watling et. al., 2010a). Larvae have also shown increased mortality and behavior changes upon exposure to extracts (Watling et. al., 2010b). While much is still left unknown about the impacts of invasive species on amphibian communities, it is clear that most of these impacts offer cause for concern.
Restoration concerns aside, it is also important to ask, what can amphibians tell us about the wetlands in which they live? For one, they are often overlooked but important parts of ecosystems, both terrestrial and aquatic. Surprisingly, amphibians occupy a large amount of biomass in North American ecosystems, especially woodland salamanders (Welsh and Droge, 2001). Their high biomass within local systems means they are large contributors to energy cycling, acting as a large food source and also as predators themselves (Welsh and Droge, 2001). Amphibian species have different tolerances and habitat requirements, allowing them to be used as reflective bioindicators of their environment and its health or quality. They can be especially sensitive to environmental changes due to their physiology and life histories. For example, woodland salamanders breathe through their skin via cutaneous respiration and are therefore highly sensitive to pollutants and changes in microhabitat (Welsh and Droge, 2001). They tend to react quickly to ecosystem disturbance and are also longer lived, making them a good representation of longer-term conditions and a signal species for environmental changes (Welsh and Droge, 2001). Some species are easy to sample due to their high abundance and biomass, like woodland salamanders. Other species are more difficult to sample due to their life histories and seasonal preferences. Many amphibians are highly sensitive to urbanization and water quality changes due to their biphasic lifestyle (Barrett and Price, 2014). Finally, as touched on throughout, amphibians typically exhibit a biphasic life history. There is often an aquatic phase of vulnerable eggs and developing larvae followed by a terrestrial adult phase, with some variations on this theme. Due to the use of both wetland and upland habitat throughout their lifetime, amphibians can reflect the quality and condition of both habitat types (U.S. EPA, 2002). Additionally, environmental sensitivity is increased because amphibians are typically unprotected at all life stages due to their gelatinous eggs, aquatic young, and sensitive adults (U.S. EPA, 2002). Therefore, they can act as “canaries in the coal mine” for habitat degradation and disturbance both in wetlands and beyond their boundaries (U.S. EPA, 2002).
Ohio has been a pioneer in the establishment of biotic indices and wetland assessment tools over many decades, and assessments for amphibians are not left out. Each tool suits a different purpose, with some surveys focusing on plants (Vegetative Index of Biotic Integrity), surveys focusing on habitat quality (Qualitative Habitat Evaluation Index), and yes, even some surveys focusing on amphibians (Amphibian Index of Biotic Integrity, or AmphIBI). The very popular Ohio Rapid Assessment Method (ORAM) is a survey method for quickly and efficiently generating a wetland category rating (Mack, 2001). Although more work-intensive than ORAM or other methods, the AmphIBI when used appropriately can also generate a wetland category (Micacchion, 2011). In a case where pond-breeding amphibian populations or their habitat is the main concern, the AmphIBI is a great tool to produce a wetland category and conduct an in-depth survey of local amphibian populations in tandem (Micacchion, 2011). In short, the method involves placing 10 funnel traps mostly underwater and evenly spaced around the edge of the studied wetland, typically an ephemeral wetland (Micacchion, 2011). The traps are left for 24 hours and then retrieved, with the study individuals identified and released, or preserved for lab identification if needed (Micacchion, 2011). This process is done 3 times - the first sampling round should coincide with the first migratory night for spotted salamanders and wood frogs, the second round should come 6 weeks later, and the third round 6 weeks after the second (Micacchion, 2011). The method works best for ephemeral wetlands, as these provide the most appropriate pond-breeding habitat for many species (Micacchion, 2011). The wetland category is calculated based on several factors; the amphibian quality assessment index, the relative abundance of both sensitive species and tolerant species, the number of pond-breeding salamander species, and the presence of spotted salamanders or wood frogs (Micacchion, 2011). Sensitive and tolerant species are determined based on their coefficient of conservatism scores (Micacchion, 2011). As detailed above, surveys like the AmphIBI can provide good indications of overall system health. Positive results indicate a healthy wetland and intact upland
habitat, allowing the AmphIBI to be reflective of habitat quality beyond the wetland edge (Micacchion, 2011).

In addition to field surveys, community science data can be obtained via popular websites like iNaturalist in order to quantitatively assess ecosystem health. As the database in iNaturalist grows, researchers are increasingly using this tool as a way to examine species distribution, richness, and other metrics as additional data sources when field surveys are not feasible (Marsh et. al., 2023). Frankly speaking, databases like iNaturalist and HerpMapper have amassed more observations than many field surveys could hope to acquire, and their utility should not be underestimated. Surveys using these databases have verified trends previously found in field studies, such as forest cover being a predictor of amphibian richness (Marsh et. al., 2023).

Materials and Methods

This study sought to investigate amphibian communities at natural, restored, and created wetlands at 6 sites in Northeastern Ohio, with sites residing in Bath Nature Preserve and Summit Metro Parks. Species were surveyed with multiple sampling methodologies to best understand these communities and how they may differ. Before surveys began, it was hypothesized that created and restored wetlands would be utilized by less sensitive species, and natural wetlands would support more sensitive species, with sensitivity being measured by coefficient of conservatism scores. Limitations regarding resources, time, and personnel required scaled down field surveys for this review. Regardless, field surveys were carried out in the spring 2023 and these surveys still had utility and function, including verifying breeding populations at study pools, gaining experience with field techniques, and observing hydrology and seasonal changes at sites over the study period. To help compensate for limited field surveys, presence-absence data was also collected from historical records from the University of Akron Field Station (UAFS) and Summit Metro Parks (SMP), as well as iNaturalist data. Each
of these data collection approaches are described in detail below, along with site history information for the study pools discussed here.

**Site History**

In an effort to protect these sensitive habitats and the amphibians that utilize them, the study sites described here will not be identified by name or specific location. All iNaturalist observations made during data collection were obscured, to further protect these organisms from poaching, increased human disturbance, or otherwise negative outcomes. The six field sites studied were chosen based on access provided by the UAFS and SMP, history of monitoring data available, site history information available, and habitat suitability. In addition, the sites were chosen to represent natural, restored, and created wetlands, for the purpose of this comparative survey. ‘Natural’ is a difficult definition to pin down in the highly development landscape of Northeast Ohio, but for the purposes of this survey ‘natural’ pools are those that came to exist as a result of undisturbed geophysical and biological processes, and these pools are not known to have been created by humans or intentionally modified by them. ‘Restored’ pools are those that were once natural wetlands, but were degraded by humans (ex. ditched/tiled for agriculture) but later these degradations were reversed to restore natural processes to the site. ‘Created’ pools are those that were intentionally dug out and established by humans.

