Occurrence of Frogs and Toads in Relation to Habitat Quality at Capital University's Primmer Outdoor Learning Center in Hocking County, Ohio

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Abstract: Amphibian species are declining worldwide due to threats including habitat loss, global climate change, and disease. FrogWatch USA is a citizen science project through which people learn how to identify frogs and toads based on their breeding calls and then participate by collecting and submitting field data to an online database. The purpose of this project was to collect accurate and detailed information on the presence of frogs and toads at Capital University's Primmer Outdoor Learning Center in Logan, OH. Data on the occurrence and calling intensity of frogs and toads were collected at six locations surveyed twice a week during the spring and summer of 2023, and water samples were collected from seven locations once a month. Over the course of fifty-seven sessions, ten out of the fourteen possible Ohio species were detected, including two sensitive species. Water chemistry testing showed that phosphate levels were elevated in the wetland, and the streams contained high concentrations of nitrate and chloride. Nonetheless, the amphibian index of biotic integrity (AmphIBI) designated Primmer at the highest level as a Category 3 Superior Wetland Habitat. It is recommended that the trails going into the woodlot where the streams are located and along the wetland be closed to visitors to help maintain habitat quality. By participating in community science, we can learn more about amphibian biodiversity and make progress towards protecting amphibians and their habitats.

Keywords: biodiversity, FrogWatch USA, citizen science, nitrate, bioindicator, AmphIBI

INTRODUCTION

Human pressures are causing amphibian populations to decline worldwide due to threats including habitat loss, global climate, and disease. There is substantial evidence that we are in the midst of the sixth mass extinction event (Wake and Vredenburg 2008). Amphibians are more at risk than either birds or mammals, with over a third of species categorized as threatened (Stuart et al. 2004). In aquatic habitats, their thin, well-vascularized skin makes them particularly vulnerable to changes in water quality, including increasing pollutants and changing thermal regimes (Wake and Vredenburg 2008). Hundreds of species are expected to go extinct over the next few decades (Stuart et al. 2004). In Ohio, amphibians have experienced declines and extinctions even in some of the most highly protected areas, but more research is needed (Pfingsten et al. 2013). Chytrid fungus is one of the leading causes of amphibian declines throughout the world (Vredenburg et al. 2010) and has been reported as widespread in Ohio (Korfel et al. 2021). Previous studies have used frogs as bioindicators of habitat quality (Parmar et al. 2016).

Citizen science projects rely on volunteers to collect data on scientific matters that are rapidly growing and in need of greater study (Silvertown 2009). This helps scientists to record more data than they could by themselves, which can result in greater conservation efforts for a particular problem (Conrad and Hilchey 2011). In addition to the advantages that researchers gain from these projects, the volunteers who participate are able to gain awareness in the respective field (Bonney et al. 2016). FrogWatch USA is one such project in which participants are trained to identify frog and toad breeding calls, learn more about wetlands and conserving amphibians in their area, and submit their findings to an online database (FrogWatch USA 2024). It was started by the National Wildlife Federation in partnership with the USGS (Kelhart 2007) and is now managed by the Association of Zoos & Aquariums. To date, there have been over 15,000 participants to help monitor local frog populations, detect rare and invasive species, and track shifts in species diversity, range, and seasonal timing (FrogWatch USA 2024). FrogWatch USA participants can also help identify new study sites with particular species present not previously known to scientists and professionals (Inkley 2006). While there are studies that have reported their findings on amphibian biodiversity in Ohio (e.g., Weyrauch and Grubb 2004, Lipps 2005, Pfingsten et al. 2013, Micaccion et al. 2015), there are few articles that have published their findings from the FrogWatch USA project along with other data on habitat quality.

