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Biodiversity of Salamanders in Central and Southeastern Ohio

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Abstract

Biodiversity and the presence of particular species can serve as bioindicators of ecosystem health. Monitoring the amphibian communities of aquatic and terrestrial sites can be an effective way to determine their habitat quality. Specifically, the assessment is based on the relative abundance of sensitive species, relative abundance of tolerant species, and other scores including the overall number of species. The goal of this study was to determine the quality of habitats in Blacklick Woods Metro Park and Capital University's Primmer Outdoor Learning Center in the Hocking Hills region of southeastern Ohio by surveying for salamanders in different habitats. Surveys were conducted in March 2010 and again in July 2017-May 2018 by turning rocks and logs over in moist wooded areas and in the banks of springs, and live-trapping using minnow funnel traps. Collection included obtaining a small tissue sample for future genetic analyses. Individuals were also swabbed to test for chytrid fungus disease. Results include finding three red-spotted newts (*Notophthalmus viridescens viridescens*), fifteen northern dusky salamanders (*Desmognathus fuscus*), and fourteen southern two-lined salamanders (*Eurycea cirrigera*) at Capital University's Primmer Outdoor Learning Center, which are all considered mid-range sensitivity. Twelve spotted salamanders (*Ambystoma maculatum*) were detected at Blacklick Woods Metro Park, which is a sensitive species. Primmer Outdoor Learning Center obtained an AmphIBI score of 33 with a rank of Superior Wetland Habitat. It was also determined to have Class III-PHWH streams, the highest level, which is relatively uncommon. Blacklick Woods Metro Park also obtained a rank of Superior Wetland Habitat, the highest category, with an AmphIBI score of 43. Future work includes supplemental sampling, lab analysis, and evaluating the habitats where particular salamanders were absent to try to determine why the species were not present in those locations. The implications of this work are that it can aid in conservation and management efforts to protect these species.

Keywords: Biodiversity, Disease, Salamanders

1. Introduction

Amphibians including toads, salamanders, tree frogs, and true frogs, are often times considered the connection between aquatic and terrestrial habitats in the landscape as a multitude of these species utilize both types of habitats[1]. Some species dwell in the water year-round such as the common mudpuppy (*Necturus maculosus maculosus*)[2] while others only utilize the aquatic habitat for breeding like the Jefferson salamander (*Ambystoma jeffersonianum*), the larval life stage, or feeding[3]. The green salamander (*Aneides aeneus*) is a species that relies on the moisture from terrestrial habitats and does not use aquatic habitats at all[4]. Although these varieties exist, amphibians in general rely on water for survival and are found in a wide range of habitats including wetlands, streams, vernal pools, rivers, ponds, lakes, and moist woodlands in Ohio. While most amphibians eat small invertebrates like earthworms, others like the eastern hellbender may eat crayfish, fish, and even other amphibians in addition to invertebrates[5]. The most influential threat to salamander populations is habitat loss and degradation caused by humans[5].

Biodiversity of the amphibian community can serve as a bioindicator of ecosystem health[1,6]. Specifically, the assessment is based on the relative abundance of sensitive species, relative abundance of tolerant species, and other scores including the overall number of species[7]. A study from 2014 used the Ohio AmphIBI (Amphibian Index of Biotic Integrity) assessment to determine the quality of wetland habitats in specific landscape conditions and results showed that fewer agricultural disturbances increased the quality of wetland habitats[7]. Another similar study explains the importance of decreasing the amount of physical changes to a habitat in relation to increasing the quality of amphibian populations[8]. Salamanders are sensitive to stressors in the environments they reside in due to their physiological dependence of their habitats, and therefore can be utilized as bioindicators[9,10]. They depend on their habitats for food resources, high moisture levels to avoid desiccation, cutaneous respiration, and the regulation of their body temperatures. Because of this, their presence and distribution indicate the quality of a habitat[9].

Chytridiomycosis was first explained in 1998 and has continued to affect amphibians in North America, Central America, Europe, and Australia[11].Chytridiomycosis is caused by chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), and is believed to be the cause of recent amphibian decline worldwide[12]. *Bd* infects amphibians through their skin, cause the skin to become thick, and therefore affects their supply of both oxygen and required salts. Due to this interaction, *Bd* impacts the way amphibians breathe and how their organs function[12,13]. In recent studies, chytrid fungal diseases has been reported to vary in susceptibility across individuals and species[14]. It is also reported that the disease did not always result in mortality of the individual, and that those individuals (or humans) might be responsible for moving it around the landscape[12]. Some individuals were even able to recover from the disease or simply be a vector of the disease and show no symptoms[14].