**Site 1 - Natural wetland, UAFS**

Site 1 is quite unique in that it is a permanent, year-round pond that sustains populations of predatory fish (central mudminnows, *Umbra limi*, and various sunfish, genus *Lepomis*) as well as predatory amphibians (green frogs, *Lithobates clamitans*, American bullfrogs, *Lithobates catesbeiana*, and eastern newts, *Notophthalmus viridescens*). However, this pond has a long-standing breeding population of spotted salamanders, including other breeding amphibians that typically use ephemeral vernal pools (Ramer, 2005). Despite this atypical hydrology and predation risk, thousands of breeding spotted salamanders have been documented over
decades of drift-fence surveys, and mass migration events and high productivity are still characteristic of this site. This pond is located within mixed deciduous and coniferous successional upland forest, and it is in close proximity to other wetlands that show more ephemeral flooding cycles, all within a similarly forested landscape. Nearby wetlands differ significantly in their features, including a highly ephemeral bog influenced by groundwater seeps, and a button-bush dominated wetland, with a large open-water center, that is undergoing hydrology changes as the result of beaver activity. Due to intact forest and significant canopy cover, and minimal roads or other migration barriers, it is likely that breeding occurs within these surrounding wetlands, and dispersal of breeding adults and newly metamorphosed individuals connects subpopulations at this site.

**Site 2 - Natural wetland, SMP**

The second natural pool in this study resides in a mainly deciduous successional forest landscape that includes a highly interconnected wetland complex. Wetlands in this area vary widely in hydrology, with many exhibiting ephemeral hydrology from seasonal flooding, and others experiencing year round saturation or standing water. A nearby brook impacts hydrology in this area, as rainfall events allow this brook to broach its bank and access its floodplain many times in a year, contributing to flooding of the wetland complex. Surprisingly, the surveyed pool had a population of central mudminnows, perhaps as a result of the highly interconnected wetland complex and flooding from the nearby stream, which could theoretically move species between pools. Year round saturation is also a possibility, considering the hydrology of this area. Although the wetlands are natural, the stream has undergone restoration efforts to reverse ditching and restore sinuosity, riffle-pool-run development, and access to the floodplain, among other things. The area also features multiple groundwater seeps, and unique stone ledges as a result of glaciation. Overall, this site is a uniquely intact and protected wetland for Northeast Ohio, and the presence of floodplain hydrology and habitat diversity provides a highly interconnected, often saturated, wetland habitat.
Site 3 - Restored wetland, UAFS

Adjacent to site 1 is site 3, a restored bog with highly seasonal flooding and a unique plant community. This wetland is a glacier-created peatland that was once ditched and drained as part of agricultural modifications to the property. As part of an ongoing restoration effort, now entering its 10th year, the wetland hydrology has been restored to the site, and an Agri-Drain has been implemented to control water levels. Restoration efforts have also included the removal of invasive plant species and the planting of Tamarack trees, a species often found in this rare habitat in Northeast Ohio. Similar to site 1, surrounding habitat includes intact successional deciduous forest and other wetlands nearby. Groundwater seeps are also present near the edges of the wetland. This site occasionally supports predatory amphibian species (American bullfrogs, green frogs, and eastern newts) likely as a result of a nearby permanently inundated pond, which would provide overwintering habitat for these species.

Site 4 - Restored wetland 1, SMP

Site 4 is a forested wetland that was once ditched and tiled as a result of agricultural modifications to surrounding land. Restoration efforts included breaking the tile and plugging the ditches to restore wetland hydrology, which occurred in 2009. Many upland tree species moved into the site when it was originally drained, and many of these trees later died as a result of flooding, leading to large volumes of standing dead trees and dead fall. Native plants emerged from the seed bank once wetland hydrology was restored. Surrounding habitat includes successional deciduous forest and wet meadow/dry meadow habitat, much of which is currently undergoing reforestation efforts. This site undergoes ephemeral flooding patterns, although year-round saturation and some standing water is not uncommon. Vernal pool specialists like fairy shrimp are commonly found in samples at this site. Uniquely, Summit Metro Parks took the step to introduce spotted salamander eggs to this site. It was believed that this wetland after restoration would support a breeding population of spotted salamanders, so eggs from nearby
populations were introduced to this wetland during the breeding seasons of 2014, 2015, and 2016. Breeding adults were first documented at this site in 2019.

**Site 5 - Restored wetland 2, SMP**

For the purpose of this study, site 5 is classified as a 'restored wetland', but it may be better classified as a recovering or rewilding wetland. This area was used for many years as an ice skating pond, with treated city water pumped into the site each fall/winter. Historically, the area would be drained in the warmer months to create a field for park visitors to utilize for recreation. However, this practice halted in recent years, with the pond no longer being filled or drained for recreation. Wetland vegetation has emerged as a result of allowing natural flooding patterns to occur, and this wetland has since been used by pond-breeding amphibians, among other wetland species, including fish and waterfowl. In the summer of 2022, the first year without draining, a very large population of recently metamorphosed American toadlets emerged, resulting in concern from visitor and park/trail closures to prevent mortality. Interestingly, summer of 2023 did not see this same mass emergence of toadlets, perhaps as a result of predators cueing into this new wetland and resulting food source. Surrounding habitat in this area includes patches of mixed deciduous and coniferous successional forest, but more fragmentation is present at this site due to roadways, railway tracks, a walking path, and heavy human traffic both on foot and by bicycle. Additionally, the pond remains in a mowed field, so canopy cover in the direct vicinity is little to none. An established natural pool with ephemeral hydrology sits in a small sliver of brush/forest nearby.

**Site 6 - Created wetland, SMP**

Finally, this study sought to include created wetlands in addition to restored wetlands, but the creation of wetlands with seasonal flooding patterns is not common in our area. Man-made, permanent ponds are very common in this area, but those do not provide suitable habitat for vernal pool specialists, and so were not considered as part of this survey. As a result, one created wetland that exhibits ephemeral hydrology was surveyed as part of this
comparative effort. That wetland is a small vernal pool located within a mainly deciduous successional forested landscape. Roughly 15 years ago it was hand dug by staff and volunteers for the park district, with the goal of creating a vernal pool for pond-breeding amphibians. Over time, leaf litter has built up in the pool, supporting populations of macroinvertebrates like dragonflies, which may be predatory to amphibian larvae. Some permanent ponds reside in the landscape nearby, but no predatory amphibian species have been noted in this created vernal pool, so it is likely that these species have yet to find the pool or avoid it due to its ephemeral nature. The area immediately surrounding the pool is quite flat, but there are natural ravines with creeks and streams close by. Unlike the other created/restored pools surveyed, this pool was established with the intention of having ephemeral hydrology and acting as a breeding site for vernal pool specialists, which makes it a unique wetland in both this survey and many other surveys of constructed wetlands.

Figure 1. Site 6 during May, 2023.

Additional site - Restored wetland, SMP

One additional site was sampled but ultimately replaced with site 5, due to a lack of proper habitat and hydrology. This pool is located within a recently restored golf course, and part of a larger wetland complex surrounded by meadow habitat. Ultimately, a sampling of this site only yielded predatory amphibians and fish, and indicated permanent open water, without ephemeral hydrology. Additionally, this area has been largely deforested and reforestation
efforts have not yet been successful, so the nearby habitat around this pool has little to no canopy cover or upland forest. Other pools within this restored golf course may have yielded vernal pool specialists, but site 5 was chosen as a replacement due to its unique position as a currently ‘recovering’ or ‘rewilding’ pool, and the interesting, albeit amusing, American toadlet situation from the year prior.