The main goal of this project was to collect accurate and detailed information on the occurrence of frogs and toads based on the intensity of their calls at multiple locations at a university field research site in Logan, OH. Additionally, water samples were collected for water chemistry analysis including pH, chloride, nitrate, and other measurements, and the amphibian index of biotic integrity (AmphIBI) was calculated to assess habitat quality on site (Micacchion 2011). This acoustic monitoring project at Capital University's Primmer Outdoor Learning Center adds to our understanding of the occurrence, distribution, and ecology of amphibian species in Ohio. By participating in community science, we can learn more about amphibian biodiversity in Ohio and strides can be taken to protect them and their habitats.

METHODS

Study Site. This project was completed at Capital University's Primmer Outdoor Learning Center in Hocking County during spring and summer of 2023, from February through August. The study site is located in the Hocking Hills region of Appalachia in Logan, Ohio. It is a 74-acre property containing seven ecosystems, including a 15-acre wetland; an area where groundwater seeps into the woodlot, feeding into three small streams; restored prairie habitats; and frontage along the Hocking River (Fig. 1). It was established to foster biological and related research experiences and to promote creative learning opportunities for students and the general public (Capital University 2024).

Frog and Toad Acoustic Monitoring. Amphibian surveys were completed twice a week, on average, at six locations across the study site (Fig. 1). Data were collected following the methodology from FrogWatch USA (FrogWatch USA 2024). This included recording air temperature during the visit and in the previous 48 hours, wind speed using the Beaufort wind scale, precipitation during the visit and in the previous 48 hours, and the calling intensity of each species of frog and toad. The calling intensity index was defined based on the following: 1 = individuals could be counted with space between calls, 2 = calls of individuals could be distinguished but with some overlapping of calls, and 3 = full chorus with constant, continuous, and overlapping calls. Monitoring began at least thirty minutes after sunset and was not recorded during high winds due to difficulties in accurately hearing the calls. All data were uploaded to the FrogWatch USA fieldscope website. These are the first data reported from southeastern Ohio to FrogWatch USA (FrogWatch USA 2024).

Water Collection and Testing. Water samples from each of the three streams, three locations around the wetland, and the river were collected in 125 ml polyethylene bottles monthly, placed in a cooler, and transported to the laboratory. Samples were frozen at -20ºC until testing could be completed, and all analyses were completed within 48 hours. pH was measured in the field (Weyrauch and Grubb 2004) using a PASCO pH meter. Temperature was also collected in the field using an electronic probing thermometer set to degrees Celsius. The same thermometer was used for recording the air and water temperatures throughout the project. LaMotte and CHEMetrics kits were used to test for levels of chloride, ammonia, and phosphate to assess water quality (Minnesota Department of Natural Resources 2024). Nitrate was tested using nitrate ion analysis in the laboratory. Measurements were averaged across time for each site.