The goal of this study was to determine the quality of habitats in Blacklick Woods Metro Park in central Ohio and Capital University's Primmer Outdoor Learning Center in the Hocking Hills region of southeastern Ohio by surveying for salamanders. Each amphibian species is given a coefficient of conservatism (C of C) which is a key to the level of sensitivity for each species, numbered one through ten, with ten being the most sensitive in the index. The salamanders of Ohio have a wide range of levels of sensitivity, ranging from a three, low sensitivity, to a ten, which is considered the most sensitive[7]. Calculating the AmphIBI using these sensitivities is an effective way of determining the quality of a wetland by using salamanders as bioindicators[7]. A second goal of this study was to identify the presence of *Bd* using conventional nested Polymerase Chain Reaction (PCR) and real time PCR in order to determine the number of individuals and species infected within a given field site. Because Primmer is privately owned, it was hypothesized that more sensitive species of salamanders would have greater abundance and that the habitat would be less disturbed than in Blacklick Woods due to public access. Analyzing the quality of habitats that salamanders reside in will allow a better understanding of how to conserve and protect species in central and southeastern Ohio.

2. Methods

2.1. Field Methods

Surveys for salamanders were completed in March 2010, July - October 2017, and March - May 2018 at Capital University's Primmer Outdoor Learning Center in the Hocking Hills region of southeastern Ohio (Fig. 1(a)) and Blacklick Woods Metro Park in Reynoldsburg in central Ohio (Fig. 1(b)). Both wetland and spring/stream habitats were surveyed at Primmer, in addition to the wetland at Blacklick Woods. Surveys were completed a total of nine times at Primmer and six times at Blacklick Woods (Appendix 1). The salamanders and newts were either live-trapped with minnow funnel traps, hand caught, or captured using a dip net in the wetland, the banks of springs, or in moist wooded areas. Once an individual was located, the length of snout to vent, vent to tail, and the width of their abdomen were measured in mm (Table 1). Collection also included a small tissue sample from the tip of the tail for genetic analysis (<2mm) and two swab samples for analysis of chytrid fungal disease *Bd* presence. The salamander was swabbed on the ventral surface 20 times and the top left foot ten times. Adult frogs and toads, along with tadpoles, were also swabbed at these sites in a related study to test for the presence of chytrid fungal disease. Gloves were changed between each specimen to reduce the risk of cross contamination between samples and the production of false positives/negatives. After the samples and pictures were collected, the species was identified using the Ohio Department of Natural Resources amphibians field guide and measurements were compared (Table 1) to the average sizes for each species found in the field guide[15]. A picture was then taken and the organism was released.