**Survey Materials**

Collapsible nylon minnow traps were obtained from the University of Akron Field Station. Traps were cleaned, sanitized (see below), and repaired prior to the initial use, and after subsequent uses. An extendable dip net was also obtained for the second round of sampling, and it was similarly cleaned and sanitized after each site visit. In order to comply with permits and prevent tampering, all traps were tagged with labels that read “**Scientific study in progress, do not disturb** Miranda Florent, University of Akron Field Station, 4240 Ira Rd, Akron, OH, 44333, Permit Number: SC220052”. Ultimately, no tampering occurred during the study period. Waders were borrowed from UAFS and buckets, gloves, bleach, and alcohol wipes were obtained for transporting materials and sorting study animals, as well as disinfection.

**Survey Methods**

Permits were acquired via The University of Akron Field Station and Summit Metro Parks in order to trap vertebrates for this field survey. Special use permits, provided by SMP, were carried at all times during field sampling, in addition to the objective statement and methodology that accompanied the SUP application (see appendix A). Permits via the University of Akron were provided from the Institutional Animal Care and Use Committee (IACUC) and the required online trainings were completed prior to any field sampling efforts.

In total, 8 field sampling days occurred, between March 23rd, 2023 and June 14th, 2023. Over that period, at least one round of amphibian trapping occurred at each study site. One site was excluded (‘additional site’) and later replaced with site 5, while site 3 was trapped twice due
to a low capture rate on the first attempt, and ease of access to make another attempt. The second round of sampling involved dip netting at each site to survey for any remaining breeding adults and aquatic larvae present. For each site visit, arrival time and date were noted, as well as any species observed but not caught, any changes or notable site conditions, and the condition of traps when retrieved.

The trapping protocol was modified based on the protocol described in Micacchion, 2011. During the month of March, site 1 was visited on rainy nights above 45°F to see if amphibians were migrating to the breeding pond. Once a mass migration was witnessed (the night of March 22nd, 2023), trapping began at the study sites. Due to a limited quantity of traps, and the large size of many study sites, the suggested 10 traps placed evenly around the boundary of the wetland (Micacchion, 2011) was not achievable. Instead, 3 traps were placed per wetland, in order to accommodate multiple site visits in a day with a limited number of traps. Traps were placed in areas that created natural ‘funnels’ to try and increase capture rates, and traps were tied off with white cord to secure trees/debris in order to prevent full submersion in water. Traps were always left for less than 24 hours to adhere to The University of Akron’s IACUC permit requirements, typically set in the afternoon, left overnight, and retrieved in the morning. Study animals sorted in buckets and trays in order to be recorded and identified. Identification was made via a field key created from multiple sources and laminated for in-field use (see appendix B, field key, and subsequent references).

In order to gain experience with a different methodology, dip-netting was used for the second round of sampling. This second sampling round was based off of the timing suggested by Micacchion, 2011, but more closely followed the sampling methodology of Denton and Ritcher, 2013. Each site was dip-netted between three and five times within a sampling period in order to accommodate for some netting attempts that did not yield study organisms. Larval salamanders and frogs were attempted to be identified in the field, using the created sampling key (appendix B). However, in-field identification of many larval amphibians is challenging for
even experienced surveyors, and this work proved very difficult for a novice. Due a lack of confidence in larval identification during this study, larval data was not included in the results discussed later on. However, additional site visits did contribute to the data set via visual observations of new species, as well as general information of each study site, including hydrologic and vegetative changes throughout the season. These second visits still provided value and contributed to the ideas and further questions in the discussion section below.

Historical species data was acquired from UAFS and SMP. Both UAFS and SMP provided data via email from past surveys of these breeding pools, which added new species to the datasets. Historical data from SMP included information from volunteer monitoring efforts and park surveys, and historical data from UAFS included information from long term monitoring and surveying of the two UAFS sites.

Finally, community science data was gathered from iNaturalist. Community science data was also requested from HerpMapper, but that request went unanswered. iNaturalist observations were acquired by searching ‘amphibians’ and drawing a polygon around each study area. It is important to note that the use of iNaturalist in this case is limited, as highly cryptic species may be less likely to have observations, and the study sites listed here are small and have limited data entries compared to larger areas that provide larger datasets. Unlike field records and historical records, iNaturalist data had more uncertainty. Some species were only observed once, many years prior. Many observations were not ‘within’ the study pool, and it is unclear if that individual was traveling to that breeding site or another, adjacent site. Some photos showed obvious breeding activity in the study pools, while other observations only suggested a species may reside in the general area, but perhaps utilize another pool.

Due to the uncertainty arising from iNaturalist data, results were examined with two ANOVA tests. One test utilized a more limited dataset of individuals, only those that were confirmed to breed in the study pools. The second test also considered possible breeding species, those observed in the area but not confirmed to breed in the study pools. ANOVA
calculations were carried out on both the more limited and more expansive data sets. Coefficient of conservatism scores for each species present at a given site were entered as values in the ANOVA tests, as a means to compare average CoC scores for breeding species between sites.

**Sanitation**

It is important to discuss sanitation, especially as it relates to wetlands and the sensitive species that reside within them. During field surveys, equipment including waders, rubber boots, minnow traps, dip nets, buckets, and all other equipment that made contact with the wetland, water, or study individuals was sanitized following Northeast Partners in Amphibian and Reptile Conservation protocols (NEPARC, 2022). The main causes for concern in wetland work is the transmission of Ranaviruses and Chytrid funguses, as well as the spread of non-native seeds from one site to another (NEPARC, 2022). Per NEPARC protocols, a 10% bleach solution was used to disinfect equipment after mud, soil, and seeds were removed by scrubbing and brushing (NEPARC, 2022). Equipment was then rinsed and left to dry while traveling to the next field site. These protocols align with other best management practices for the protection of amphibians and wetlands as a whole (Whittaker & Vredenburg, 2011). Additionally, non-latex, powder-free nitrile gloves were used during the handling of all study individuals, to prevent the transmission of irritants and toxins from human skin to the study individuals (Whittaker & Vredenburg, 2011). Extra care was taken with captured frogs, and gloves were changed between each individual that was captured separately from another (Whittaker & Vredenburg, 2011).

![Image of Ambystoma maculatum and Ambystoma jeffersonianum](image)

**Figure 2.** *Ambystoma maculatum* (left) and *Ambystoma jeffersonianum* (right) in a gloved hand.
**Effort**

As a means to quantify investment of time and energy for the field survey portion, rough calculations for effort have been made. In the case of this survey, effort was calculated as the number of target study organisms captured per hour spent on site. This is an oversimplification, as countless variables can increase or decrease capture success rate including, but not limited to, trap/net placement, weather conditions, pool size, migration timing (often differs with species/sex), unknown migration direction, baited/non-baited traps, and pure luck. Still, effort calculations can provide a rough idea of how many in-field hours need to be invested to acquire desired sample sizes or datasets for future work. Effort calculations will be detailed in the results portion and discussed in comparison with other methods, and their respective ‘effort’, in later sections.

