2020

Amphibian Species Richness and Distribution in Vernal Pools at Glover's Ledge, Langdon, NH

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December 2020

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A Thesis
Presented to the Department of Environmental Studies
Antioch University New England

In Partial Fulfillment
of the Requirements for the Degree of
Master of Science

By Kimberly Snyder
December 2020

ACKNOWLEDGMENTS

I would like to thank my thesis committee: Liz Willey, Peter Palmiotto, and Brett Thelen for their support, guidance, and assistance in putting together this project and final thesis report. Thank you Liz for taking me in and responding to my many requests for assistance starting out in a new field! Thank you Peter for introducing me to this project and helping me out with the gritty details and last minute equipment needs! And a special thank you to Brett for always making time for my many questions and for training me in amphibian and egg mass identification techniques.

I also wish to thank Stoff Scott, Megan Ormsby and Ally Gelanis for their assistance with equipment set-up and monitoring of the pools and Audrey Boraski and John Crockett for their expertise in audio recording. Additional thanks to John Crockett for his generous assistance with field equipment testing and loaning. Thanks to Steven Lamonde for all his assistance in helping develop pool perimeter and area measurement protocols as well as the spatial analysis methods used to map pool perimeters and areas.

I wish to extend special thanks to Dr. Rachel Thiet and Megan Ormsby for their help in planning and researching this project and fine-tuning the proposal as well as listening to my complaints throughout the project!

This project was funded by the Glover's Ledge Research and Education Grant.

ABSTRACT

Vernal pools are important breeding grounds for forest amphibians and vital habitat for many populations of species. With the goal of better managing Glover's Ledge (GL) for its amphibian communities, the objectives of this study were to assess the current hydrologic profile of the GL vernal pools over the duration of the breeding season, identify richness and distribution of amphibian species utilizing vernal pools, and provide baseline amphibian data for future monitoring and management at GL. Egg masses of *Lithobates sylvaticus* (wood frogs) and *Ambystoma maculatum* (Spotted salamanders) in three pools on the site (SWP, LL, and SW) were monitored weekly over 20 weeks from March through August of 2020. Hydrological data on the trends of pool depth, extent, temperature, and pH were also sampled. All pools contained egg masses for 7 weeks before larvae hatched (except for SW, which dried up prior to larval emergence). The LL pool supported the greatest number of *A. maculatum* egg masses with a maximum number of 63 egg masses counted. This study is only a single-year snapshot of the GL vernal pool system, so it is too early to draw conclusions about population health or trends from these data alone. However, these baseline data may prove important in beginning to understand the GL amphibian community and reveal areas where we can focus our efforts to improve future studies and management efforts.

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INTRODUCTION

Amphibians are a critical trophic connection in terrestrial and aquatic ecosystems and rely on specific habitats for breeding in the Northeast. Frogs and salamanders provide a conduit between invertebrate sources of energy and vertebrate consumers and consume a wide diversity of prey, from vegetation and detritus to invertebrates (Stebbins & Cohen, 1995; Semlitsch et al. 2014; Walker et al. 2018). Amphibian breeding in New England is a phenological event, occurring every spring in very specific conditions and locations. For example, many New England mole salamander species and frog species rely on unique, ephemeral systems to lay their eggs — vernal pools. Vernal pools are temporary bodies of water, forming wetlands in spring and drying up during summer or autumn. Most of their water comes from precipitation and snowmelt but groundwater, subsurface flow, and natural springs may also contribute to seasonal filling (Colburn, 2004; Calhoun et al. 2014). Several species of adult amphibians migrate to the pools during brief spring windows to mate and lay eggs before retreating back to the uplands.

Amphibians have become a visible and much-adored sign of spring in the Northeastern United States. Many people are starting to recognize their importance in forest ecosystems and the need to document vernal pools to protect populations (Colburn, 2004; Colbert et al. 2011). Vernal pool surveys have become increasingly popular in New England states in the past 15 years, with some states and nature centers even implementing very popular citizen science and school programs based around amphibians (Tappan & Marchand, 1997; Jansujwicz et al. 2013; B.A. Thelen, Science Director, Harris Center for Conservation Education, personal communication). While many new pools have been documented throughout the region, not all are studied, monitored, or understood to the same degree due to limitations in personnel, funding, or access. Despite the plethora of student work on other taxa and communities at the Glover's

Ledge (GL) property in Langdon, NH (Littleton & Frauenhofer, 2014; Kinsella, 2016; Ferrario, 2018), the GL vernal pool communities have not been the subject of any research since a 2014 natural resources inventory (Littleton & Frauenhofer, 2014). The amphibian population at GL is understudied and the species' population trends and habitat use are poorly understood.

Study goals and objectives

In an effort to better understand the amphibian community of the GL property and with the goal of better managing the property for its amphibian species, the objectives of this study are:

1. Assess the current hydrologic profile (depth, temperature, pH, extent) of the GL vernal pools over the duration of the 2020 breeding season.
2. Identify the distribution and richness of amphibian species using the vernal pools at GL.
3. Monitor amphibian egg masses and juvenile development over the course of the 2020 breeding season.
4. Provide baseline amphibian data for future monitoring and management at GL.

LITERATURE REVIEW

Amphibia under threat

Class Amphibia encompasses over 4,500 defined species of salamanders, frogs, and caecilians (Stebbins & Cohen, 1995). Characterized by glandular skin, metamorphic maturation, and amphibious breathing abilities, frogs and salamanders are an integral part of New England forest ecosystems. Amphibians serve as the primary vertebrate predator of invertebrates in freshwater and moist upland ecosystems and exert predatory control over fungal and insect communities in the soil (Stebbins & Cohen, 1995; Semlitsch et al. 2014; Walker et al. 2018). For example, Walker et al. (2018) observed that *Plethodon cinereus* (red-backed salamander) predation on insects created a strong top-down control on the functional diversity of soil fungal communities — and therefore an indirect impact on soil nutrient cycling and storage. Amphibians are also major food sources for birds, mammals, and fish, providing a direct trophic link between invertebrate soil communities and above-ground biomass (Stebbins & Cohen, 1995; Welsh & Droege, 2001). Because of this important linkage, amphibian populations are a key part of many aquatic and terrestrial ecosystems.

Having permeable skin and a diverse set of habitat requirements subjects amphibian populations to a plethora of threats. Deforestation and development pose threats to upland habitat for salamanders and frogs. Where vernal pools are present, degradation of the forest is of particular concern, since estimates suggest a 500m buffer of healthy forest surrounding breeding pools is necessary for their conservation (Scott et al. 2013). Vernal pools may also be filled in or impacted by development activities, altering their hydrologic regime and changing their biological community (Colburn, 2004).

Connectivity between vernal pools and upland forest habitats is an important consideration for amphibian conservation. Since salamanders and frogs require a mosaic of wetlands and uplands for their life cycles, fragmentation of these habitats can cause local population declines. Clustered vernal pools are more diverse and have greater relative abundance of each species than isolated pools (Van Dyke et al. 2017). Roads also fragment and separate habitats, creating new obstacles for moving amphibians. Road crossing is a significant factor in localized amphibian mortality, with 17% of salamander species in California ranked at high risk of individual mortality when roads are near their habitat (Brehme et al. 2018). Where roads cross salamander paths to breeding pools, even moderate road mortality can be a significant mortality factor (Gibbs & Shriver, 2005). Gibbs & Shriver's (2005) study on road mortality in Massachusetts found that annual road mortality risk of 10% or higher could lead to local amphibian population extirpation unless preventative measures such as culverts, tunnels, and road closures were strategically employed. Citizen science efforts such as Big Nights, Bucket Brigades, and Salamander Crossing Guards are another effective way to reduce local road mortality of migrating amphibians (Sterrett et al. 2019; B.A. Thelen, Science Director, Harris Center for Conservation Education, personal communication, unpublished data).

Amphibians are also threatened by chemical changes to their environments. Because of their amphibious life cycle, absorptive skin, and metamorphic periods, frogs and salamanders are susceptible to dangerous impacts from pesticides and accumulated toxins from agricultural and road runoff (Stebbins & Cohen, 1995; Turtle, 2000). Acidic water conditions (low pH) resulting from increased pollution are also a risk factor for breeding amphibians. Embryonic development and larval growth are negatively impacted by low pH levels in breeding pools (Turtle, 2000; Barth & Wilson, 2010).

Fungal infection, particularly the chytrid *Batrachochytrium dendrobatidis* (Bd) fungus is another global threat to amphibians, credited as a driving force in the world-wide decline of amphibians (Wake & Vredenburg, 2008). While pool-breeding amphibians in the northeastern United States have been documented as being asymptomatic to Bd, high infection rates exist among New England frog populations, particularly in bull frogs (*Lithobates catesbeianus*) and green frogs (*L. clamitans*) (Longcore et al. 2007; Richards-Hrdlicka et al. 2013), both of which can coexist alongside vernal pool obligate species in the Northeast. While Bd has not been documented as a cause of widespread mortality in New England, the high infection rate and corresponding lack of knowledge may mean losses have gone undetected, or that northeastern populations have some advantage over the fungus (Longcore et al. 2007). Alongside all these risks, climate change is expected to exacerbate declines in local populations, mainly with regard to changes in winter conditions, exacerbation of Bd infections and altering the availability of water during the breeding season (Miller et al. 2018).

In the Northeast, amphibians are particularly vulnerable during their breeding cycle (March-April), when they are traveling across multiple habitats and over roads to vernal pools to mate and lay eggs. These critical few weeks between egg laying and hatching are an advantageous time to assess the annual population size of amphibians in a given habitat and make predictions about juvenile recruitment to the local population (Egan & Paton, 2004; Baldwin et al. 2006).

<u>Vernal pool protection for amphibians</u>

Amphibians have historically been underrepresented in forest biomass surveys. Burton & Likens' (1975) study of terrestrial amphibian biomass in the Hubbard Brook Experimental Forest revealed 2,950 red-backed salamanders (*P. cinereus*) per ha – representing more than double the biomass of all resident birds at the height of migration. In a particularly telling study, *P. cinereus* occurred in average densities of 3m^{-2} in a forest survey in Virginia — accounting for more biomass than all the birds and mammals estimated to utilize the same habitat, combined (Mathis, 1991). Even today, amphibians as a whole may be underestimated in forest surveys. In Missouri, Semlitsch et al. (2014) reported estimates of 7,300-12,900 *P. cinereus* ha^{-1}, an estimate 2-4 times larger than they had initially expected. Semlitsch et al. (2014) speculated from their biomass survey results that the role of salamanders and other amphibians in terrestrial carbon retention, invertebrate control, and biomass concentration may be vastly underestimated. Vernal pool breeding species have not been studied as well in this regard but when they have been, isolated wetlands have generated a large magnitude of mobile biomass in the form of juvenile amphibians (Gibbons et al, 2006). Because of this, the study and understanding of vernal pool systems is a vital field of research for the health of forest ecosystems and as an estimate of amphibian species diversity, distribution, and abundance.

Over the last 20 years, the role of vernal pools in forest ecosystems became an avenue of study and a new conservation concern to forest managers. Three states in New England (Massachusetts, New Hampshire, and Connecticut) have been regulating the filling of isolated depressions that contain surface water for at least 2 continuous months in the spring or summer for over 20 years (Tappan & Marchand, 1997) and most New England states' water quality policies have language regulating development and activities around vernal pools (Colburn, 2004). When protecting wetlands for conservation, wetland size is typically considered the most important factor, the presumed theory being that a larger wetland area will act as an umbrella for protecting many species. Hydroperiod (annual period of water inundation) of the wetlands in question is considered far less often, even though many studies have shown hydroperiod to be more important to amphibian species richness than wetland size (Snodgrass et al. 2000; Paton & Crouch, 2002; Babbitt, 2005; Baldwin et al. 2006; Tournier et al. 2017).