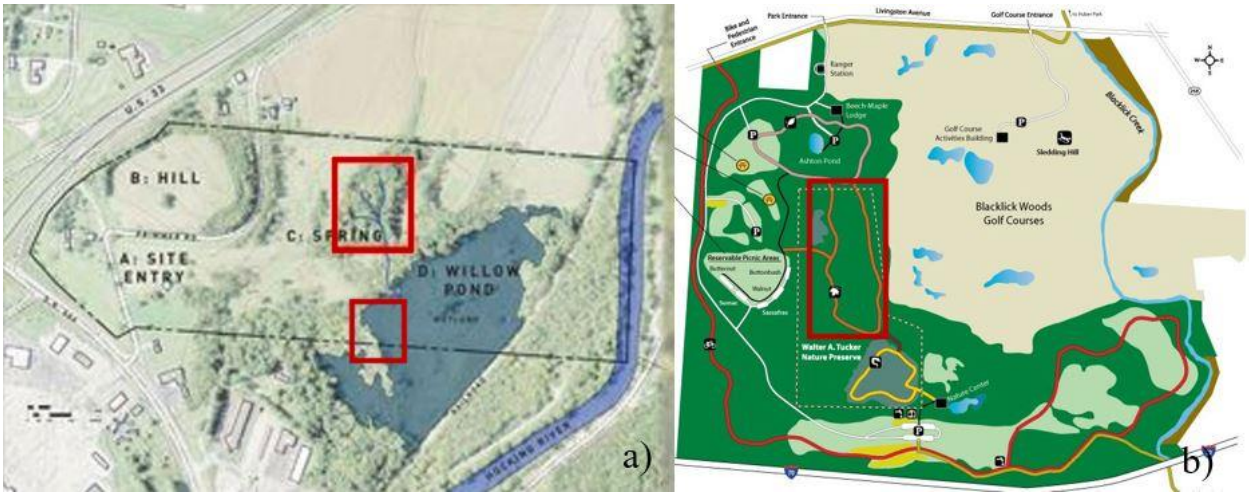

Figure 1 (a). An overview map of (a) Primmer Outdoor Learning Center in Logan, OH and (b) Blacklick Woods in Reynoldsburg, OH. The red boxes show where the salamanders and newts were found in each site.

Table 1. Mean and standard deviation values of snout to vent length, tail length, and body width categorized by each species at the two field sites.

	Primmer			Blacklick Woods
Measurements	Northern Dusky	Southern Two-Lined	Red-Spotted Newt	Spotted
SVL Mean (mm)	27.2±15.1	31.6±11.4	38.0±6.2	64.8±25.8
Tail Length Mean (mm)	23.9±14.8	31.5±13.2	N/A	67.4±25.6
Width Mean (mm)	4.9±3.1	5.4±2.6	N/A	15.8±5.1

2.2. Lab Methods

Tissue samples were placed in a 95% ethanol solution and stored at -20 deg C until genetic analysis while swab samples were placed in tubes and stored at 4 deg C. DNA was extracted from the tail tissue and one swab sample using the Qiagen DNeasy blood and tissue kit with the following modifications: (1) samples were placed in the water bath overnight at 65 deg C, and (2) were eluted to 100 ul. The eluted DNA was tested using the NanoDrop One spectrophotometer for quality and quantity. Singleplex and nested conventional PCR were performed using the Qiagen Multiplex PCR kit and the forward and reverse primers Bd1a and Bd2a, respectively were used[16]. Both singleplex and nested PCR mixtures were put under the same thermocycler conditions, including an annealing temperature of 55°C[3]. The products of nested PCR were imaged using gel electrophoresis with 3% agarose gels. The first well was loaded with a 50 bp DNA ladder (New England Biolabs). The gel was stained with ethidium bromide before being imaged under UV light. A fluorescent band at 300 base pairs would indicate the presence of chytrid in a sample[17]. Twelve salamander swab samples (ten from Primmer and two from Blacklick Woods), and additional swab samples from adult frogs and tadpoles in a related project, were analyzed for the presence of chytrid using real time PCR at the San Diego Zoo Amphibian Disease Laboratory for a more sensitive test.

2.3. Data Analysis

By using data collected in the field, the wetland at each site was given an AmphIBI score. The calculation of each score is determined by considering five factors. The first factor is the Amphibian Quality Assessment Index (AQAI), which determines the overall C of C for that specific site by utilizing the C of C of each species and taking the average of combined individuals found at each site. The AQAI result was then compared to a table in the published field manual to assign 0, 3, 7, or 10 numerical value to use toward the AmphIBI score[7]. The next factor analyzed was the Relative Abundance of Sensitive Species which was determined by calculating the percentage of species present that score a C of C from 6 to 10, then comparing those results to the table to assign 0, 3, 7, or 10 numerical value[7]. The third factor is the Relative Abundance of Tolerant Species. This is similar to the previous factor but only consider C of C from 1 to 3, and using the table again to score a 0, 3, 7, or 10 numerical value toward the AmphIBI score[7]. The fourth factor is the number of pond breeding species present at the field site. This factor also could score a 0, 3, 7, or 10 based on this number to score toward the AmphIBI score[7]. The fifth and final factor in calculating the AmphIBI score is the presence or absence of wood frogs or spotted salamanders. If neither of these species are found, they receive a numerical value of 0 toward the score. If either of these species are present, the numerical value of 10 is given toward the score[7]. Once each of the five factors are considered and numerical values are given, the values are added together to reach a total numerical value. This value is then compared to a different table to determine if this habitat is a Limited Wetland Habitat (<10), a Restorable Wetland Habitat (10-19), a Wetland Habitat (20-29), or a Superior Wetland Habitat (30-50)[7].