**Results**

**Table 1.** Coefficient of conservatism scores by species (Celebrezze, 2010).

<table>
<thead>
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<th>C of C</th>
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<tr>
<td><em>Ambystoma complex</em></td>
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</tbody>
</table>

This survey utilized coefficient of conservatism (C of C or CoC) scores to compare trends in species sensitivity across the study sites. The CoC scores of each species surveyed are listed below in table 1, with CoC’s also included in subsequent tables. CoC scores were obtained from the *Ohio’s Hidden Wonders* field guide (Celebrezze, 2010). *Hyla versicolor / Hyla chrysoscelis* is used in this table, and subsequent tables, because gray treefrogs and Cope’s gray treefrogs are indistinguishable without DNA sequencing, which this study did not undertake. Similarly,
*Ambystoma complex* is used to denote the all female, unisexual ambystomatid complex, of which *Ambystoma jeffersonianum* can contribute to, however *Ambystoma jeffersonianum* can often be distinguished by eye with a high degree of certainty, so the complex and species are not combined in these results.

The following data table highlights presence/absence species data for each of the surveyed sites, and allows visual comparisons between site data. It is important to note that some iNaturalist survey data may only indicate species presence in an area, and not confirmed breeders in the study pool. In the affected cells, these possible breeding species are noted with a red X rather than a black X. That consideration will be discussed later in this section. Sites are grayed out in alternating column groupings to help with readability.
Table 2. Presence/absence data for the surveyed sites, divided by data source.

<table>
<thead>
<tr>
<th>Common name, Latin name (CoC)</th>
<th>Site 1 (Natural)</th>
<th>Site 2 (Natural)</th>
<th>Site 3 (Restored)</th>
<th>Site 4 (Restored)</th>
<th>Site 5 (Restored)</th>
<th>Site 6 (Created)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unisexual mole complex, <em>Ambystoma</em> complex (5)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Jefferson salamander, <em>Ambystoma jeffersonium</em> (6)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Spotted salamander, <em>Ambystoma maculatum</em> (8)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>American toad, <em>Anaxyrus americanus</em> (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Four-eyed salamander, <em>Hemicryptobates scutatum</em> (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Gray treefrog, <em>Hyla versicolor</em> <em>Hyla chrysocelis</em> (4)</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>American bullfrog, <em>Lithobates catesbeiana</em> (2)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Green frog, <em>Lithobates clamitans</em> (1)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pickerel frog, <em>Lithobates palustris</em> (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern leopard frog, <em>Lithobates pipiens</em> (4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood frog, <em>Lithobates sylvaticus</em> (7)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Eastern newt, <em>Notophthalmus viridescens</em> (6)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring peeper, <em>Pseudacris crucifer</em> (3)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Western chorus frog, <em>Pseudacris triseriata</em> (4)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Utilizing the data listed above in table 2, one-way ANOVA calculations were run on the CoC’s for each species present at each site (see table 3). To illustrate, data for site 1 was entered as “5, 6, 8, 1, 2, 1, 7, 6, 3” to represent the CoC’s for each species present at the site (see table 2). Importantly, two ANOVA calculations were run to account for presence/absence uncertainty. The first test utilized more limited data, only including CoC scores for verified breeders. This survey defines verified breeders as species where adults were found breeding in the study pool during field surveys, historical surveys, or those featured in iNaturalist observations that show obvious breeding activity (GPS coordinates within the study pool, adults in the water, etc.). The second ANOVA test expanded the data set to include possible breeders, namely individuals from iNaturalist observations within the immediate area, but not confirmed or pictured breeding within the study pools. These individuals may utilize the study pools, or simply be traveling through to access other breeding sites, and therefore are only considered possible or potential breeders. The addition of these potential breeders only affected data sets for sites 2, 3, and 5, and the species are noted with red X’s in the table above.

The results of these two ANOVA tests did not yield statistically significant results, failing the f-test needed to carry out a post-hoc Tukey HSD test. The p-values found are listed below in table 3.

**Table 3.** P-values from ANOVA tests examining CoC differences.

<table>
<thead>
<tr>
<th>P-value (verified breeders)</th>
<th>0.1534</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-value (possible breeders)</td>
<td>0.5301</td>
</tr>
</tbody>
</table>

Finally, table 4 shows effort calculations for this survey. These calculations are simplifications of the true effort and time invested and should be considered rough estimates. The effort calculation for ‘identifiable adults’ only considers survey individuals that contributed to the final data set, namely those individuals that could be identified with certainty during field surveys. This calculation also does not consider individuals that were observed visually during
site visits, only captured adults. Therefore, the first effort calculation is a more conservative estimate of possible organisms captured per hour on site. The second effort calculation for ‘all captured and observed’ organisms, includes all study organisms captured and observed, including the larval organisms that did not make it into the final data set due to lack of certainty on identification. As such, this calculation represents a more optimistic scenario when it comes to effort investment. As stated, these calculations are rudimentary at best, but they may act as a rough estimate for those considering field work like this in the future.

Table 4. Effort calculations for the field survey portion.

<table>
<thead>
<tr>
<th>Effort (identifiable adults)</th>
<th>2.04 organisms/hour in field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effort (all captured and observed)</td>
<td>9.75 organisms/hour in field</td>
</tr>
</tbody>
</table>

Discussion

Ultimately, this survey did not demonstrate a significant difference in the CoC between natural, restored, and created wetlands, so the null hypothesis should be accepted. However, the limited survey techniques and small sample sizes, along with other potentially limiting factors like single season sampling, did not make this an ideal study design. Therefore, this question should still be pursued and investigated by future work. A number of anecdotal observations were made that also deserve further investigation. For example, site 5 is a wetland that deserves continued attention as recovery and restoration efforts unfold. Many questions remain unanswered and deserve proper investigation, such as whether or not the adjacent, mature vernal pool is preferred by vernal pool specialists, and whether this preference may shift as site 5 recovers. Additionally, collecting data on hydroperiod length, water levels/fluctuations, and canopy cover for these study pools may help determine habitat suitability and ephemeral qualities, and if/how these factors affect breeding populations of amphibians. It seems that restored and created vernal pools show great potential for supporting highly-sensitive breeding amphibians, as long as care is taken to achieve ephemeral hydrology, extensive canopy cover,
and natural wetland processes with minimal disturbance. Species reintroduction is also a process that should be considered in our area, especially as habitat loss increases. Relocating species by anthropogenic means can help improve genetic diversity and contribute source/sink populations to lessen local extinction risk. Anecdotal considerations aside, this section will discuss findings from other works that could explain some of the observations made here, considerations for future studies that may generate more reliable and accurate results, and implications for conservation will be discussed as they relate to our current wetland protections and restoration practices in Ohio.