Ephemeral pools with longer hydroperiods are correlated with both increased amphibian species richness (Snodgrass et al. 2000) and larger population sizes of obligate species (Paton & Crouch, 2002; Baldwin et al. 2006). For example, in both the Southeast (Pechmann et al. 1989; Snodgrass et al. 2000) and the Northeast (Baldwin et al. 2006), pools with hydroperiods greater than 130 days had significantly higher numbers of individuals and diversity of amphibian species. Having a longer hydroperiod also means larvae have a longer window of time to mature before migrating from vernal pools. In some cases, proper hydroperiod is essential to reproductive success. For instance, Semlitsch et al. (1996) monitored a pond in South Carolina over a 16-year period to observe the structure of the amphibian community. The observed pool was inundated an average of 170 days annually. Years with shorter than average hydroperiods (< 100 days inundation) resulted in total reproductive failure for the local amphibians, whereas years with longer hydroperiods (> 200 days inundation) tended to have the greatest diversity and productivity. Hydroperiod timing can also affect the length of the larval period of some species, with larvae shortening their maturation time to migrate prior to drying (Semlitsch & Wilbur, 1988).

Breeding success is also tied to mean pool temperature and pH variation. Vernal pool temperatures can fluctuate dramatically over the course of the season and within the pool itself. Seasonal variation can range from 8-30℃ from April to late summer, and a 10℃ difference between surface and benthic temperatures is common (Colburn, 2004). Amphibian embryo and juvenile development is dependent on temperature, with faster growth and higher survival rates occurring at higher temperatures (Stebbins & Cohen, 1995; Davis et al. 2018). However, if temperatures are consistently too high, amphibian growth is reduced. Mean water temperature and egg mass density follow a quadratic relationship, with the greatest densities occurring around 15°C (Davis et al. 2018). For Spotted salamanders (*Ambystoma maculatum*), average water temperature in vernal pools influences the duration of egg mass incubation, thereby affecting breeding success (Brodman, 1995). Levels of pH of New England vernal pools can vary across habitats depending on surrounding forests and soil type, but tend toward slightly acidic conditions (Colburn, 2004). Permanent and semi-permanent pools have more stable pH measurements across seasons (Freda & Dunson, 1985b). Very low pH levels (3-5) during the breeding season can stunt embryonic development and larval growth of amphibians (Barth & Wilson, 2010). While hydroperiod has been shown to have a significant impact on amphibian abundance and distribution (Pechmann et al. 1989, Snodgrass et al. 2000, Babbitt et al. 2003), pH and temperature could have compounding impacts on species abundance within a pond when considered alongside wetland hydrology.

Given the consistent results seen, it is no surprise that wetland researchers are advocating for a change in how wetland regulation is conducted. Snodgrass et al. (2000) suggested that hydroperiod be included as a primary regulation criterion to help create a landscape approach to management that considers the small-scale details of each pool and how they impact local amphibians. Baldwin et al. (2006) agree, suggesting that particular emphasis be placed on pools at the longer end of the hydroperiod gradient. In some cases, conservation efforts and laws on the municipal level, where intimate knowledge of local conditions exists and landowner input can be considered, may prove more effective for vernal pool protection (Colbert et al. 2011). Colbert et al. (2011) provide an interesting case study of vernal pool conservation in Maine, bringing developers and private landowners together for a more inclusive attempt at vernal pool conservation. They combined stakeholder interviews with biological surveys to determine incentives and a best course of action for amphibian conservation on the property in question. Within this case study, developer willingness to incorporate wildlife habitat on the property and landowner understanding of and desire for nature made them open to the idea of creating a conservation subdivision where the land was managed for amphibians using available biological data. Given the prevalence of sprawl in New England and the importance of minimizing divisions between amphibian habitats, Colbert et al.'s (2011) case study could prove to be a valuable tool for landowner-conscious conservation.

Amphibian surveys in New England

Studies conducted in different areas of New England have generally agreed with the larger herpetology community about the importance of hydroperiod to amphibian species richness, species diversity, and reproductive success in vernal pools (Babbitt, 2005; Skidds & Golet, 2005; Tarr et al. 2005; Brooks & Colburn, 2012). Typical hydroperiod of New England ephemeral ponds ranges from 2-44 weeks each year depending on canopy cover, basin depth, and specific conductance of surface water (Skidds & Golet, 2005). In Rhode Island, Paton & Crouch (2002) observed that four to nine months was the optimal hydroperiod for amphibian reproductive success, with the optimal length per pool depending on the species using the pool to breed. Similarly, the hydroperiod of 103 separate wetlands in southern New Hampshire had a significant effect on both species richness and occurrence patterns of individual species (Babbitt, 2005). Richness was higher in wetlands with intermediate (greater than 4 months inundated) and long (permanently inundated) hydroperiods, so long as fish were absent from the wetlands. Babbitt (2005) also observed that wetland size had species-specific impacts on obligate species, but the relationships were not strong in one general direction. For every species surveyed by Babbitt (2005), additional studies showed that hydroperiod consistently had a stronger influence on richness and occurrence than wetland size (Tarr et al. 2005; Brooks & Colburn, 2012).

Babbitt's (2005) study provides a good overview of pool dynamics in southern New Hampshire, encompassing a variety of pools across diverse habitat types. However, many pools remain yet undocumented and as a result, are under-studied for similar environmental and ecological factors. Different environmental and human factors in the immediate area around a pool can impact the trends encountered and the species present. Little can be done for conservation of upland forest vernal pools if unique pool conditions and species use patterns are unknown.

The Glover's Ledge Vernal Pool System

The GL property in Langdon, NH, owned and managed by Antioch University New England, is the focus of many student class projects and theses (Hansen et al. 2015). Four vernal pools were confirmed present on the property during two separate studies. Obligate vernal pool species spotted salamanders (*A. maculatum)*, Jefferson salamander complex (possible *A. jeffersonianum/A. laterale* hybrids) and wood frogs (*Lithobates sylvaticus*) have been documented in and around the pools through egg mass/larvae presence surveys (Littleton & Frauenhofer, 2014) and with audio recordings (A. Boraski, MS student, AUNE, personal communication). Since Littleton & Frauenhofer's (2014) work, four vernal pools and one possible vernal pool were confirmed on the property by an Antioch student during a wetland delineation and assessment conducted in 2016 (Kinsella, 2016).

Much remains unknown about the vernal pool community at GL. Typical hydroperiod and seasonal variance in pH, temperature, depth, and extent of the pools are currently not known

(P. Palmiotto, Core Faculty, ES, AUNE, personal communication) and the pools have never been officially classified according to NH protocols (Tappen & Marchand, 1997). Species occupation and use of the pools has been documented in short snapshots, but not over the length of the breeding season, between seasons or over other extended periods of time. Littleton & Frauenhofer (2014) recommend that the pools present on the property be inventoried further to understand obligate amphibian species' relative abundance and how it is impacted by changing environmental parameters (water depth, pool size, pH, dissolved oxygen, turbidity, and temperature) throughout a typical vernal pool phenological cycle. With a better understanding of the amphibian community on the property, managers could improve amphibian habitat, document abundance and richness trends over time, and better understand the connectivity of the different habitats present.

METHODS

Species Profiles

Mole salamanders (*Ambystomatidae*) and wood frogs (*L. sylvaticus*) are categorized as obligate vernal pool species since they require pools without predatory fish to maintain their populations (Hopey & Petranka, 1994). *L. sylvaticus* are often the first species to immigrate to pools in New England, arriving between late February and mid-April depending on the weather (Paton & Crouch, 2002; Colburn, 2004) and marking their arrival with a deafening duck-like chorus. They range from 3.7-7cm in length, with a tan to dark brown body and a distinct dark eye mask (Powell et al. 2016) (Figure 1a). Females deposit up to 3,000 eggs in a single thick, gelatinous mass close to the shallows of the pool. A single adult female typically deposits one egg mass per year (Crouch & Paton, 2000). Juveniles typically leave the pool in early summer depending on the hydroperiod of their natal pool (Baldwin et al. 2006; Tournier et al 2017).

A. maculatum arrive slightly after *L. sylvaticus,* the two often jockeying for position within the same pools when their timing overlaps. These salamanders can reach lengths of up to 20cm and are black or grey with up to 50 bright yellow spots arranged into unique patterns across their backs (Tappan & Marchand, 1997; Powell et al. 2016) (Figure 1b). After the females deposit their eggs (averaging around 100 per mass per female) on submerged twigs and vegetation, they return to the uplands. Each female deposits 2-4 egg masses every 1-3 years (Petranka, 1998). Juveniles are benthic feeders and remain in the pool until late summer or early fall, when they metamorphose and disperse from their pools back to the uplands (Colburn, 2004).

The Jefferson complex of salamanders refers to a subset of salamander hybrids and mixed-genotype individuals that are able to reproduce with each other. In the Northeast, members of this group are typically unisexual individuals with some mixture of Jefferson salamander (*A. jeffersonianum*) and Blue-spotted salamander (*A. laterale*) genetic information (Bogart et al. 2007). Most individuals are unisexual females who mate with a host of either species to stimulate egg fertilization and produce genetically identical offspring (Charney et al. 2014). Members of this complex can be difficult to distinguish as offspring may look similar to either parent or display intermediate characteristics, and unisexuals may even contain some DNA from their host father species (Tappan & Marchand, 1997; Colburn, 2004; Bogart et al. 2007). Jefferson complex salamanders vary greatly but range between 10-18cm in length and are dark brown to gray with some displaying flecks of white or pale blue along their body (Powell et al. 2016) (Figure 1c). They typically breed a few days or weeks prior to *A. maculatum* and concentrate their breeding to a length of only a few days depending on weather. Females deposit highly variable amounts of egg masses — depending on the hybridization — typically ranging from 6-30 eggs per mass (Tappan & Marchand, 1997).

Figure 1a: Wood frog (*Lithobates sylvaticus*), a common obligate vernal pool amphibian in New England. Peter Paplanus, Creative Commons 2.0, Wikimedia commons.

Figure 1b: Spotted salamander (*Ambystoma maculatum*), a common obligate vernal pool amphibian in New England. Tom Tyning, Public domain, Wikimedia Commons.

Figure 1c: Jefferson salamander (*Ambystoma jeffersonianum*), a common obligate vernal pool amphibian in New England. Albert Herring, Creative Commons 2.0, Wikimedia commons.

Site Description

GL is a 0.4km^2 parcel situated northwest of NH Route 123 and southwest of NH Route 12A in the town of Langdon, NH (Figure 2). Average annual temperatures range from 1 – 15^{o}C and average annual rainfall is 118cm (US Climate Data, 2020). Since 2014, the property has been owned and managed by Antioch University New England as an outdoor classroom and living laboratory for conservation. The main forest type of the area is hemlock, beech, oak, and pine (Sperduto and Kimball, 2011) ranging in age-class from early to late successional (AUNE, 2019). The property also contains outcroppings of bedrock, streams, a pond, a hemlock-cinnamon fern swamp, three defined vernal pools, and two probable vernal pools (Figure 2). Through multiple studies, students and professionals have documented over 400 different species within the borders of GL including breeding evidence of three obligate amphibian vernal pool species (Littleton & Frauenhofer, 2014). Exact hydroperiod of the local pools is not currently known but other studies in New England have found pools in similar habitats to range 2-44 weeks each year (Skidds & Golet, 2005; Tarr et al. 2005) depending on canopy cover, basin depth, and specific conductance of surface water (Skidds & Golet, 2005).

This study encompasses three vernal pools: two (Lookout Lane and Swamp) are located near the center of the property in a swampy depression and the third (Stone Wall) abuts the northern boundary of the property. Lookout Lane (LL) and Swamp (SWP) occupy the same wetland basin and are separated only by a marshy swamp area. There is a seasonal stream that can provide a hydrologic connection between the two during wet years. Both are surrounded by hemlock (*Tsuga canadensis*), cinnamon fern (*Osmundastrum cinnamomeum*) and beech (*Fagus grandifolia*) forest. SWP is suspected to be a permanent pool as it feeds a small stream and has been previously seen filled late into the summer months (personal observation). LL may be permanent or semi-permanent, as it occupies the same wetland but sits uphill of SWP. LL contains much downed and submerged woody debris, an important component for obligate species as they rely on such material as attachment sites for their eggs. Stone Wall (SW) is much smaller than the other pools and has been reported to be a temporary pool (P. Palmiotto, Core Faculty, ES, AUNE, personal communication). An old stone wall forms the northern edge of this pool and the pool feeds a small seasonal stream. The 2014 NRI of the property confirmed that each of these pools contained one or more of the three obligate amphibian species on site: *L. sylvaticus*, *A. maculatum*, and Jefferson complex salamanders (Littleton & Frauenhofer, 2014).