To determine the Primary Headwater Habitat (PHWH) stream classes for the springs found at Primmer, the data collected in the field was evaluated. Classes were evaluated by determining what biotic factors are present in each stream. Each species found in Ohio that uses primary headwater streams for steps in their reproduction processes are included in the evaluation for determining stream class. The stream can only be classified in the PHWH stream classes if larvae, eggs, or a mixture of juveniles and adults are found in the stream[18]. The species are used to classify the stream as PHWH stream class I, II, or III. If there were multiple species found in the same stream, the species that was classified with the highest value was used to determine the PHWH stream class[18]. Class I-PHWH streams are ephemeral because they are typically dry, having water only during times of runoff or the result of melting snow, and therefore do not typically have any aquatic life using this class as a habitat. Class II-PHWH streams have warm-water dwelling organisms such as fish, salamanders, or macroinvertebrates that live there either year-round or only during select seasons. Class III-PHWH streams, which are very uncommon, have cool-cold water dwelling organisms such as lungless salamanders, fish, or benthic macroinvertebrates that use this stream year-round[18].

3. Results

Results include catching three red-spotted newts (*N. viridescens viridescens*) in the wetland at Capital University's Primmer Outdoor Learning Center, as well as fifteen northern dusky salamanders (*D. fuscus*), and fourteen southern two-lined salamanders (*E. cirrigera*), including both adults and juveniles, in the banks of springs (Appendix 1). A salamander was found each day that the site was surveyed. Eggs were found at Primmer attached to logs in the banks of the springs, found with adults under the same logs, and a gravid female was found underneath a log. Twelve spotted salamanders (*A. maculatum*) were found in moist woodlands at Blacklick Woods Metro Park (Appendix 1). Four spotted salamander egg masses were also seen at Blacklick Woods Metro Park. Although this site was surveyed six times, salamanders were only found three of those six days.

Spotted salamanders, considered a high sensitivity species[7], were found in moist woodlands at Blacklick Woods Metro Park. In this study, Blacklick Woods' AmphIBI score is 43 based on (1) Amphibian Quality Assessment Index of 8, (2) Relative Abundance of Sensitive Species of 100%, (3) Relative Abundance of Tolerant Species of 0%, (4) one pond-breeding species, and (5) the presence of spotted salamanders. A score of 43 indicates that Blacklick Woods is a Superior Wetland Habitat (SWLH) because it scores between 30-50 (Table 2). In 2010, three red-spotted newts (*N. viridescens viridescens*), a mid-range sensitive species[7], were found at Primmer in the wetland area. Finding this species indicates an AmphIBI score of 33 based on (1) Amphibian Quality Assessment Index of 6, (2) Relative Abundance of Sensitive Species of 100%, (3) Relative Abundance of Tolerant Species of 0%, (4) one pond-breeding species, and (5) the absence of spotted salamanders. A score of 33 indicates that Primmer Outdoor Learning Center is a Superior Wetland Habitat (SWLH) because it scores between 30-50 (Table 2).

Table 2. AmphIBI scores, Categories, and Aquatic Life Use for the wetlands at each field site.

Site	AmphIBI Score	Category	Aquatic Life Use
Primmer Outdoor Learning Center	33	3	Superior Wetland Habitat
Blacklick Woods Metro Park	43	3	Superior Wetland Habitat

Because there was evidence of reproduction in the salamanders found at Primmer, the springs had the ability to be described with PHWH stream classes. The presence of northern dusky salamanders categorizes the springs as Class II-PHWH streams, but the presence of southern two-lined salamanders categorizes the springs as Class III-PHWH streams, and therefore the springs are considered Class III-PHWH streams[18].

In a related project, zero of the 26 chytrid swabs from frogs and tadpoles tested positive from Primmer with real time PCR conducted at the San Diego Zoo Amphibian Disease Laboratory, while Blacklick Woods had positive samples found on nine frogs and tadpoles from 2014 to 2017 out of 37 total samples collected. In this study, recent results from the San Diego Zoo using real time PCR indicate two positive chytrid results out of ten salamander samples from Primmer and zero positive results out of two salamander samples from Blacklick Woods (Figure 2 & 3). Based on results using conventional nested PCR in the laboratory, one positive sample was confirmed, and another positive sample from Primmer was found (Figure 3). Two of the three positive chytrid samples were found on northern dusky salamanders, and the third positive result was found on a southern two-lined salamander. All three individuals were found within the same stream at Primmer. Additional bands present also indicate that further optimization of methods will be conducted (Figure 3).