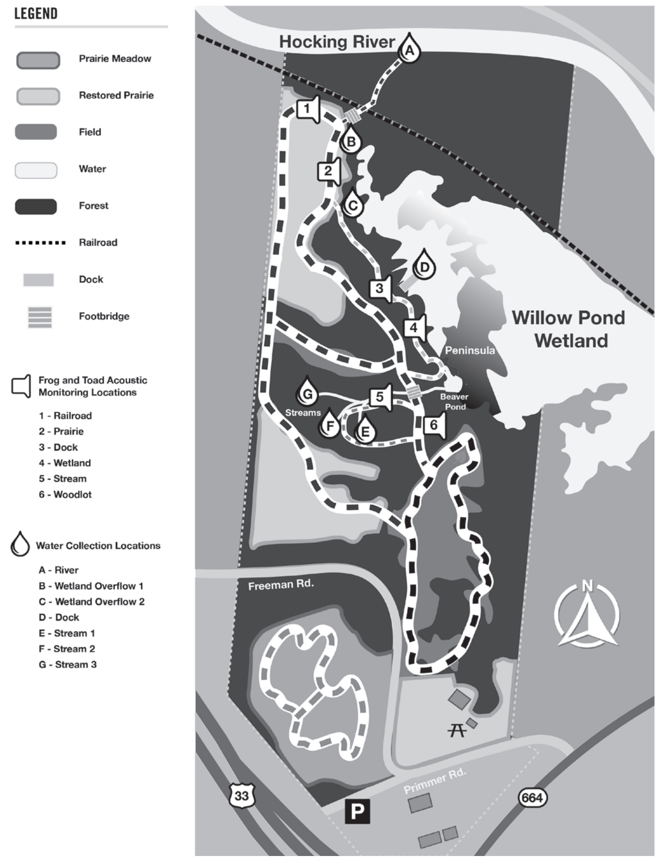

Fig. 1. Map of the Primmer Outdoor Learning Center in Hocking County. The six frog and toad acoustic monitoring locations and the seven water collection locations are labeled.

Habitat Quality Assessment. The amphibian index of biotic integrity (AmphIBI) of the study site was calculated using the frog and toad data in addition to salamander observations to assess habitat quality as described in Micacchion (2011). The AmphIBI score is based on summing five individual metrics including the Amphibian Quality Assessment Index (AQAI), the relative abundance of sensitive species, the relative abundance of tolerant species, the number of pond-breeding salamander species, and the presence of spotted salamanders and/or wood frogs (Micacchion 2011).

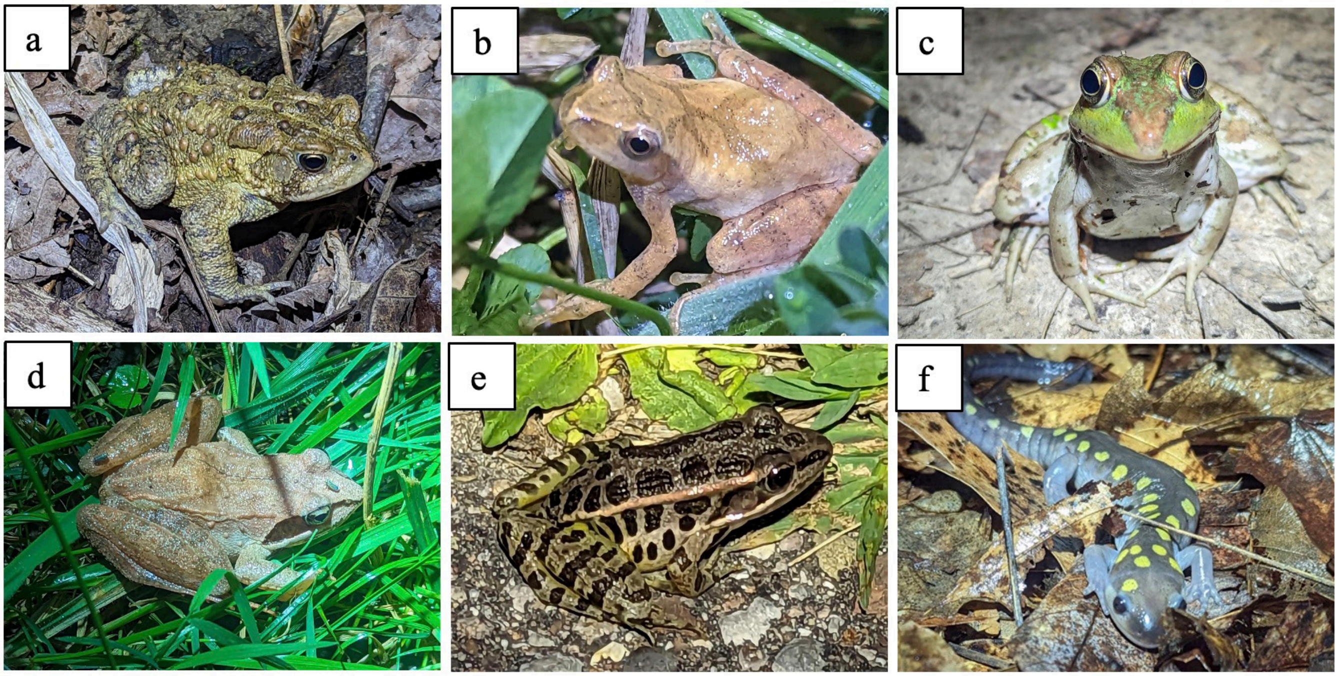

Fig. 2. Photos of six of the species visually observed at the Primmer Outdoor Learning Center in 2023. These included the eastern American toad (a), northern spring peeper (b), northern green frog (c), wood frog (d), northern leopard frog (e), and spotted salamander (f).