Experimental Design

I sampled amphibian breeding activity and pool conditions once weekly for 20 weeks in the 2020 breeding season (March 15-July 27, 2020). On March 15th, 2020 I inserted hydrologic depth stations in each of the three pools to monitor water depth (cm) throughout the season. These stations provided consistent locations for depth readings (cm) each week and served as relative markers for collection of pH and temperature data throughout the season.

I collected water and air temperature (degrees C) and water pH readings weekly from the shore and 1m off-shore of each pool to limit foot traffic inside the pool. I measured the weekly perimeter of each pool using GPS tracks around the edges of each pool to track changes in pool shape and extent throughout the growing season. Pool edges were defined as areas connected to open water without barriers of vegetation or large stretches of exposed mud that would prevent amphibian larvae movement (i.e., swampy areas that lacked open water were not counted as part of the ‘pool’). Weekly rainfall amounts (cm) and air temperature (degrees C) data for the site were gathered using a Rainwise unit (RainLog, RainWise Inc, Bar Harbor, ME) and a Kestrel unit (Drop2, NK, Boothwyn, PA) placed on an open-canopy tract near the pools.

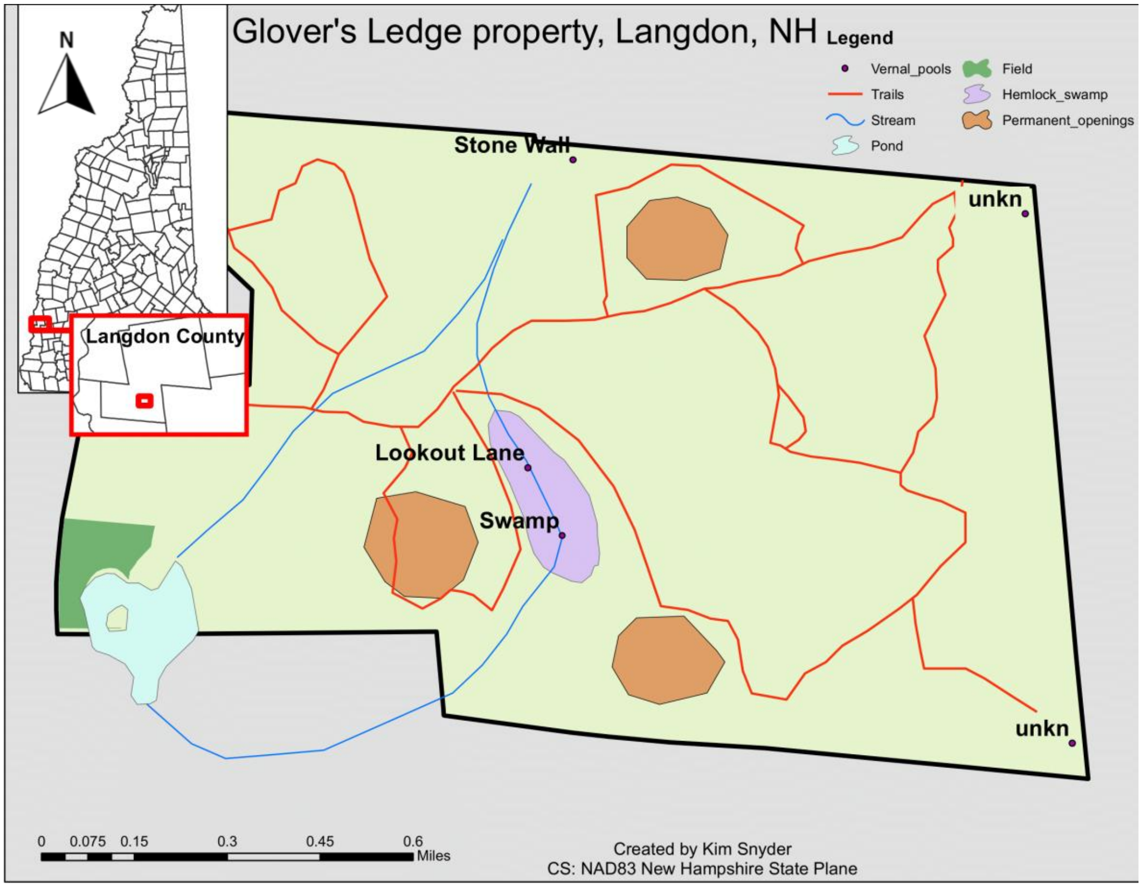

Figure 2: Map of Glover's Ledge property with main features marked, Landon, NH. "Unkn" pools are locations where vernal pools are probable but have not been confirmed. Map created by Kim Snyder.

Spatial analysis of pool extent was calculated using ArcGIS 10.8 software (Environmental Systems Research Institute, Redland, CA). GPS waypoints from each pool were connected and turned into shapefiles that provide an aerial extent of each pool each week. Each perimeter polygon was overlayed to create a time-lapse visual of pool perimeter changes across the season.

I characterized amphibian breeding data through weekly auditory surveys and weekly visual egg mass surveys during the breeding season (March 15-May 3, 2020). When used in conjunction with density studies such as egg mass counts, auditory surveys can provide frog density estimates of the immediate habitat around target areas (Heyer, 1994). AudioMoth 1.0 recorder units (Open Acoustic Devices, Southampton, UK) were placed near the pools to record at 2-minute intervals every 15 minutes from 8:30pm to midnight EST as recommended by A. Boraski (personal communication, 2020). One unit was placed at SW 5m from the north end of the pool. Another unit was placed in the swamp between SWP and LL roughly 20m from the edge of each pool. This second placement was due to the lack of a third unit available for use. Intensity of calling activity was categorized from the recordings using the 0-3 scale from FrogWatch protocols (AZA, 2020), where 0 is no calling and 3 is a continuous chorus of overlapping calls. The scores from each weeks' recordings were averaged to determine an average weekly metric of calling intensity and create a timeline of frog activity at each pool.

Each week from mid-April to June, I recorded the number of egg masses of each species visible in the pools and marked the location of each individual mass within each pool on a pool sketch. The previous week's sketch was consulted after the next week's count to verify if any egg masses were missed or new ones spotted. Visual sighting and identification from land was necessary to ensure minimal disturbance to the substrate, developing eggs, and any hatched larvae (Heyer, 1994; Tappan & Marchand, 1997). Surveys of egg masses continued from the first visual encounter of breeding adults or egg masses (April 12) until two consecutive weeks of no visual encounters of egg masses (June 14). No egg masses were encountered prior to April 12th or after June 14th, 2020. When possible, any egg mass that was close enough to reach from the shore was handled once while submerged to aid identification while limiting disturbance to the eggs (See *Appendix B – Egg Mass Handling Guide*).

As egg masses began to hatch, I used weekly dip net surveys of larvae in place of egg mass surveys (June 14 - July 27). Every week, each pool was sampled from the shore along 3 new randomly-placed 1m line transects to limit substrate disturbance (Heyer, 1994). Transects were parallel to shore and the 1mm mesh net was dragged once along the pool bottom. All substrate and other materials collected were transferred to a small bucket for processing. Any individuals caught were observed in a vial of vernal pool water briefly to confirm taxaidentification and count and then gently released. I compared the frequency of each species encountered across the pools to estimate abundance (individuals m^{-2}) of each species per pool. Dip net surveys continued until the pools dried up in late July.

Data Analysis

I used general linear models to assess the effects of vernal pool dimensions and water depth, pH, and temperature on weekly egg mass counts per each pool separately. A forward selection approach was used to compare models and determine which variables or combination of variables has the most influence on the response variables of interest. I also used a Poisson regression to determine when and how many *A. maculatum* egg masses were likely to be encountered in each pool. All statistical analyses were conducted with R software (R Core Development Team, 2017), and statistical significance was determined at alpha = 0.05 unless otherwise noted.

RESULTS

Weather

New Hampshire had a dry year in 2020, with total rainfall from Jan-Sept totaling only 152.5cm (US Climate Data, 2020). May and September were particularly dry months with only 12.6cm and 2.52cm of rainfall, respectively, as recorded by the nearby North Walpole, NH weather station. July saw the maximum temperature of 36 ^{o}C and February recorded the minimum temperature of -25 ^{o}C. Average temperature from Jan-Sept was 13.5 ^{o}C, within the range of a typical year in this region (12-15^{o}C).

Audio analysis

Audio recordings from the pools revealed no *L. sylvaticus* activity. In-person dusk surveys conducted on May 2nd to supplement recordings revealed a high number of spring peepers (*Pseudacris crucifer*) utilizing the pools and only one or two individual *L. sylvaticus* utilizing the LL pool. This limited use by *L. sylvaticus* was further evidenced by the low number of egg masses encountered overall. The May survey also noted a multitude of *L. sylvaticus* calling from the pond on the southwest end of the property (magnitude of 3 via AZA protocols from 6pm to midnight). Several *L. sylvaticus* adults were individually heard or encountered in and around the LL pool (n = 7) in April and May.

Pool Profiles

Pool monitoring occurred over 20 weeks from March through August of 2020. Of the three pools, only Swamp (SWP) was sampled all twenty weeks, as it was the only pool that did not dry up before then. Stone Wall (SW) dried up after 12 weeks of sampling (early June) and Lookout Lane (LL) after 19 weeks (late July). The SWP pool still contained water during the last week of monitoring and into August, although the perimeter had retreated considerably.

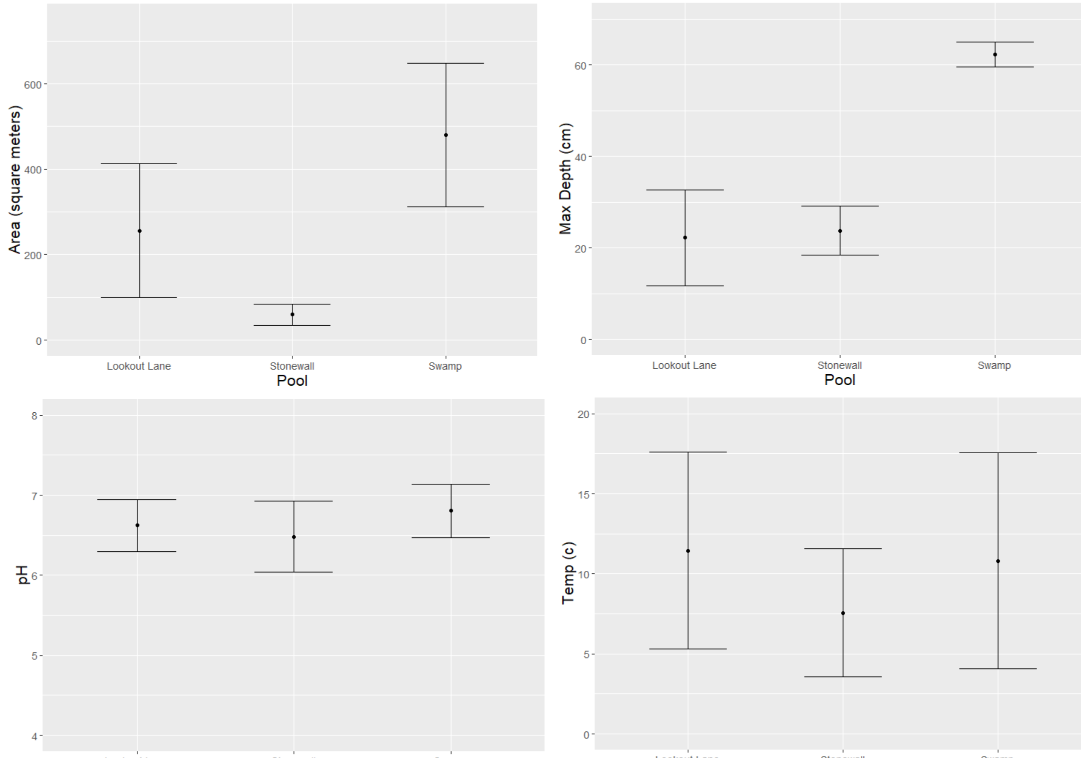

Figure 3 A-D: Profiles of each pool sampled. A) Mean and range of area per pool: SWP mean = 480.5m; LL mean = 256.2m; SW mean = 59.7m.
B) Max depth mean and range per pool: SWP mean = 62.25cm; LL mean = 26.36cm; SW mean = 23.73cm. C) pH mean and range per pool: SWP mean = 6.8; LL mean = 6.6; SW mean = 6.5. D) Temperature mean and range per pool: SWP mean = 10.8 C; LL mean = 11.4 C; SW mean = 7.6 C

Area varied per pool with a sizable range for the larger pools (SWP and LL) and a smaller range for the smaller pool (SW). SWP varied in area from $795.9m^2$ – $261m^2$ (mean = $480.5m^2$). LL was slightly smaller with area ranging over the course of the monitoring from $533m^2$ – $79m^2$ (mean = $256.2m^2$) before drying up. SW was the smallest and had the smallest range from $81.9m^2$ – $6.6m^2$ (mean = $59.7m^2$) before drying up (Figure 3A, Figure 5). Maximum depth for pools ranged from 65.5cm (SWP) to 27.8cm (SW) with mean depths ranging from 62.25cm (SWP) to 23.73cm (SW) (Figure 3B). SWP was markedly deeper than the other pools and was the only one to not dry up by the time monitoring ceased. SWP showed the least variation in depth change (65cm – 57cm). Levels of pH varied least of all the parameters sampled, with all pools ranging between 7.5 and 6.1 (Figure 3C). SWP was the most consistent overall (mean = 6.8). Temperature profiles per pool were very consistent (Figure 3D), with all pools having similar temperatures week to week (Figure 5). SWP was consistently the coldest pool but only by a few tenths of a degree.