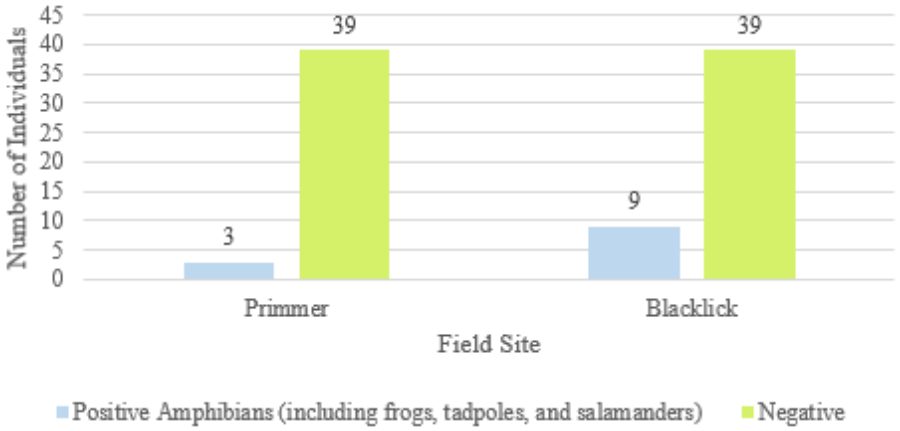

Figure 2. The number of amphibians swabbed for chytrid fungus versus individuals that tested positive at Primmer (in SE OH) and Blacklick Woods (in central OH).

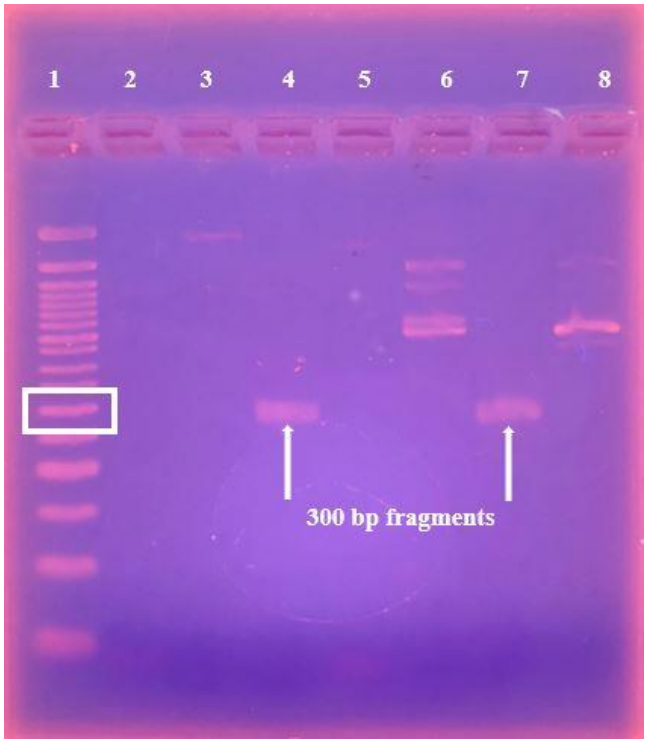

Figure 3. Agarose gel with 50 bp ladder in Lane 1 and conventional nested PCR products in lanes 2-8. The white box shows the 300 bp location. Two lanes show bands at 300 base pairs indicating positive chytrid results. The positive result in lane 4 confirms real time PCR results conducted elsewhere.