Based on the values for each metric, scores are 0, 3, 7, or 10. For these analyses, we conservatively estimated that an acoustic monitoring calling intensity of one was equivalent to one individual, an intensity of two was equivalent to three individuals, and a calling intensity of three was equivalent to five individuals. Some species were also visually observed during the surveys, including at least ten spring peepers, ten spotted salamanders, two southern two-lined salamanders (*Eurycea cirrigera*), and one northern slimy salamander (*Plethodon glutinosus*). In related work, we hand-caught four northern dusky salamanders (*Desmognathus fuscus*) at Primmer in 2023 (Dion and Anderson, in prep.; Gibbs et al., in prep.), and previously live-trapped three red-spotted newts (*Notophthalmus viridescen*s) in the wetland (Musial 2013).

RESULTS

Over the course of fifty-seven sessions, from 9 February to 31 August 2023, ten species were recorded based on their calls (Table 1). These included the eastern American toad (*Anaxyrus americanus*), gray treefrog (*Hyla versicolor*), northern spring peeper (*Pseudacris crucifer*), mountain chorus frog (*P. brachyphona*), western chorus frog (*P. triseriata*), American bullfrog (*Lithobates catesbeianus*), northern green frog (*L. clamitans melanota*), wood frog (*L. sylvaticus*), northern leopard frog (*L. pipiens*), and pickerel frog (*L. palustris*). The majority of the frogs and toads recorded were also observed visually (Fig. 2). The spring peepers, wood frogs, and western chorus frogs were the first species detected in the study in February, and spring peepers were heard at a high calling intensity (3) for four straight months (Table 1). American bullfrogs were heard in April, June, July, and August, but not May, and had the lowest calling intensities along with pickerel frogs (Table 1). Only four species of Ohio frogs and toads were not detected in this study.

Water chemistry was tested monthly at each of the seven water collection locations at Primmer (Fig. 3). Average (±SE) water temperature collected on site ranged from 13.3ºC±1.5 to 18.6ºC ±2.1 (Fig. 3a) across the locations, with temperatures increasing slightly from spring into summer. The pH levels stayed relatively constant from February to August, and the averages ranged from 6.4±0.09 to 7.5±0.1(Fig. 3b). Average chloride levels (in ppm) were lowest at the wetland overflow 2 site (60.0±9.9) and the dock (60.0±12.2), but much higher in stream 2 (170.9±14.0; Fig. 3c). Ammonia concentrations (in ppm) were low across all sites (Fig. 3d), but phosphate levels were above 0.1 ppm on average at the three sites in the wetland (Fig. 3e). Average nitrate levels (in ppm) were above 2.0 ppm in the wetland (e.g., ≈3.7 at both overflow 2 and the dock), and above 10 ppm in stream 3 (12.9±2.7), stream 2 (14.0±2.2), and the river (14.2±2.7; Fig. 3f).

The AmphIBI was calculated to indirectly assess habitat quality based on the frog and toad species recorded during the acoustic monitoring sessions, and on visual observations of salamanders on site. As described in Micacchion (2011), the AQAI is essentially an average coefficient of conservatism (c of c) score for all individuals detected. The AQAI for Primmer was 4.43 (Table 2). The c of c ranges from 1 to 10, and was determined by experts based on species sensitivity or tolerance to stressors in the environment (Micacchion 2011). The most

Table 1. The frog and toad species that were detected during acoustic monitoring sessions at the Primmer Outdoor Learning Center in 2023. The numbers represent the greatest calling intensity across sessions within each month. Dashes indicate that the species was not detected. A calling intensity of 1 indicated that individuals could be counted. Intensity 2 represents individuals that could be distinguished with some overlapping of calls, and an intensity of 3 indicates a full chorus.