Area of all pools fluctuated weekly (Figure 4 and Figure 5) with only SW remaining fairly consistent. Each pool hit a point in the season where area dropped precipitously; both SW and LL dried up 3-4 weeks after this drop. SWP was the only pool not to dry up after this decline but it had a similar precipitous drop in the penultimate week of sampling (Figure 4). In all but the SW pool, this drop followed the appearance of flower buds on trees in the surrounding forest. The August 29th check on the SWP pool revealed enough water for obligate species to occupy it but the pool edges had shrunk considerably.

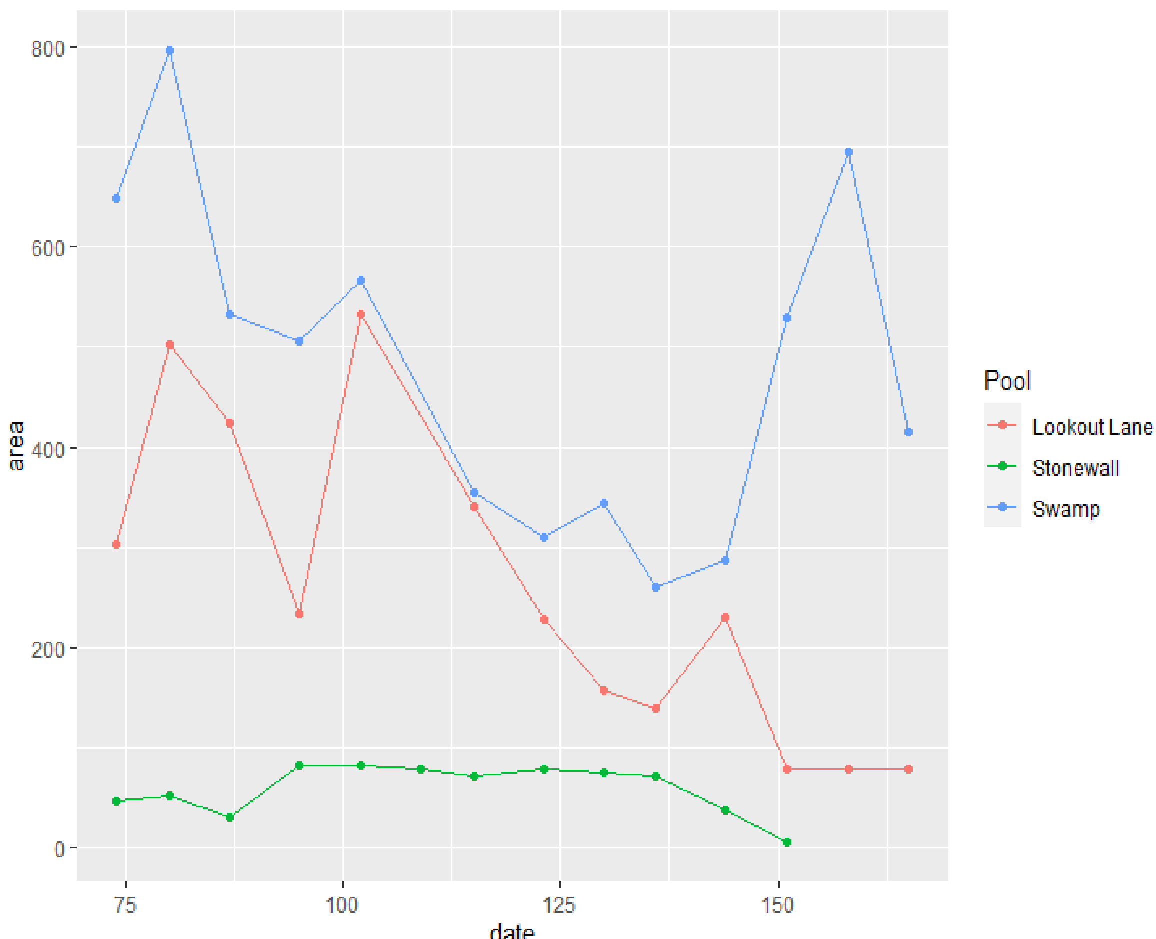

Figure 4: Area (m^2) of pools sampled over the duration of monitoring. Date represents day of the year from January 1st.

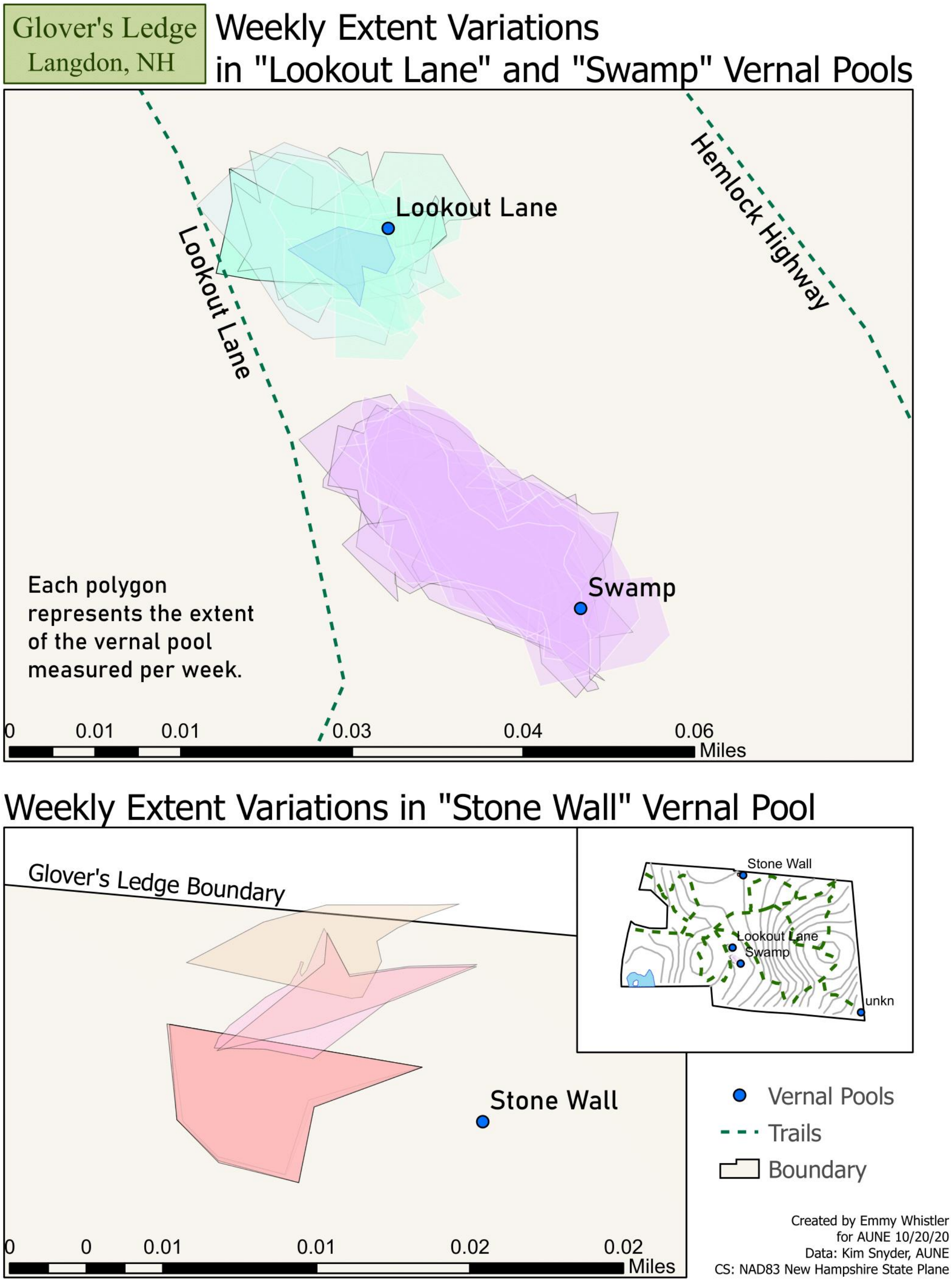

Figure 5: Map of changing pool areas (m^2) over the duration of monitoring. The shifting appearance of the SW pool is due to inaccurate GPS readings in the first few weeks of data collection. The area was too small for the GPS to accurately capture so we switched to stick and tape methods of measurement. Map created by Emmy Whistler using Kim Snyder's data.

Temperature and pH over the duration of monitoring followed similar season-long upward trends with a few weekly dips (Figure 6 and Figure 7).

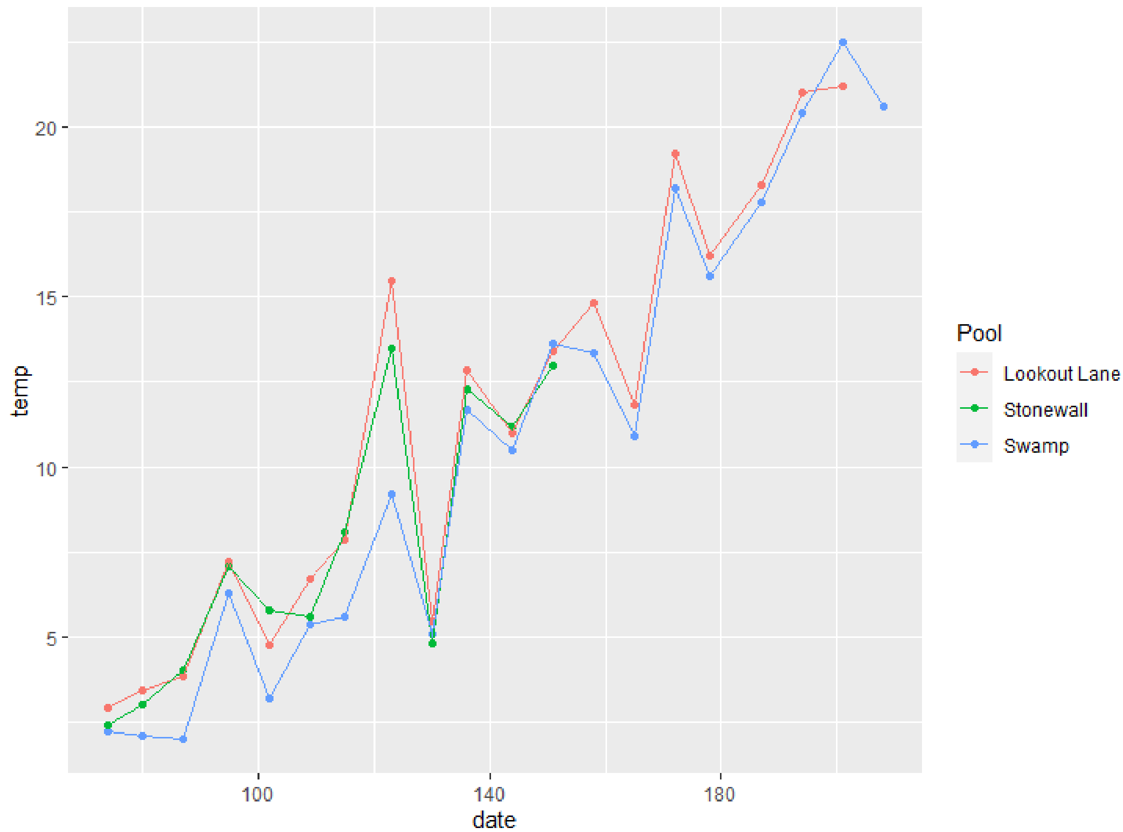

Figure 6: Temperature (C) of pools sampled over the duration of monitoring.
Date represents day of the year from January 1st.