4. Discussion

At Primmer Outdoor Learning Center, 32 salamanders were found including red-spotted newts, southern two-lined (Figure 4 (b)), and northern dusky salamanders (Figure 4 (c)), as well as indications of reproduction due to eggs and juveniles. All three of these species are classified as mid-range sensitive species[7]. As shown in Figure 1 (a), Primmer Outdoor Learning Center is bordered by farmland, highway US-33, railroad tracks, Hocking River, and a Walmart. The farmland is higher up on a hill compared to the springs, and so these surroundings have the potential to affect the soil and water due to runoff contamination. However, southern two-lined and northern dusky salamanders are known to reside in flowing, cool water habitats such as springs[7,15], which could also suggest that this is why they were found in these springs. These results also conclude that the streams are classified as Class III-PHWH streams which usually run into larger streams and rivers considered Cold Water Habitats[19]. Because Class III-PHWH streams are infrequent in Ohio, the same protection that is given to Cold Water Habitats should be given to these streams as well. They should also be protected from modifications and disturbances that would affect the flow of these streams[18]. The red-spotted newts found indicate that the wetland at Primmer is a Superior Wetland Habitat. Because Primmer is on private property and is used by Capital University primarily for research and outreach, there has been minimal disturbance of this habitat by humans. Care should be taken to continue to minimize disturbance to maintain the diversity of sensitive salamanders found here.

A total of twelve spotted salamanders (Figure 4 (a)) were found at Blacklick Woods Metro Park describing this to be a Superior Wetland Habitat. Results were surprising considering this is a public park, where humans have the ability to be present and disturb this ecosystem. The salamanders were found mostly near the concrete paths under logs where people could cause more disturbance, rather than further into the woodland. The vernal pools were scattered and large, and used the majority of the woodlot, except the areas near the paths. Adults only reside in vernal pools when breeding[5], and therefore the logs near the path could have been the only place with preferred moisture levels in the soil. The trail (Maple Loop) where the spotted salamanders were found is also inside of the Walter A. Tucker Nature Preserve (Figure 1(b)). The presence of the spotted salamanders could indicate that the wetlands in this area are likely

minimally disturbed. According to a study published in 2003, the quality of habitat where spotted salamanders reside can be determined by their hormone concentration and their ability or lack of ability to react to certain stressors in their environment and can indicate whether a population is in danger of declinations. The results from this study showed that once a habitat was disturbed, the number of individuals located decreased, possibly from migration to a habitat with better quality[20]. Another study in 2015 supports the idea that minimal disturbance increases the quality of vegetation in wetlands and amphibian populations[21]. Based on the results of these studies, it should be recommended that the Walter A. Tucker Nature Preserve should continue to be designated as a preserve to minimize disturbances and stabilize spotted salamander populations.

Figure 4. Photographs of a spotted salamander (a), a southern two-lined salamander (b), and a northern dusky salamander (c) found during surveys at the two field sites.

Unfortunately, chytrid fungus has been detected at the Blacklick Woods site as well as at Primmer (Figures 2 & 3), which could likely be the result of foot traffic in both Blacklick Woods Metro Park and Primmer. As stated previously, Blacklick Woods Metro Park has the potential to have humans walking through areas and causing disturbance to these habitats. Until this study, *Bd* had not been detected at Primmer, but has now been confirmed positive on three individuals. Two of the three positive results were found on northern dusky salamanders and the third was found on a southern two-lined salamander. Because these springs flow into the wetland, it is surprising that positive results have not been found on individuals living in that habitat, including on frogs and tadpoles. This result could suggest that *Bd* was introduced after the negative samples were taken. In comparison, Blacklick has had nine samples with confirmed positive *Bd* results. The heavier foot traffic could be a likely reason for the spread of this disease, however there are other factors that could affect this. Certain species of salamanders seen in recent studies do not show symptoms of the disease, even when they are purposefully infected. In one study, blue spotted salamanders were infected with *Bd* and seemed to not be affected and had no mortalities because of the disease, although they carried the fungus on their skin[22]. This could be an indication that individuals could be a vector for *Bd* and transport it from one habitat to another. Since many species of salamanders use vernal pools for breeding sites[5], they have the potential to carry *Bd* from one to another, infecting other individuals including other salamanders and even frogs that use the same vernal pools that are more susceptible to the effects of this disease.