When restoring or creating wetland habitat for pond-breeding amphibians, it is important to consider the trade-off between fast results and natural results. There are potentially many ways to create a wetland or vernal pool, and many people might seek techniques that are more like gardening or landscaping due to their high disturbance levels and quick turnaround time. These techniques could include large earth moving efforts, native planting, or highly artificial hydrology changes like damming and ditching. While these techniques absolutely have a time and a place, and they return results much faster than other techniques, perhaps there is something to be said about slower, more natural efforts. A stand out example of these slow and steady efforts would be study site 6. This man-made vernal pool was created in the simplest way - hand digging a depression within a forested landscape. This depression allowed for seasonal wetland hydrology to take hold, slowly changing the soils, and eventually the plant community, at this site. Leaf litter brought nutrients to the system, which supported seasonal benthic macroinvertebrate communities, and eventually provided food sources to the larval amphibians that came to breed in this pool. This site had the smallest number of confirmed breeding species during field surveys (only *Ambystoma jeffersonianum* and *Lithobates sylvaticus*, see table 2 and figure 3) however both of these species are considered sensitive vernal pool specialists. Interestingly, small positive associations between these two species and spotted salamanders have been found, perhaps indicating similar habitat requirements, which
could be the case at site 6 (Davis, et. al., 2018). Excitingly, field surveys for this project confirmed breeding Jefferson salamanders, which were suspected the year before but not confirmed, and which are a new breeding species in this pool. By taking an exceptionally simple approach and allowing natural wetland processes to occur with time, this constructed breeding pool has matured into a highly ephemeral and productive pool that mimics natural wetlands and supports specialist breeders with high CoC’s.

**Figure 3.** *Ambystoma jeffersonianum* (left) and *Lithobates sylvaticus* (right) from site 6.

One consideration in the ‘slow and steady’ approach is that many sensitive species of pond-breeding amphibians exhibit philopatry and have high site fidelity. This is especially well studied in mole salamanders (genus *Ambystoma*). Many studies have found high site fidelity among breeding adults, with as many as 96.4% of breeding adults returning to the same pools each season (Gamble, et. al., 2007). It seems that most individuals that disperse to new ponds are new metamorphs or first year breeders, which can undergo migrations to/from breeding pools in excess of 1,000 meters (Gamble, et. al., 2007). Therefore, it seems quite likely that newly restored and created wetlands may remain unproductive for an extended period, until a few individuals decide to set off for new breeding pools. After all, migrating to new ponds yields substantial risk, and it is thought that maintaining site fidelity helps ensure successful breeding and limits the physiological and ecological risk of new, uncertain migration patterns (Sinsch, 1990). Despite the risk, some believe that these far dispersing individuals help create sources
and sinks in a metapopulation structure of pond-breeding amphibians (Gamble, et. al., 2007). The individuals that strike out for new pools can help offset the effects of genetic drift and inbreeding depression in otherwise small, rather localized populations (Gamble, et. al., 2007).

Considering high site fidelity and migration limitations, it is possible that some wetlands considered in this survey are too ‘new’ to be utilized by these high site fidelity species. Perhaps the most likely to experience this effect is site 5, which is very early in the process of recovery, in a fragmented landscape, with an established breeding pool nearby. Whether it be site fidelity, landscape barriers, inappropriate habitat/hydrology, the presence of predators, etc. this site does not yet serve as a breeding site for high CoC/sensitive species, but maybe it could with time.

One consideration for overcoming site fidelity delays in wetland restoration/creation efforts could be headstarting efforts or species reintroductuion, like that carried out at site 4 in this survey. Although many species may quickly find new wetland habitat on their own, pond breeding amphibians are limited in migration capabilities when compared to other groups like birds or mammals, and high site fidelity can further impact their ability to find new breeding habitat (Ramer, 2005). Head starting efforts for amphibians are popular and well underway, such as the Amphibian and Reptile Conservancy’s efforts with the highly imperiled frosted flatwood salamander (Ambystoma cingulatum) (ARC, 2023). In an effort to preserve genetic diversity and increase vulnerable populations, species reintroductuion and headstarting seems to be at the forefront of amphibian conservation, and it fits quite well with restoring/creating wetland habitat.

Beyond the results of this survey, it is important to consider amphibian conservation and wetland restoration in Ohio more broadly. As discussed earlier, restoration methods are often imperfect, but there are ways they could be improved, such as increasing wetland protections overall. For example, preserving quality upland habitat is very important for many pond-breeding species but this fact is often overlooked. Work has been done in this arena to quantify how wetland buffer requirements could be improved to more efficiently protect core habitat for
amphibian and reptile species (Semlitsch and Brodie, 2003). This upland habitat is vital for a number of activities including overwintering, foraging, and nesting (Semlitsch and Brodie, 2003). To best protect wetlands and their pond-breeding communities, core habitat should be determined for local species by examining their maximum dispersal distance and land use (Semlitsch and Brodie, 2003). From there, land managers can then determine how much upland habitat should be preserved to protect 90-95% of the species population (Semlitsch and Brodie, 2003). For Missouri amphibians, core habitat was calculated to range between 159 meters to 290 meters depending on species, which is significantly wider than a traditional 30-60 meter wetland buffer (Semlitsch and Brodie, 2003). This method has also been utilized to examine theoretical survivorship at different wetland buffer distances for the California tiger salamander (Ambystoma californiense), a mole salamander with similar upland habitat requirements as our native mole salamanders (Trenham and Shaffer, 2005). Due to their extensive upland habitat use, especially as subadults that have not yet reached breeding age, preserving 95% of the population at the study site would require a buffer zone of 630 meters (Trenham and Shaffer, 2005). This pales in comparison to even the typical best-case buffer zone of 60 meters, which would preserve less than 10% of the upland habitat the California tiger salamander vitally depends on. In the case of a 60-meter buffer, the model predicted that California tiger salamanders at the study site would be extinct within 100 years, or only present at rates of 1% or less if in an interconnected landscape allowing for new migrations (Trenham and Shaffer, 2005). In a similar study focused on a species native to Ohio, the marbled salamander (Ambystoma opacum), it was estimated that up to 100% of the studied individuals migrated beyond a distance of 30 meters when leaving their breeding pools (Gamble et. al., 2006). Some metamorph individuals went as far as 1,230 meters from their natal pools, and although these dispersal distances may be outliers, they are likely very important for metapopulation connectivity and genetic contributions that offset the effects of inbreeding depression and genetic drift (Gamble et. al., 2006). It is evident that current wetland buffers neglect to consider
extensive upland habitat use, including outlier dispersal distances, which provide important genetic contributions to largely isolated subpopulations.