<u>Species abundance and distribution</u>

L. sylvaticus eggs were observed only in one pool (LL) and counts were minimal (max = 9). They were excluded from the analysis due to this low sampling return. LL was also the only pool with Jefferson complex (*A. jeffersonianum/A. laterale* hybrids) salamander eggs visible. A maximum of 4 egg masses were seen on April 19th. They were also excluded from this analysis due to low sampling. Figures 8A and 8B illustrate the range of *A. maculatum* egg mass counts per pool and when egg masses were encountered.

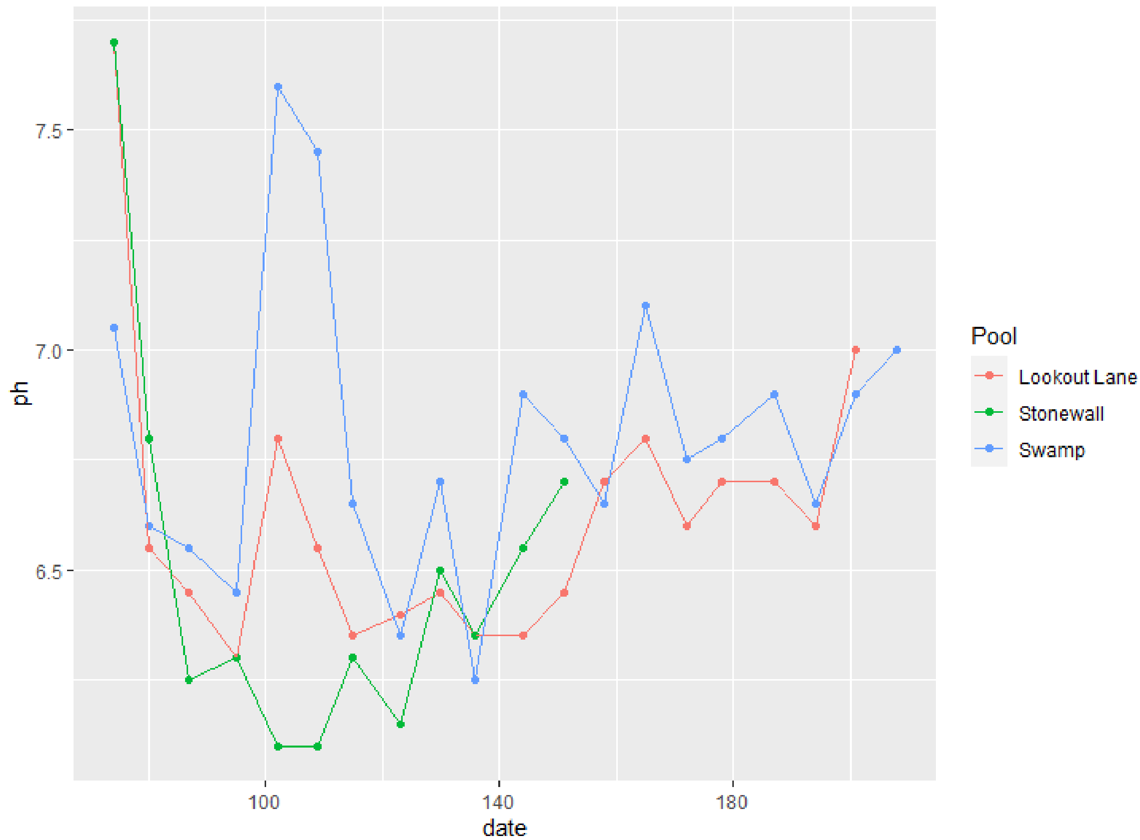

Figure 7: pH of pools sampled over the duration of monitoring. Date represents day of the year from January 1st.

In addition to being the only pool to host *L. sylvaticus* eggs, the LL pool supported the greatest number of *A. maculatum* egg masses with a maximum number of 63 egg masses counted on May 10th, 2020. SWP had a maximum of 46 *A. maculatum* eggs counted on April 25th and SW contained a maximum of 23 *A. maculatum* egg masses on May 16th. All pools contained egg masses for 7 weeks before larvae hatched (except for SW, which dried up prior to larval emergence). Results of the poisson regression model predicted counts of *A. maculatum* egg masses using date for each pool can be seen in Figure 9. Forward selection revealed that the strongest model included pool, date, and area as predictor variables, and abundance decreases significantly with both date and area ($z = 2.897$, $df = 33$, $P = 0.007$). The model showed a range of expected egg masses per day per pool with the median upper limit being 12.4 egg masses and the median lower limit 8.8. A goodness of fit test showed no evidence for lack of fit and the poisson model was significant ($z = 21.491$, $P = 2e-16$).

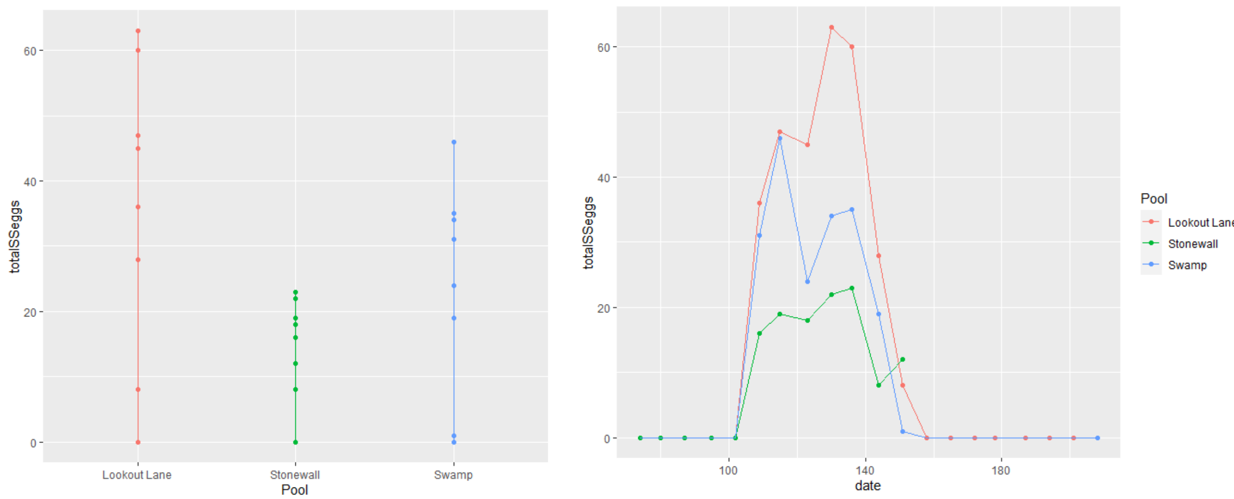

Figure 8 A-B: A) Range of *A. maculatum* egg mass counts per pool across all weeks of sampling. B) Number of *A. maculatum* egg masses encountered in each pool over the course of the survey. Date represents day of the year from January 1st.

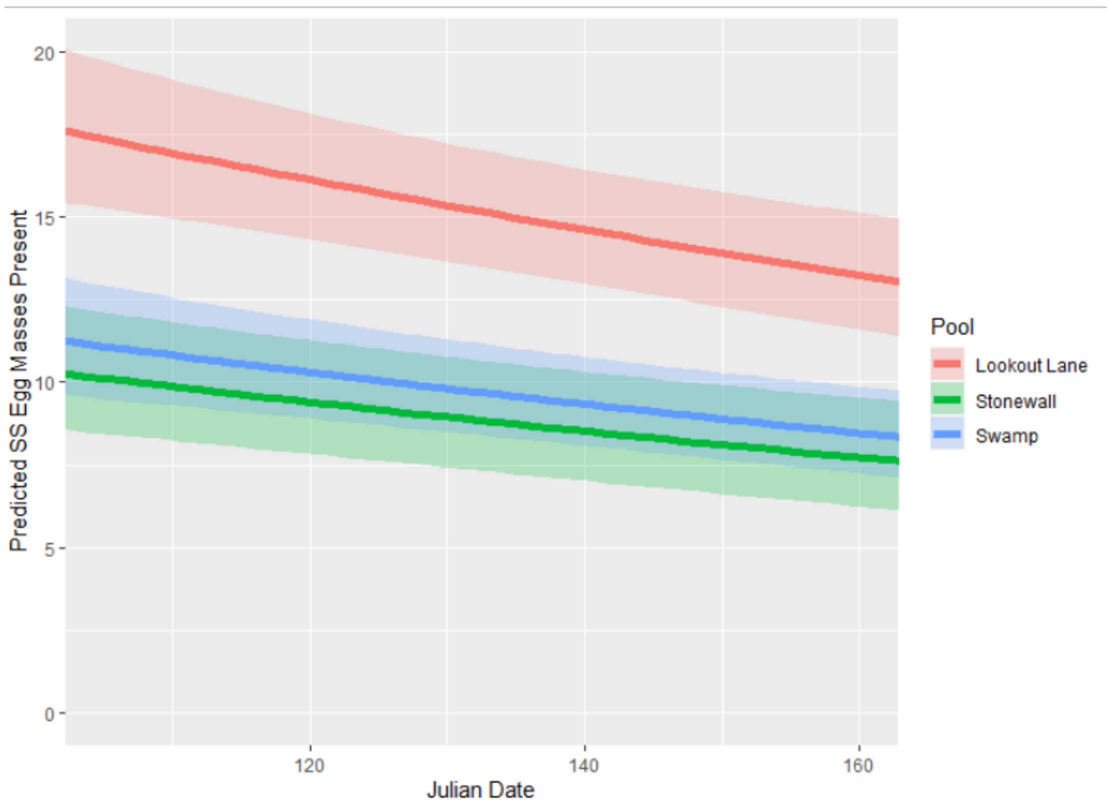

Figure 9: Poisson regression of when *A. maculatum* egg masses are most likely to be encountered for each pool and expected range of how many will be present on that date.