More research needs to be done to protect amphibians and reduce the spread of chytrid fungal disease. One strategy to reduce the risk of spreading chytrid is to educate people about the disease and how they can impact the spread of the disease. Before going into a new place, people should be informed that they should clean their shoes and boots and/or traps with a bleach solution to kill any fungus and/or fungus spores, so the fungus is not transported from one place to another. In a study published in 2003, it was concluded that using household bleach with a concentration greater than and equal to 1% sodium hypochlorite is effective in killing the fungus[23]. Although not the most effective, household bleach is likely one of the easier products for people to get. Because the positive chytrid results have been found only recently at Primmer, it is likely that foot traffic from researchers in the field could have caused the presence of chytrid if boots or traps were not cleaned properly prior to exposure. Moving forward, it would help to understand other techniques to assess habitat quality such as water quality and the presence of macroinvertebrates and vegetation. Once all factors are included in a larger assessment, an analysis of the presence of certain species can be explored, habitat quality can be assessed, and actions can be taken to protect the habitat and conserve organisms.

Additional field work at these sites and additional Metro Parks provides the opportunity to calculate a more accurate and precise AmphIBI score for each field site. Future work also includes analysis of macroinvertebrates and water quality based on collaboration with other students' research in the same places. Future lab analysis includes additional testing for the presence of chytrid fungus using real time PCR and confirmation of each species using DNA sequencing. Surveys in different times of the year in different seasons will also allow for a better understanding of habitat quality in relation to bioindicators. Another direction for field surveys will include the construction and use of leaf litter bags to aid in the detection of juveniles and rare species[24,25].

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7. Appendices

Appendix 1. The specific dates, locations, method of capture, species found, temperature, and precipitation during field surveys at both sites. The coefficient of conservatism of each species is also included for reference[7].

Date	Site	Location in Site	Method of Capture	Species	Temperature (°C) High/Low	Precipitation (cm)	C of C
03/18/2010	Primmer	Wetland	Live trap	Red-Spotted Newt	-1.1/17.2	0	6
03/18/2010	Primmer	Wetland	Live trap	Red-Spotted Newt	-1.1/17.2	0	6
03/19/2010	Primmer	Wetland	Live trap	Red-Spotted Newt	0.56/17.8	0	6
7/25/2017	Primmer	Stream	Hand catch	Northern Dusky	28.3/15.0	0	4
7/31/2017	Primmer	Stream	Hand catch	Southern Two-Lined	28.3/12.8	0	5
7/31/2017	Primmer	Stream	Hand catch	Southern Two-Lined	28.3/12.8	0	5
8/1/2017	Primmer	Stream	Hand catch	Northern Dusky	28.9/15.0	0	4
9/10/2017	Primmer	Stream	Dip net	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Dip net	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Hand catch	Southern Two-Lined	20.6/9.4	0	5
9/10/2017	Primmer	Stream	Hand catch	Southern Two-Lined	20.6/9.4	0	5
9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Hand catch	Southern Two-Lined	20.6/9.4	0	5
9/10/2017	Primmer	Stream	Hand catch	Southern Two-Lined	20.6/9.4	0	5
9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Hand catch	Southern Two-Lined	20.6/9.4	0	5
9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4

9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4
9/10/2017	Primmer	Stream	Hand catch	Northern Dusky	20.6/9.4	0	4
10/9/2017	Blacklick	Wetland	Hand catch	Spotted	23.3/17.8	0.508	8
10/9/2017	Blacklick	Wetland	Hand catch	Spotted	23.3/17.8	0.508	8
3/29/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
3/29/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
3/29/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
3/29/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
3/29/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
4/9/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
4/9/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
4/9/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
4/9/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
4/9/2018	Blacklick	Wetland	Hand catch	Spotted	16.1/6.1	1.7	8
5/1/2018	Primmer	Stream	Hand catch	Southern Two-Lined	21.1/-1.1	0	5
5/1/2018	Primmer	Stream	Hand catch	Southern Two-Lined	21.1/-1.1	0	5
5/11/2018	Primmer	Stream	Hand catch	Northern Dusky	26.7/13.3	0	4
5/11/2018	Primmer	Stream	Hand catch	Southern Two-Lined	26.7/13.3	0	5
5/11/2018	Primmer	Stream	Hand catch	Southern Two-Lined	26.7/13.3	0	5
5/11/2018	Primmer	Stream	Hand catch	Southern Two-Lined	26.7/13.3	0	5
5/11/2018	Primmer	Stream	Hand catch	Southern Two-Lined	26.7/13.3	0	5
5/11/2018	Primmer	Stream	Hand catch	Southern Two-Lined	26.7/13.3	0	5
5/18/2018	Primmer	Stream	Hand catch	Northern Dusky	25.0/17.8	0.38	4