Since wetland buffer requirements could be yielded as a tool to protect pond-breeding amphibian communities throughout all life stages, it is important to discuss Ohio's requirements. It appears that there are no statewide requirements for wetland buffers, although there are Ohio EPA suggestions, but each jurisdiction can establish its own regulations on the topic. The Ohio EPA suggests a setback of “greater than 100 feet” for habitat protection, which is roughly 30 meters (Ohio EPA, n.d.). The largest riparian setback mandated by Summit County, via the Summit Soil and Water Conservation District, is a width of 300 feet (approx. 91 meters) but only in the case of a stream with a drainage basin in excess of 300 square miles (SSWCD, n.d.). For riparian wetlands, an extra 50-foot setback (approx. 15 meters) is added for category 3 wetlands, and a 30-foot setback (approx. 9 meters) is added for category 2 riparian wetlands (SSWCD, n.d.). Even the largest setback mandated by SSWCD only covers a fraction of the upland habitat needed to protect highly mobile pond-breeding species like mole salamanders. It is important to note that only riparian systems are given these extra protections, and therefore vernal pools/ephemeral wetlands in forested areas would only receive the standard 30 meter setback. In the case of native species like the spotted salamander, studies have found an average migration distance of 150 meters (Douglas and Monroe, 1981). If 150 meters is used as a core habitat estimate, the suggested ‘habitat protection setback’ of 30 meters would cover only 20% of their core habitat, leaving much of the population vulnerable. As discussed above, this setback would mean imminent extinction for the California tiger salamander, and similar concerns can be extended to our native mole salamanders (Trenham and Shaffer, 2005). Little is known about the terrestrial upland use of many of our pond-breeding amphibian species, which makes these estimates difficult to apply. That said, there is a very good chance that our current wetland setback measures do not protect enough upland habitat for pond-breeding amphibian species. This is especially true for under-valued vernal pools, which are of great
importance for many pond-breeding amphibians due to their ephemeral nature and lack of predatory species of fish, amphibians, etc. However, vernal pools are often overlooked or unnoticed, especially in the dry season when they show little indication of their wetland status to an untrained eye (U.S. EPA, 2023). If changes could be made to the setback scheme in Ohio and beyond, with additional vernal pool protections, critical habitat could be preserved for many ecologically important and valuable species.

Another consideration for amphibian conservation is a popular topic - wildlife corridors. Research has shown that amphibian communities do exist as metapopulations, rather than isolated communities (Marsh and Trenham, 2001). Therefore, pursuing restoration on a landscape scale and providing connectivity between suitable habitat and breeding ponds can help prevent local extinctions and genetic depression (Marsh and Trenham, 2001). As such, it stands to reason that amphibians can benefit from further habitat connectivity both for breeding ponds/streams and for upland habitat. Additionally, as metapopulations, they are potentially vulnerable to issues like habitat fragmentation and isolation (Purrenhage et. al., 2009). Some amphibian species, like spotted salamanders and the California tiger salamander, have relatively high movement and migration rates, which have genetic importance as discussed above (Purrenhage et. al., 2009). Species like spotted salamanders have the potential to travel great distances (respectively) but only with suitable habitat available (Ramer, 2005). Like many amphibians, the species tends to avoid grasslands and areas with less than 30% canopy cover (Gibbs, 1998). In a human-dominated landscape overwhelmed by roads and lacking in canopy cover, metapopulations may suffer from these dispersal barriers. Efforts have been made to provide wildlife corridors and increased connectivity for pond-breeding amphibian species. Using data-driven, focused assessment tools like landscape connectivity models can yield positive results with minimal investment (Wade et. al., 2023). These models can identify areas that are migration barriers, and calculate the highest increase in connectivity that can be achieved with the fewest barriers removed or altered (Wade et. al., 2023). In practice, focusing
on smaller scale restoration (10 culvert upgrades, as opposed to 428 possible upgrades) provided a significant increase in habitat connectivity for four-toed salamanders (*Hemidactylium scutatum*) in Tennessee at less than 20% of the possible cost (Wade et. al., 2023). These data-driven, scientific modeling techniques can make otherwise overwhelming efforts feasible and realistic, allowing for critically important increases in connectivity and reductions in habitat fragmentation. Overall, the metapopulation model of amphibians speaks to the need for conservation and restoration to be considered on a wider scale. Additionally, the potential for longer migration distances and the avoidance of unsuitable habitat speaks to the need for habitat corridors and the potential benefits they could bring to amphibians, among other wildlife.

Another approach to lessening habitat fragmentation in a human-dominated landscape is more community-based, and born from the care and commitment of citizens. There are countless organizations across the globe with volunteers that help amphibians cross roads during migrations. For example, the Amphibian Migrations and Road Crossings Project in New York assists amphibians in their nightly mass migrations, ensuring they can access breeding ponds safely and with minimal car strike losses (Amphibian Migrations and Road Crossing Project, n.d.). Volunteers wear reflective vests and headlamps to help amphibians cross the road and volunteers also act as community scientists, collecting data on the amphibians they are helping to protect (Amphibian Migrations and Road Crossing Project). This is but one example of community science practices, which are growing in both popularity and ecological value across the world.

For those looking to continue this work in the future, there are many things to consider. For one, this work is very resource and time intensive, and having multiple sampling seasons with more researchers would be highly beneficial. In order to obtain the most complete understanding of breeding populations at study ponds, drift fences can be utilized. However, these fences require daily checks for the length of time in which they are operating, which should ideally be most of the breeding migration period. Surveying multiple sites during a
season with this technique is difficult, but it would provide robust population data to examine a much wider variety of trends, and additional analysis could be completed via the application of the Shannon-Weiner diversity index and many others. It is likely that diversity indexes and other metrics will provide a better measure of sensitivity and community health than CoC scores and presence/absence surveys, as abundance and biodiversity can also be considered. CoC comparisons were chosen in this survey due to attainability limitations, but other metrics should be used going forward. Additionally, drift fences that completely encircle a breeding pond greatly increase the capture rate of study organisms, and therefore greatly reduce possible error and increase accuracy and certainty. This highly increased capture rate would provide much larger datasets and be more efficient. However, drift fences can be invasive and require human disturbance in the environment, and they may not suit all sites. In that case, having a larger team of people to administer significantly more minnow traps, perhaps over more sampling days, would be another improvement in this survey method. A consideration for trap deployment could be using ‘bait’ in the form of light, or glow sticks, which has been found to increase capture rate for both breeding adults and larvae (Grayson & Roe, 2007). Additional site data should also be gathered, such as ORAM scores, hydroperiod, water depth, canopy cover, egg mass counts to estimate productivity, etc. If resources were not limited, perhaps the ultimate investigation into this topic could involve experimental creation/restoration of vernal pools in our local landscape, with long term monitoring of pool development and key abiotic factors, in addition to surveys for breeding amphibian populations. More realistically, if future studies can increase sample size, collect more site data, and implement more effective survey measures, the relationship between local restoration efforts and pond-breeding amphibians can be given the attention it truly deserves.