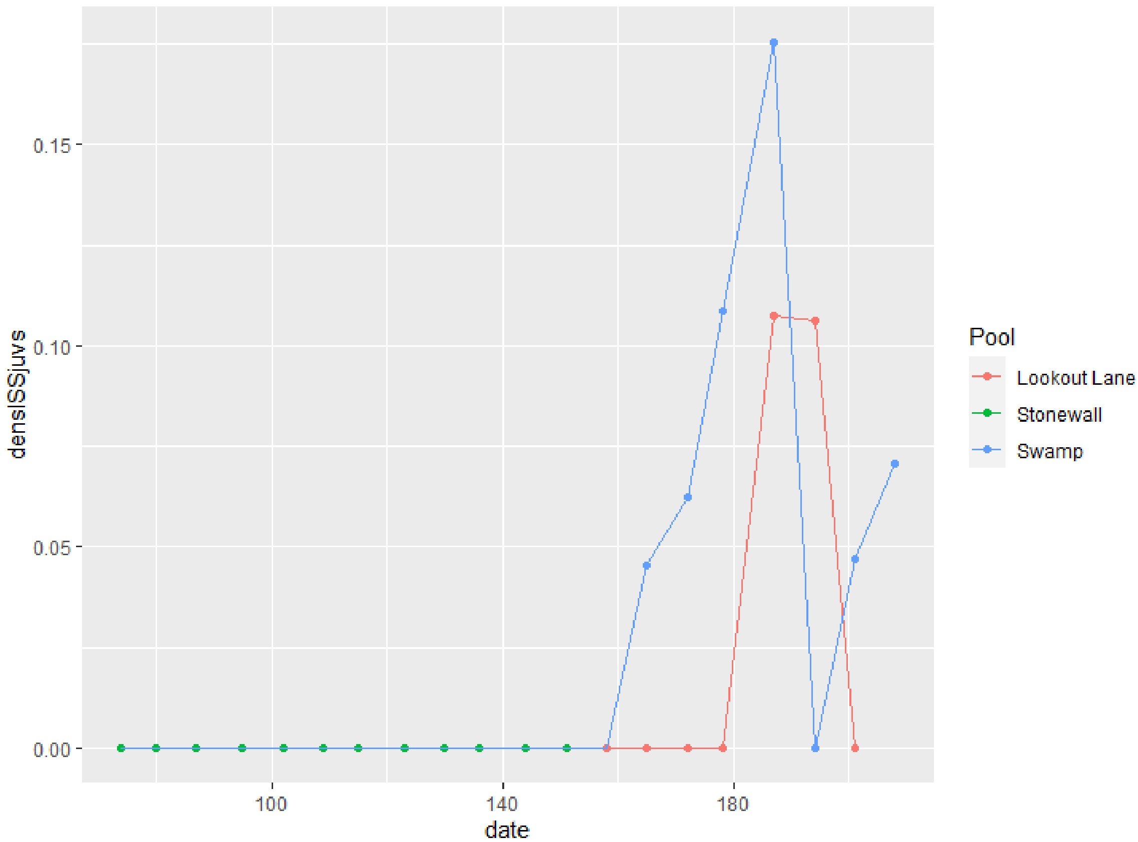

Figure 10: *A. maculatum* juvenile density encountered in each pool by date. SW is excluded because no *A. maculatum* egg masses were able to hatch from that pool before it dried up. Date represents day of the year from January 1st.

Juvenile density of *A. maculatum* peaked at July 13th for LL and July 20th for SWP (Figure 10). The SW pool also contained fairy shrimp (*Anostraca*) for several weeks in March and April — the first documented sighting of these obligate vernal pool invertebrates at Glover's Ledge.

DISCUSSION

This study is only a single-year snapshot of the GL vernal pool system, so it is too early to draw any conclusions about population health or trends from these data alone. However, these baseline data may prove important in beginning to understand the GL amphibian community and reveal areas where we can focus our efforts to improve future studies and management efforts.

<u>Pool Profiles and Implications for Obligate Species</u>

The GL pools displayed typical profiles to other New England vernal pools. The range of pH levels (6.1 - 7.5) were slightly higher than expected for pools located in the Connecticut River Valley (4.75 – 6.82) (Colburn, 2004). The hydroperiod of GL pools was 12-20+ weeks, while typical hydroperiod of New England ponds ranges from 2-44 weeks each year depending on canopy cover, basin depth, and specific conductance of surface water (Skidds & Golet, 2005). Longer hydroperiods are correlated with both increased amphibian species richness (Snodgrass et al. 2000) and larger population sizes of obligate species (Paton & Crouch, 2002; Baldwin et al. 2006).

Across all three pools, the GL ponds could support amphibian larval growth for a period from 12-20+ weeks each year. Every vernal pool on the property, even the one fed by a natural seepage (SWP), reached a point in the year where it was completely dry. For the smallest pool (SW), this occurred in mid-June, before any egg masses could hatch. For the larger pools, drying occurred in late July (LL) and late August (SWP). This was most likely a reflection of the very dry year that New Hampshire experienced in 2020, as well as high summer temperatures.

Pond drying time and speed are important factors in larval amphibian survival. Semlitsch & Wilbur (1988) tested the effect of drying speed on larval survival of the mole salamander *(Ambystoma talpoideum)* and discovered a positive correlation between drying speed and the number of larvae to metamorphose. Pond drying was an important influence on larval survival, as larvae that were able to match their growth process to rising pool temperatures and complete their metamorphosis before drying had better chances of survival in variable ponds. In a later study, Semlitsch et al. (2015) determined that intermediate pond sizes of 100-1000m^2 were most likely to produce larvae, recruit juveniles to adulthood and generally be more diverse than smaller or larger pools. For the GL pools, average pool area of LL (256.2m^2) and SWP (480.5m^2) fell within Semlitsch et al.'s (2015) criteria. Of the two, the smaller pool on average (LL) had higher diversity (3 species) and higher egg mass counts. SW (average area 59.7m^2) was below Semlitsch et al.'s (2015) intermediate size threshold.

Based on their late-May hatching — characterized by the sharp drop in egg mass counts and corresponding increase in larvae density — larvae from the LL and SWP pools would have been ready to leave the pools around late July, if they'd reached their minimum metamorphosis sizes (Colburn, 2004). In New England, most vernal pool obligates require 4-9 continuous months of pool inundation (typically March – August) to allow 95% of metamorphs to successfully leave pools (Paton & Crouch, 2002). Since LL and SWP retained their water until late July, I suspect that some of the faster-growing larvae would have been able to escape the pools before they dried. Larvae density peaked 4-5 weeks after the egg mass counts declined, with larvae displaying limb metamorphosis and gill reduction as weeks progressed. There is no scale for what a 'typical year' looks like for the GL pools but if we take the assumption that this study represented a dry year, LL and SWP probably retain water into August in 'normal' years and perhaps even into September in wet years. Given how early SW dried up, I suspect that juveniles emerge from that pool only in very wet years when it is able to retain water into summer.

Obligate Species Use of Pools

In the Northeast, March and April are the months when vernal pool obligate egg masses are expected to be encountered, but they may be encountered in New Hampshire as late as July or August depending on pool characteristics and seasonal climate variation (Tappan & Marchand, 1997). Larvae of all three target amphibian species are typically encountered anytime between May and September, with metamorphosis occurring between late July and early December (Tappan & Marchand, 1997). In the GL pools, eggs were first encountered in mid-April and larvae last found in late July with some signs of metamorphosis occurring.

The only species that the GL pools supported in significant numbers was *A. maculatum*. While *L. sylvaticus* was observed in large numbers in the large pond on the southwest edge of the property, only 9 egg masses were encountered within the study pools. I deemed these data insufficient for richness and abundance comparisons as only one pool (LL) contained multiple amphibian species.

Baldwin et al. (2006) used reproductive effort as a relative indicator of breeding population size and consequently pool and terrestrial habitat quality. Based on the egg masses I encountered and using the Crouch & Paton (2000) guideline that 1 egg mass represents 1 *L. sylvaticus* female, it appears that the upland population of *L. sylvaticus* at GL is only about 20-30 individuals (assuming there are 1-2 males for every breeding female (Colburn, 2004)). This is consistent with my audio and night surveys, which revealed little auditory evidence of breeding *L. sylvaticus* around the pools. In-person audio surveys in May along with a low sample size of egg masses across pools indicated that the local *L. sylvaticus* population might mainly breed in the pond in the southwestern section of the property rather than in the vernal pools. The frogs had a much louder presence at the pond on the property, so the pond will need to be studied in the future for an accurate assessment of the *L. sylvaticus* population at GL. Why the frogs would prefer to breed in the pond rather than the upland pools is a question for further study. Multiple years of data are necessary to estimate *L. sylvaticus* populations reliably and they must account for all breeding pools, as the frogs may shift pool use year to year (Raithel et al. 2011).

For the upland *A. maculatum* population, the best I can determine is a probable range of 33-66 females based on the maximum number of egg masses spotted (n = 132 from combining all pools) and the Petranka (1998) guideline that each *A. maculatum* female lays 2-4 egg masses every 1-3 years. While these estimates can serve as a baseline, they should not be considered accurate population estimates without further study.

Littleton & Frauenhofer (2014) reported egg masses from Jefferson's complex salamanders in their initial survey, but did not indicate which pool they encountered them in or when they surveyed for them. I recorded some Jefferson's egg masses in the LL pool but saw no evidence of juveniles emerging from that pool.

Anostraca were only encountered in the SW pool in late March and again in late April. These obligate invertebrates had never been previously recorded in Glover's Ledge pools and their presence indicates seasonal wetlands (Colburn, 2004). *Anostraca* typically inhabit and rely on pools with shorter hydroperiods (< 4 months inundation) to support their breeding. These short-hydroperiod pools can also support unique community assemblages, compared to permanent wetlands or pools with longer hydroperiods (Gibbs, 1993).

Management implications

If drought conditions continue at GL and in the Northeast, we may need to assess how to increase pool hydroperiods to support local amphibian populations. The major threats to vernal pool health include physical destruction or filling, loss of their surrounding habitat, hydrologic alterations from changes to the watershed or surrounding landscape, pollution, and isolation of pools from other nearby pools (Stebbins & Cohen, 1995; Tappan & Marchand, 1997; Colburn, 2004; Scott et al, 2013). Thankfully, due to the conservation easement goals of GL, destruction, pollution and isolation are very unlikely to occur. In order to protect proper hydrology for the pools, GL staff should take steps to determine the water sources for each pool and, if necessary, work alongside neighboring landowners to ensure the health of the pools.

Managers at GL often create wildlife openings to maintain a diversity of habitat types and to control for invasive species. There are two such cuts uphill just west and southeast of the SWP and LL pools (Figure 2). For future cuts or maintenance of these openings, staff should monitor the pools to determine if nearby forest management practices impact pool hydrology or species presence in pools.

A. maculatum egg mass counts in Rhode Island were positively associated with the presence of upland forest area within 1 km of the pool (Skidds et al. 2007). A similar study in Massachusetts determined thresholds for obligate species persistence in forested habitat surrounding their breeding pools that may provide a helpful baseline for future cuts (Homan et al. 2004). Such thresholds for *A. maculatum* were ~30% forest cover at a buffer of 100m or less from the pond edge, 41% cover at 500m, and 51% habitat cover at 1000m. Thresholds for the presence of *L. sylvaticus* were 88% habitat cover at 30m from the pond edge, declining to 44% habitat cover within a 1000m buffer (Homan et al. 2004).

In addition, climate change promises to exacerbate current threats to amphibians and alter regional climate patterns (Miller et al. 2018), which could make years like this one with hot, dry summers typical. GL staff should determine how important precipitation is to pool volume and hydroperiod and what, if anything, can be done to sustain pool hydrology.

Recommendations for Future Work

Because this is only a single year of data, it is hard to draw any definite conclusions about the health of the amphibian population at GL or whether this year was representative of a typical year or not. These data could serve as a baseline for future work and be included in longer-term studies of the property. With a better understanding of the amphibian community, managers of GL could improve amphibian habitat, document abundance and richness trends in the pools over time, and better understand the connectivity of the different habitats present. Future studies of the GL vernal pool system should also consider factors such as pool distance from roads, canopy cover, and microtopography for the impact they may have on juvenile or egg mass density.

More research must be conducted on GL amphibians before management plans are created. But this study provides some baselines for where to start. A population estimate should be conducted, utilizing egg mass data over multiple years and live-trapping of adults and migrating juveniles. The hydroperiods of each vernal pool on the property should be monitored over several years and averaged to better understand the habitat and breeding quality of each pool.

Maintenance of a diversity of wetland hydroperiods in a landscape is a good way to protect amphibian biodiversity (Semlitsch, 2000), since amphibian species richness is influenced by wetland hydroperiod more then by wetland size (Snodgrass et al, 2000; Babbitt, 2005). While the pools at GL are at little risk of being filled in or removed, steps should be planned to ensure their continued health and presence. Forestry best management practices from New Hampshire recommend these guidelines for protecting vernal pools from sedimentation and premature drying: limit tree removal and maintain existing understory vegetation within 200ft (61m) of a pool, avoid depositing slash in pool basins, and avoid creating skid trails or surface disturbances such as roads or paths that may impede water flow or amphibian movement in and out of pools. Beyond the 200ft buffer, limit the area logged to what is necessary for wildlife objectives and retain as much existing understory and dead and down woody material as possible (Bennett et al. 2010).