Species	February	March	April	May	June	July	August
Eastern Spadefoot		—	—	—	—	—	—
Eastern American Toad	—	2	3	3	3	3	—
Fowler's Toad	—	—	—	—	—	—	—
(Blanchard's) Northern Cricket Frog	—	—	—	—	—	—	—
Gray Treefrog	—	—	1	3	3	3	2
Cope's Gray Treefrog	—	—	—	—	—	—	—
Northern Spring Peeper	3	3	3	3	1	—	—
Mountain Chorus Frog	—	2	3	1	—	—	—
Western Chorus Frog	1	3	3	1	—	—	—
American Bullfrog	—	—	1	—	1	2	1
Northern Green Frog	—	—	—	2	2	3	3
Wood Frog	3	2	1	—	—	—	—
Northern Leopard Frog	—	2	3	—	—	—	—
Pickerel Frog	—	—	1	1	—	—	—

sensitive species found at Primmer were wood frogs, pickerel frogs, spotted salamanders, and red-spotted newts, which have higher c of c scores. The most tolerant species have lower c of c scores and included eastern American toads, spring peepers, American bullfrogs, and northern green frogs. The two pond-breeding salamanders were spotted salamanders and red-spotted newts. After assigning the scores for each metric and summing across the five metrics, an AmphIBI score of thirty-three designates Primmer at the highest level as a Category 3 Superior Wetland Habitat (SWLH; Table 2).

DISCUSSION

Ten out of the fourteen frog and toad species of Ohio were found in Hocking County at the Primmer Outdoor Learning Center, including pickerel and wood frogs, which are described as sensitive (Micacchion 2011). Pickerel frogs tend to prefer cool, clear waters of streams and have been described as intolerant of pollution (Lehtinen 2013). Wood frogs, on the other hand, are highly terrestrial and can tolerate the freezing of tissues as they hibernate beneath leaf litter at the soil surface (Costanzo 2013). Not surprisingly, the extremely rare eastern spadefoot (*Scaphiopus holbrookii)* was not observed during this study. They are a state endangered species and have only been reported in Athens, Coshocton, Lawrence, Meigs, Morgan, Muskingum, Scioto, Tuscarawas, and Washington counties (ODOW 2012). Fowler's toads, which are most often found in areas of loose and sandy soils (Brune 2013), were also not expected because that habitat is largely absent from the study site. Blanchard's cricket frog (*A. blanchardi*) and Cope's gray treefrog (*H. chrysoscelis*), the other two species not heard during these acoustic surveys, have not been recorded in Hocking County since 1989 (Folt and Davis 2013; Matson 2013).

It was expected that American bullfrogs would be one of the most abundant species heard during the acoustic monitoring sessions. They are typically heard from late April through late summer, and their calls can be heard over a mile away (ODOW 2012). They are described as tolerant and can be found in a wide range of permanent water bodies including ponds and streams throughout Ohio (Micacchion 2011). However, they were completely absent during the month of May and were found at just one or two sites each session along the wetland when they were calling, and their calling intensity only reached a moderate level (2) on one occasion. At a different field site in central Ohio, vegetative cover and water temperatures were found to affect the abundance of overwintering tadpoles (Hargis et al. 2008). Because the wetland is heavily vegetated with buttonbush shrubs and water lilies, it is possible that water temperatures were not optimal for American bullfrogs during the previous breeding season at Primmer. In this study, the water temperature recorded in May across sites in the wetland was not particularly cold (i.e., ≈20ºC). In the field, frogs have been shown to select cool, moist habitats with body temperatures ranging from 15–21ºC (Köhler et al. 2011).

Northern spring peepers were the most abundant frog species detected during this study. They were found in all six locations where acoustic monitoring took place and had the highest calling intensity for the longest time. Northern spring peepers start calling early in the spring and move to moist woodlands after they are done breeding (ODOW 2012).