**Conclusion**

In closing, amphibians can be greatly impacted by restoration activities, and not always in positive ways. However, there are many ways that restoration can be improved to generate
more positive outcomes for amphibians and many additional species. This can include more ecologically-minded restoration projects with improved monitoring, management, and maintenance. Taking a watershed or landscape-wide view can make restoration more effective for amphibians and countless others. The funding and establishment of wildlife corridors can benefit both charismatic megafauna and those that are small and slimy. Increasing wetland buffer protections and making legislation more ecologically and biologically informed could be hugely beneficial. Considering and including upland habitat in wetland protections and monitoring is crucial and often overlooked. Further research into the life histories of amphibians can help land managers better protect and recover their much-needed habitat. It is well established that amphibians can act as biological indicators due to their life history and biphasic life cycle, allowing them to be informative about both terrestrial and wetland habitat quality. Ecology and legislation aside, amphibians have an innate value in ecosystems and as individual species. Despite this, many struggle to receive the attention they deserve. Herpetofauna at large is not helped by their reputation as many are outright disliked or feared. In reality, the natural world that is loved and adored by most would not look the same without amphibians, which succeed in all of Earth’s corners, from the rainforests to the Arctic, and even the harshest deserts. A group with such evolutionary success and ecological value should be advocated for and protected, and restoration can be a tool to aid in this effort if wielded correctly.
References

*Amphibian Migrations and Road Crossings Project.* New York State Department of Environmental Conservation. (n.d.).
https://www.dec.ny.gov/docs/remediation_hudson_pdf/amrchandbook.pdf


https://doi.org/10.1086/677556


https://doi.org/10.2307/1444239

https://doi.org/10.1016/j.biocon.2007.07.001


What are riparian areas and riparian setbacks? Summit Soil and Water Conservation District - SSWCD. (n.d).

https://sswcd.summitoh.net/sites/default/files/2021-06/Riparian_factsheet.pdf

Appendix A

Research Protocol - SMP, SUP Supporting Documentation
Miranda Florent
2.14.23
Goals and Objectives

Through my honors research project, I hope to assess amphibian populations in several restored wetlands using a modified AmphIBI protocol, or the Amphibian Index of Biotic Integrity, created by the Ohio EPA (Micarechion, 2011). When considering wetland restorations, there is always the question of how restored or created wetlands compare to natural wetlands, and if there are quality differences between the two. While these issues are incredibly complicated and assessing them fully would require decades of work, my honors project can make an attempt at studying differences between amphibian communities in natural and restored wetlands. Additionally, using an AmphIBI inspired protocol will assess amphibians present (namely salamanders) based on their environmental sensitivities. Therefore, populations can be compared between the different sites to examine any differences in tolerant or sensitive species present, which could indicate wetland health.

Methodology

The US EPA established the AmphIBI as a method of evaluating wetland ecosystem health based on amphibians present. The AmphIBI survey generates a habitat score between 0 and 50, which will indicate the suitability for ‘aquatic life use’ within the habitat and the category of the wetland as a whole, which corresponds to ecosystem quality. While I do not have the time or resources to complete full AmphIBI surveys on my study sites, I can use the tried and true sampling methodology to survey species that are present between the different sites.

Following a modified AmphIBI protocol, I intend to place 5 nylon minnow funnel traps in study ponds for periods of 24 hours or less. These traps will collect larval and adult caudates and anurans that may float or swim into the funnel opening. The traps will then be collected and the trapped organisms will be identified and released. The identification will occur at the study
site and be a non-invasive, visual identification. I intend to repeat this two times, coinciding with the weather when possible. The first sampling period will hope to catch spring migration of Ambystomatids and red-spotted newts, generally occurring in mid-March or early April per the AmphIBI protocol (Micacchion, 2011). Exact dates will be chosen based on weather to increase the chances of successful sampling. The second round will be roughly 6 weeks later, to survey later arriving species and the larval stages of early arriving species. AmphIBI sampling is based on coefficients of conservatism. These scores range from 0 to 10, with higher scores representing species that are more rare, sensitive, or require an undisturbed/high-quality ecosystem. By using this method, the AmphIBI protocol and my modified version will consider the abundance of sensitive and tolerant species, providing an indication of wetland health and disturbance.

Due to the modified protocol, I will not be able to generate EPA AmphIBI scores and I will not be able to determine the wetland category. That said, the sampling method will still survey amphibian populations between the studied sites. I will document all amphibians collected (both salamanders and frogs/toads), but my project will focus on salamander populations predominantly. Despite the modified protocol, the individuals found can still be examined from the lens of their coefficient of conservatism scores, which indicate habitat quality.

In total, I would like to survey 6 sites, considering both restored sites and unrestored sites as controls. The sites I intend to survey are as follows: sites 1, 2, 3, 4, 5, and 6. The sites are chosen to represent two unrestored reference sites (sites 1 and 2), a created wetland site (site 6), a young restoration (additional wetland site, later replaced with site 5), a middle-aged restoration (site 3), and an older restoration (site 4). All chosen sites have known salamander populations, which will hopefully ensure successful sampling and populations available to compare. As stated above, 2 rounds of sampling need to be completed per site, roughly 6 weeks apart. The data collection period will therefore take about 1.5 months, give or take depending on weather and scheduling. I would like to begin as soon as the weather allows,
presumably in March. Data collection would therefore be completed by May, unless the weather delays migrations.

Preventing the spread of pathogens and diseases like chytrid fungus is an important part of working with amphibians. Multiple different sanitation protocols will be followed to ensure the safety of all study animals and prevent the spread of pathogens. Visual inspection of field equipment and boots, followed by manual cleaning with scrub brushes will be performed in the field (Weldon & Hillmer, 2014). When moving between sites, rinsing shared equipment and boots with tap water after manual cleaning will be performed (Weldon & Hillmer, 2014). When practical, diluted bleach can be used for sanitation in the field (Weldon & Hillmer, 2014). When this is not practical, additional clean equipment can be brought, like an additional pair of clean boots to change into. Cleaning field equipment and boots in 10% bleach solution after use at each study site can help prevent cross contamination (Whittaker & Vredenburg, 2011). Using disposable nitrile gloves and changing between organisms, especially when handling anurans, is an additional step to prevent the spread of disease (Whittaker & Vredenburg, 2011). Thankfully, the sampling methodology will not include the capture and removal of any organisms, so the disturbance will be temporary and minimal, especially with proper safety protocols in place.