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Appendix A
Data Form for Weekly Pool Surveys

Observation Datasheet

Observer:

Weekday, Date, Year ____ Pool Observed ____

Duration of observations ____

Pool Depth N E S W Hydro station: 1m from edge:	Pool pH N E S W Edge: 1m from edge:	Pool Temperature N E S W Edge: 1m from edge:

Phenological phase of understory (circle all that apply)

Leaf Buds Furled Leaves Full-size Leaves Flower Buds

Ripe Fruits Colored Leaves Falling Leaves

Phenological phase of canopy (circle all that apply)

Leaf Buds Furled Leaves Full-size Leaves Flower Buds

Ripe Fruits Colored Leaves Falling Leaves

Weather Conditions

(Write in temperature during observation and make one selection per weather category)

Air Temperature (Indicate °C or °F):

Wind Speed using Beaufort Wind Scale:

☐ 0 ☐ 1 ☐ 2 ☐ 3
☐ 4 ☐ 5

Precipitation during visit:

☐ None ☐ Fog/Mist ☐ Light Rain/Drizzle ☐ Medium Rain
☐ Hard Rain ☐ Hail ☐ Snow

Precipitation in the past 48 hours:

☐ No Precipitation ☐ Some Precipitation ☐ Much Precipitation

The temperature during the past 48 hours has primarily been:

☐ Above Freezing ☐ Below Freezing

Beaufort Wind Scale

0 Calm: *smoke rises vertically.*

1 Light Air: *rising smoke drifts; weather vane inactive.*

2 Light Breeze: *leaves rustle; can feel wind on face.*

3 Gentle Breeze: *leaves and twigs in constant motion; small flags extend.*

Too windy for monitoring:

4 Moderate Breeze: *moves small branches; raises dust and loose paper.*

5 Fresh Breeze: *small trees in leaf begin to sway.*

Rain Gauge Reading: ___ cm

Enter egg mass data on reverse side!

Pool sketch (mark hydro station and include locations of any encountered egg masses/individuals)

N

Full pool perimeter: ________ m

	Wood Frog	Spotted Salamander	Jeff. Complex	Unknown
# of egg masses				
# of adults sighted				
# of juveniles sighted				

Visit comments:

Appendix B
Text for Interpretive Signage at the Glover's Ledge Vernal Pools

In front of you is a vernal pool.
Look carefully. Please don't step in the pools! Depending on when you are visiting, this may not look very much like a pool. Vernal pools are temporary bodies of water that fill up in the spring from snowmelt and rain and dry up in summer or autumn. If you are reading this in late summer or autumn, you may be looking at a puddle or a patch of mud. But if you are here in the spring, you are probably seeing a small pond.
No matter the season, these pools are incredible places for many of Glover's Ledge's creatures. Try using your senses to explore these vernal pools and their inhabitants!

Touch – Sphagnum moss is often found around vernal pools. Feel the soft moss growing at the pool edges. Stick your fingers in the water and feel how cold it is.

Smell – Take a deep breath. The pools may not smell so nice! This is because all of the dead leaves at the pool bottom are decaying very slowly and releasing some smelly methane gasses. In spring, see if you can smell the evergreen hemlocks nearby. In autumn, see if you can smell the dying leaves.

Hear – In the springtime, frogs gather in the pools to mate and lay eggs. Listen for a chorus of spring peepers as dusk falls and the occasional quack of a wood frog. In all seasons, listen for several different species of birds that live around the pools – ovenbirds, winter wrens, black-throated green warblers and many more in spring and summer, and chickadees and nuthatches into the winter!

See – There is always something to see at a vernal pool! In the spring, look for egg masses of salamanders and frogs. In summer, see if you can spot the salamander larvae and tadpoles swimming around. In the fall, if you are lucky, you may see a juvenile salamander crawl out of the water! Watch for frogs and birds year-round (except in the dead of winter).

Inhabitants of the vernal pool
A vernal pool is a special habitat. Since it is temporary, predators like fish are less likely to be present. Fewer predators make this the perfect place for salamander and frog larvae to grow without fear of being eaten.

Spotted salamanders – These mole salamanders spend most of their lives underground eating invertebrates, but in the spring they leave their underground homes to travel to vernal pools to breed. Larvae have to grow fast, as they need to be ready for life on land before their pools dry up. Larval salamanders eat any kind of insect or worm they can get in their mouths and rely on aquatic insects to feed themselves as they grow.

Wood frogs – Wood frogs primarily breed in small, fish-free pools. On warm spring afternoons and nights, their mating calls can sound like a flock of ducks has landed in the pool. The tadpoles eat algae, insects, and vegetation to grow as fast as they can. They leave the pools in late summer once they grow limbs and lose their tails.

Spring peepers – Peepers are best known by their loud and constant springtime calls. Males fill the spring nights with their chorus, calling to try to find mates. Peepers will breed in any type of pond but take advantage of vernal pools when they can due to the lack of predators.

Caddisflies – These little invertebrates build themselves tiny homes of leaves, sticks, and grit to carry around with them! They feed on the dead leaves in vernal pools. In summer, they will transform into winged adults and leave the pools to mate.

Mayflies – These are good sources of food for growing salamanders and frogs. Like caddisflies, mayflies feed on dead leaves and leave the pools in summer once they complete their transformation into flying adults.

Painted turtles – These common turtles can be seen in the larger vernal pools whenever they have water. They feed on vegetation, invertebrates, crustaceans, and tadpoles.

Bull frogs – These large frogs are common across ponds in the Northeast, where they feed on anything they can get in their mouth, including rodents, small turtles, and tadpoles. They frequent the pools in spring and summer.

Appendix C
Egg Mass Handling Guide

Amphibians are sensitive to diseases that can be spread from pool to pool on contaminated boots and field gear. If you are visiting multiple pools, clean your boots and gear of any mud or vegetation before traveling between pools. At the end of the day, disinfect all gear with a 4% bleach solution to kill viruses and bacteria.

All photos taken by Kim Snyder

Appendix D

High School NGSS curriculum: Activity for Visiting Students

How might climate change affect Glover's Ledge's vernal pools? A one hour, two-part data exploration lesson. *This is an inquiry-based, interactive lesson used to encourage 10-12th graders to collect observations, examine data and answer questions with that data. (Lesson may be repeated more than once if desired)*

Climate change is expected to change our seasons in New England. Summers will become hotter and longer while winters become warmer and shorter. Because of this, forests are expected to get drier over time as less snowfall accumulates and less rain falls. Vernal pools are temporary wetlands in forests that rely on snowmelt and rainfall to keep them filled from the spring to the fall. These pools are used by salamanders and frogs to lay their eggs and are important feeding grounds for the amphibian larvae throughout summer.

What do we want students to understand and be able to do at the end of this lesson?

1. Students will understand the relationship between vernal pool hydroperiod and climate.
2. Students will understand how to create a data set and make factual statements about the data set.
3. Students will be able to consider how their data set can be used to answer questions.
4. Students will be able to generate their own questions that could be explored with the data they collect.

Keywords: climate, weather, amphibian, hydroperiod, average, trend, metamorphosis, ecosystem, habitat

What will students do to develop and demonstrate this understanding?

Students will collect depth or temperature measurements from several points at the edges of the vernal pool and create a data set of their measurements. This might include date of visit, depth from each point, current weather conditions and anything else they observe. Students will create bar graphs of depth at each point they sampled and save their data for future observations.
Students will record rainfall between visits (if any) or gather rainfall data from WeatherUnderground.
Students will hypothesize about how the pools will change upon their follow-up visit.
Students will repeat data collection at a second visit (2-4 weeks after first visit) and use it to create a similar data set. This may be repeated multiple times if desired.
Students will examine their 2+ datasets and generate true statements from their data.
Students will create a graph of depth/temperature at each of their sampling points and compare the weeks they sampled. Using this graph, students will determine if their hypothesis was correct. They will also generate new questions from the data they have or observations they have made.
Students will share their observations, predictions, graphs, and questions with classmates.

Lesson Overview:

1. Goal: Help students understand link between climate and the vernal pool ecosystem.
2. What is a vernal pool? Have students read defining information from books or use the websites below:
    a. Vernal Pools | UNH Extension
    b. What Is A Vernal Pool? - WorldAtlas
3. Visit Glover's Ledge vernal pool and complete the data collection process described above. Repeat this process as many times as desired.

4. Have students generate a list of true statements they can make from these data. Focus on trends they can see, and other observations they made.
5. Come back together and share out their true statements from the data in two columns, one for each parameter: temperature and depth.
6. Using their true statements, have the students think of questions that these true statements could answer.
    a. Statement: The vernal pool temperature rose 5 degrees between visits.
    b. Question: How much did vernal pool temperature rise over 3 weeks?
7. Now examine the weather data from each location and generate true statements.
8. Have students create graphs of their data and graphs of weather data. Compare the graphs and share their thoughts with the class.

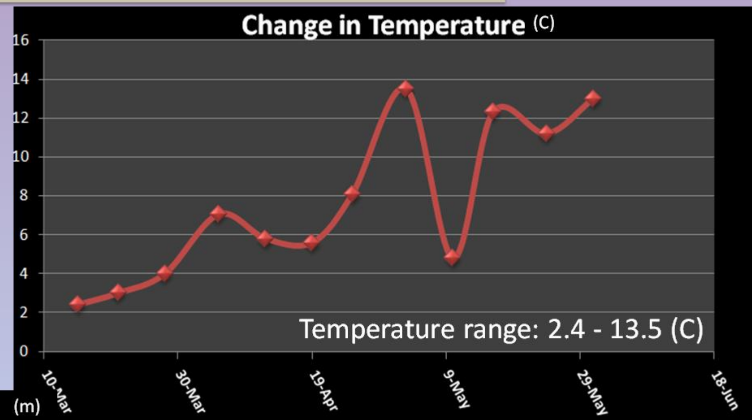
Supplies: Graph paper, rulers, thermometer, pen/pencil, WeatherUnderground website or printout.