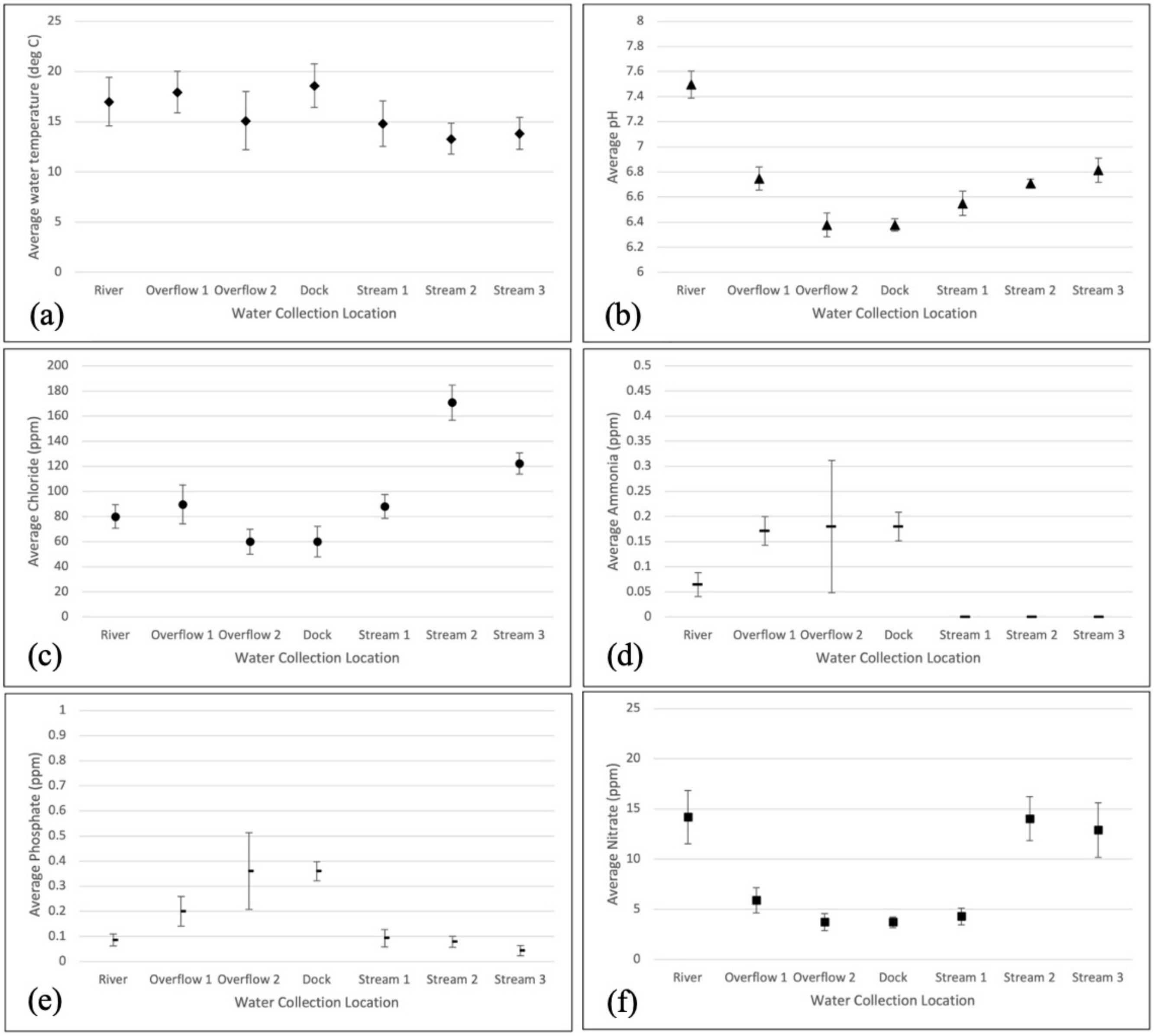

Fig. 3. Average (±SE) levels of water temperature (a), pH (b), chloride (c), ammonia (d), phosphate (e), and nitrate (f) collected monthly from the seven water collection locations at the Primmer Outdoor Learning Center in 2023.