References


### Appendix B

#### Field Key

<table>
<thead>
<tr>
<th>red-spotted newt - <em>Notophthalmus viridescens</em></th>
<th>four-toed salamander - <em>Hemidactylium scutatum</em></th>
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<tbody>
<tr>
<td>3-5”, adults are aquatic, olive green or yellowish green with yellow belly with small black spots, line of red spots circled by black on sides, keeled tail</td>
<td>Lays eggs on/near sphagnum moss overhangs</td>
</tr>
<tr>
<td>Larvae: yellowish green with mottled pattern, yellow underside, blunt snout and black stripe from snout to eye</td>
<td>4 toes on hind limbs, 2-3.5”, reddish brown with white underside with black spotting, constriction at base of tail</td>
</tr>
<tr>
<td>Adult: ![Red-spotted Newt](Ohio’s hidden wonders pg 44)</td>
<td>Larvae: head is orange, green, or yellow with dark brown/black spots that make a Y shape on the back of the head and short, wide bands from eyes to gills</td>
</tr>
<tr>
<td>![Larvae](Ohio’s hidden wonders pg 40)</td>
<td>Adult: ![Four-toed Salamander](A field guide to the animals of vernal pools pg. 42)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>smallmouth salamander - <em>Ambystoma texanum</em></th>
<th>unisexual mole salamander - <em>Ambystoma UNISEXUAL</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5-6.25”, small head, narrow short snout, small mouth, head is swollen at base, triangular shape overall, blackish, grayish, or brownish with pale gray or white markings, not bluish → <em>generally those are unisexual</em></td>
<td>Can be crosses between blue-spotted, jeffs, small-mouthed, and tiger</td>
</tr>
<tr>
<td>Larvae: heavily pigmented back, sides have dark cross bands with lighter cross bands in between, belly is pigmented but has a light, sharp, unmarked band, pale lateral stripe from behind eye to tail, below a blackish line extends</td>
<td>Noticeably swollen cloaca means male, likely not unisexual</td>
</tr>
<tr>
<td>![Smallmouth Salamander](Ohio’s hidden wonders pg 44)</td>
<td>Blue flecking on sides of body (adults, to differentiate from newly metamorphosed Jeff’s)</td>
</tr>
<tr>
<td>![Larvae](Ohio’s hidden wonders pg 40)</td>
<td>Tend to be larger than other “maximum” sizes, 5-6”</td>
</tr>
<tr>
<td>![Unisexual Mole Salamander](Ohio’s hidden wonders pg 29)</td>
<td>Larvae: most similar to blue spotted, jeffs, or small mouth, indistinguishable from those</td>
</tr>
<tr>
<td>![Moldy White Eggs](Ohio’s hidden wonders pg 29)</td>
<td>moldy white eggs on leaves or twigs</td>
</tr>
</tbody>
</table>
**Spotted salamander - Ambystoma maculatum**
- 4.5-8”, black salamander, yellow spots
- Larvae: very feathery gills, “balancers” beneath them for the first few days, a large head, older larvae have all 4 legs, brown with dark dorsal spots, pale band on side (jefferson has dark side band), pale underside, more slender than jeffersons

**Jefferson Salamander - Ambystoma jeffersonianum**
- 4.75-7”, long, broad snout, long slender toes, chocolate brown to blue black, light blue flecking on underside, newly emerged young have light blue/grey spots
- Larvae: dark pigmented, broad head, white throat, indistinct darker blotches of small, dark dots, dark line above belly, yellow flecks

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<table>
<thead>
<tr>
<th>Adult:</th>
<th>Larvae:</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.jpg" alt="Spotted Salamander" /></td>
<td><img src="image2.jpg" alt="Spotted Salamander Larvae" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult:</th>
<th>Larvae:</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.jpg" alt="Jefferson Salamander" /></td>
<td><img src="image4.jpg" alt="Jefferson Salamander Larvae" /></td>
</tr>
</tbody>
</table>
Larvae still with balancers:

Ohio’s hidden wonders pg 40, A field guide to the animals of vernal pools pg 32

<table>
<thead>
<tr>
<th>Western chorus frog - Pseudacris triseriata</th>
<th>Spring peeper - Pseudacris crucifer</th>
</tr>
</thead>
<tbody>
<tr>
<td>• A little over an inch, irregular longitudinal brown/gray stripes (3 distinctive stripes down back)</td>
<td>• 0.75 - 1.25”</td>
</tr>
<tr>
<td>• Thin white line on upper lip</td>
<td>• Light to dark brown, pinkish tinge</td>
</tr>
<tr>
<td>• Larvae: small tadpoles, gray/brown body, round shape, clear tail with white line below, black line above, small black flecks</td>
<td>• X on back</td>
</tr>
<tr>
<td>Adult:</td>
<td>• Dark line between eyes on top of head</td>
</tr>
<tr>
<td><img src="image1.png" alt="Western chorus frog - Pseudacris triseriata" /></td>
<td>• No thin white line on upper lip (chorus frogs)</td>
</tr>
<tr>
<td>Larvae:</td>
<td>• Larvae: &lt;1.3” tadpoles, greenish with gold flecks, metallic bronze underside &amp; dark tan back, emerge from water w/ tail still present</td>
</tr>
<tr>
<td><img src="image2.png" alt="Western chorus frog - Pseudacris triseriata" /></td>
<td>Adult:</td>
</tr>
<tr>
<td><img src="image3.png" alt="Western chorus frog - Pseudacris triseriata" /></td>
<td><img src="image4.png" alt="Spring peeper - Pseudacris crucifer" /></td>
</tr>
<tr>
<td><img src="image5.png" alt="Western chorus frog - Pseudacris triseriata" /></td>
<td>Larvae:</td>
</tr>
<tr>
<td><img src="image6.png" alt="Western chorus frog - Pseudacris triseriata" /></td>
<td><img src="image7.png" alt="Western chorus frog - Pseudacris triseriata" /></td>
</tr>
</tbody>
</table>
Gray treefrog/Cope’s Gray Treefrog - Hyla versicolor/Hyla chrysoscelis
- 1.25 to 2”
- Grainy skin, green, brown, or gray
- Inside of back legs are bright yellow/orange
- Larvae: large, black-flecked tail that extends up to head, stress can turn tail bright red/orange

Adult:

Larvae:

Wood frog - Rana sylvatica
- 1.5-2.5”, light tan to dark brown, terrestrial, dark mask, dorsolateral ridges
- Larvae: dark brown to blackish, .25” long when hatched, brown back and coppery flecks on belly as they mature, emerge ~June, laid in ~April, when toad and wood frog tadpoles are present, woodfrog will be large

Adult:

Larvae:

Green frog - Rana clamitans
- Green, olive, or brown in color
- Have a pair of dorsolateral ridges on back *bullfrogs do not*
- Larvae: Olive green above, white below, long slender tail with uniform spot patterns (in lines), can be large

Adult:

Bullfrog - Rana catesbeiana
- 4-8”, large
- Smooth back w/out dorsolateral ridges
- Bright green to dark olive, can have black striped hind legs
- Males have yellow throat, eardrum larger than eye
- Larvae: large, 2+ years to
**Northern Leopard frog - Rana pipiens**
- Stark white thigh, as opposed to yellow (*pickerel*)
- 2-3”, dark spots w/ pale outlines
- Tadpoles: fairly large, olive/brown/tan bodies, dark head, creamy white belly, speckled tail

**Adult:**

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**Pickerel frog - Rana palustris**
- 1.7-3”
- Dark, rectangular spots on light brown/green
- Yellow/orange underside (*vs leopard frog’s white*)
- Tadpoles: light brown to pale green, irregular brown spots, sharp pointed tail with little pigmentation, creamy white belly, gold hue

**Adult:**

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**Larvae:** metamorphosis, light green/dark olive with black or brown spots, creamy white/yellow belly

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**Adult:**
American Toad - *Bufo americanus*
- 2-4.3”, dry warty skin, brown/gray/olive, dark spots of brown/black
- Black tadpoles, swim in schools, unpigmented tail fin and rounded tip

Larvae:
References