Appendix E
Pool Profile Infographics*

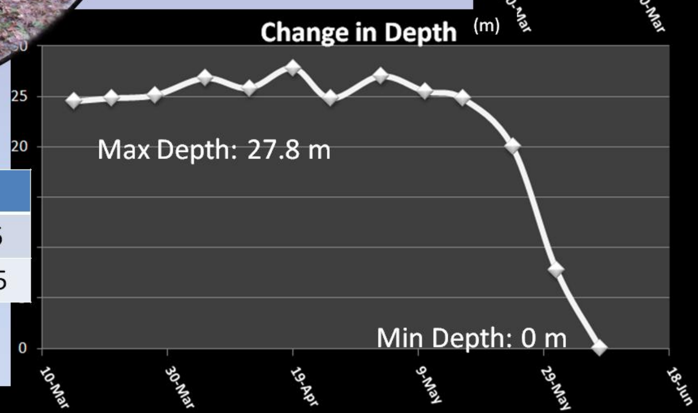
*All photos taken by Kim Snyder

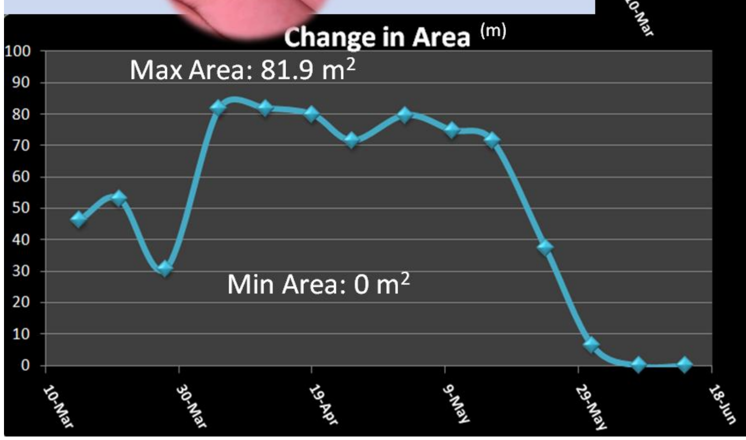
Stonewall Vernal Pool Profile 2020

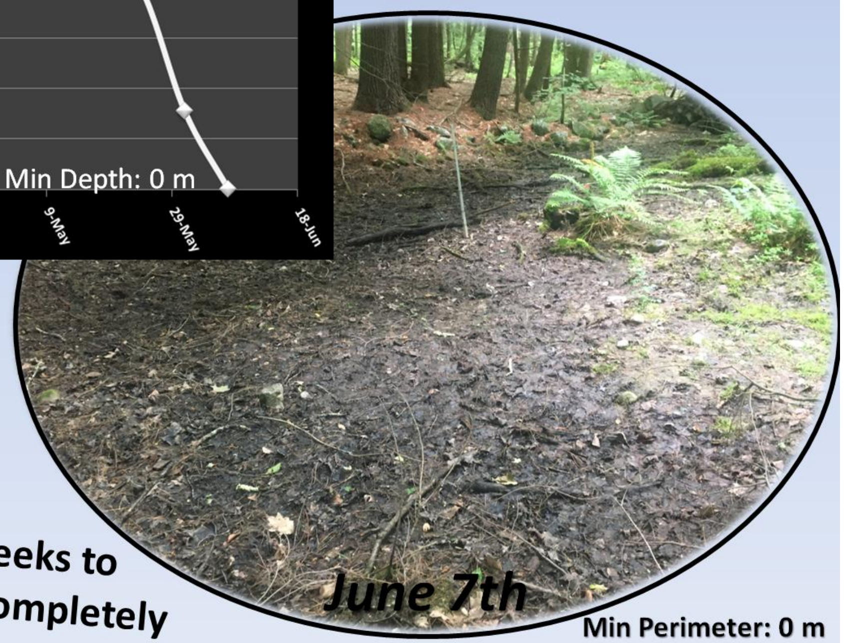
Max Perimeter: 43.5 m



Obligate Species	High Count	Date
Spotted Salamanders	23 egg masses	May 16
Fairy Shrimp	6 adults	April 25





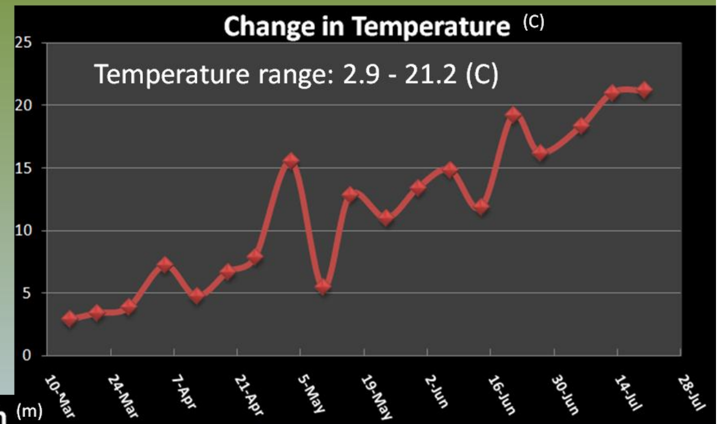


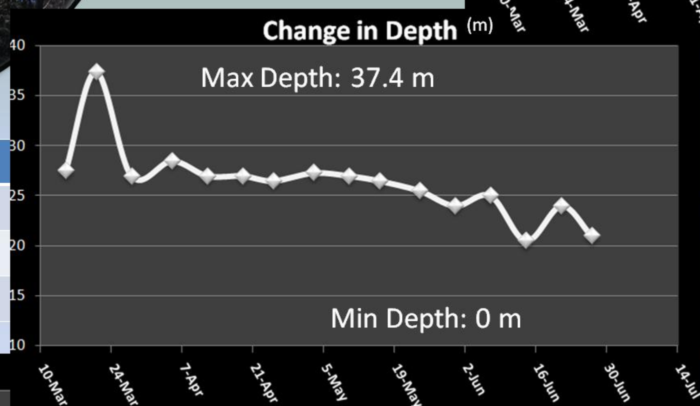
13 weeks to dry completely

Min Perimeter: 0 m

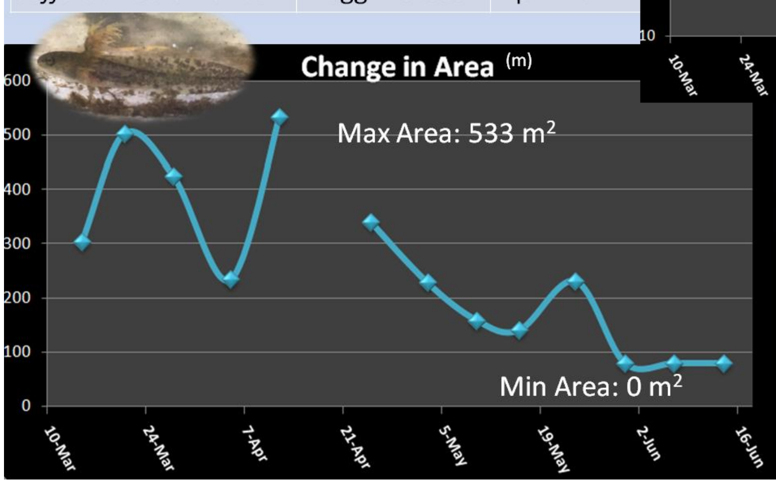
Lookout Lane Vernal Pool Profile 2020

Max Perimeter: 141 m

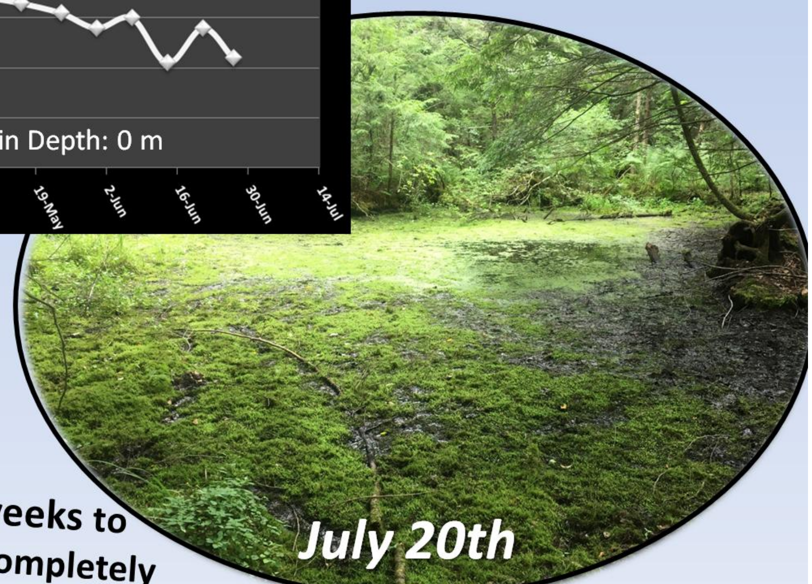




Obligate Species	High Count	Date
Spotted Salamanders	63 egg masses	May 10
Wood Frog	9 egg masses	April 25
Jefferson Salamander	4 egg masses	April 19



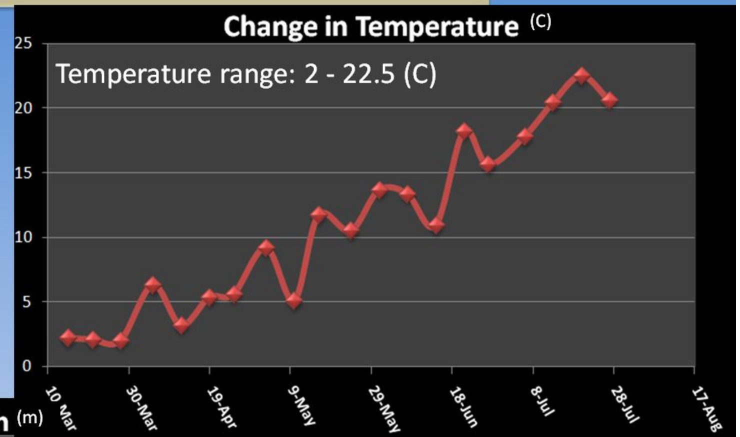
18 weeks to dry completely

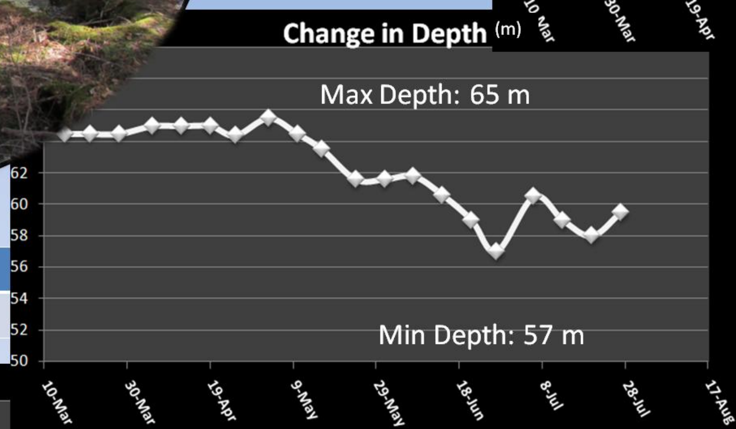


Min Perimeter: 0 m

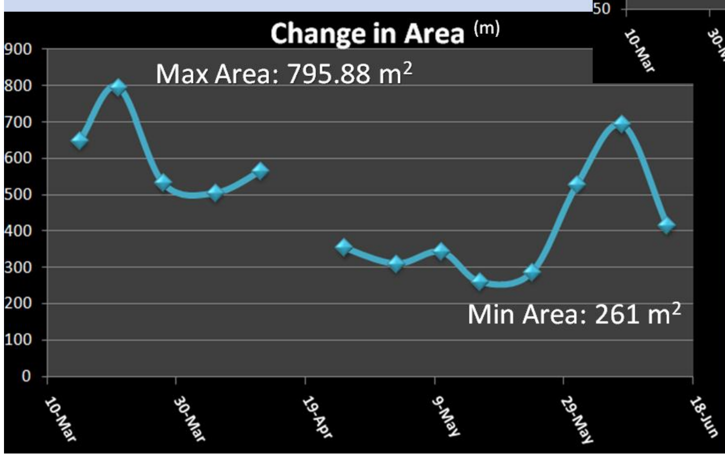
Swamp Vernal Pool Profile 2020

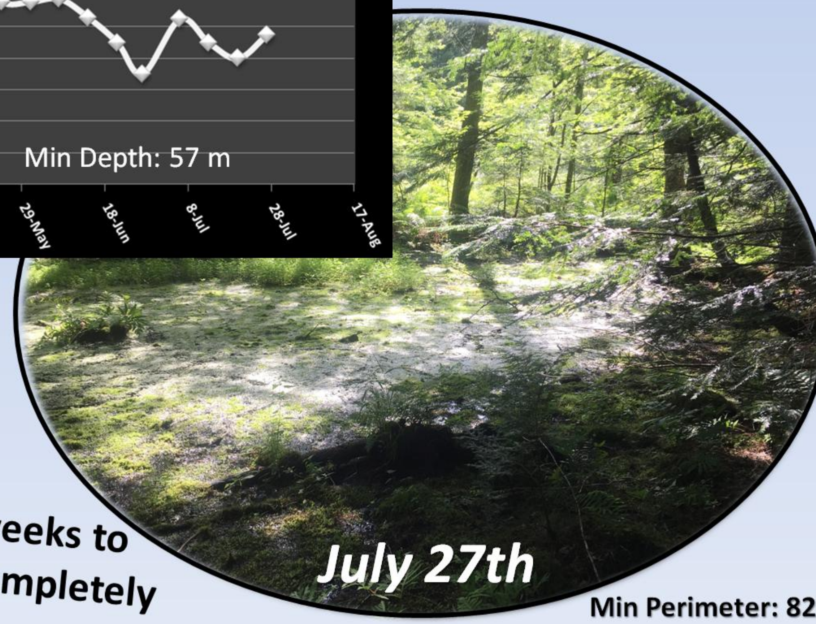
Max Perimeter: 176 m





Obligate Species	High Count	Date
Spotted Salamanders	46 egg masses	Apr 25





20+ weeks to dry completely

Min Perimeter: 82 m