The levels of pH and ammonia concentrations recorded in this study across months and sites at Primmer were within normal limits (EPA 2024). To control eutrophication, the US EPA limit is 0.05 ppm for total phosphates in streams that enter lakes, and 0.1 ppm for total phosphorus in flowing waters (Litke 1999). Values that exceeded this threshold were recorded at the three sites in the wetland. Additional water testing should be conducted in subsequent years to determine whether these levels are consistently high.

Chloride occurs naturally in groundwater but can enter a watershed through road salting, water softener discharge, and animal manure and fertilizers (Ohio Watershed Network 2024). The US standard exposure limit of chloride for aquatic life is 230 ppm to prevent chronic effects (Miltner 2021). While most of the values recorded in our study were below this limit, there was one recording in August from Stream 2 at 244 ppm for chloride. Nitrate concentrations were above 10 ppm in the river, Stream 2, and Stream 3, even at the beginning of the study in February, and all sites were typically above 2 ppm.

The US federal maximum limit of nitrate in drinking water is 10 ppm (OEPA 2022); a large review of nitrate toxicity to aquatic organisms (including amphibians) suggested this same limit, and also recommended a maximum level of 2 ppm for extremely sensitive species (Camargo et al. 2005). Previous work conducted at Primmer in 2008 also reported high nitrate levels in two out of the three streams (>30 ppm) and the river (>20 ppm) compared to the other sites tested (Hinkle et al., unpublished data). It is possible that runoff from fertilizers in surrounding agricultural fields are cycling into the streams on the property and into the Hocking River.

Table 2. Metrics and values used to calculate the Amphibian Index of Biotic Integrity (AmphIBI) for the Primmer Outdoor Learning Center.

Metric	Value	Score
Amphibian Quality of Assessment Index (AQAI)	4.4	3
Relative Abundance of Sensitive Species	27.5%	7
Relative Abundance of Tolerant Species	7.5%	10
Number of Pond-Breeding Salamander Species	2	3
Spotted Salamanders or Wood Frogs	Yes	10
	Total	**33**
	Wetland Designation	**Superior Wetland Habitat (SWLH)**

Primmer was categorized as a Superior Wetland Habitat. The Index of Biotic Integrity (IBI) top-level designation was also supported by the relatively low levels of most pollutants in the water chemistry analysis. Another study completed habitat assessments and calculated the IBI at 54 wetlands across Ohio (including shrub and forested sites in southeastern Ohio) and found support that AmphIBI has higher values in less disturbed wetlands (Micacchion et al. 2015). In order to increase ecological integrity of both plant and animal communities, the authors suggest maintaining forest buffers around wetlands and minimizing nutrient input from surrounding agricultural fields (Stepanian et al. 2015). To maintain the habitats where sensitive species were found at Primmer, we recommend that the trails be closed to visitors in the woodlot with the streams, along the wetland, and back to the river. When hosting students and the public for events and educational activities, they will clean their boots using a dilute bleach solution and water rinse to maintain the integrity of the property and prevent any contamination with chytrid fungus (MW PARC 2024). We will also investigate building a raised boardwalk along the streams to prevent habitat degradation from foot traffic in that area.

In addition to monitoring phosphate, chloride, and nitrate levels at Primmer and other nearby sites, future work should also focus on investigating patch and landscape characteristics including hydroperiod and wetland perimeter that can predict amphibian and specifically anuran species richness (Weyrauch and Grubb 2004). Future amphibian-related projects on site could include testing for *Batrachochytrium dendrobatidis*, the deadly fungal pathogen that causes chytridiomycosis (see Korfel et al. 2021), and expanding this citizen science project to include surveys at other nearby properties in Hocking County and engage more participants from the university and local school districts.

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