BIODIVERSITY IN AN ISOLATED SUBURBAN RESERVATION: AMPHIBIANS IN THE MIDDLESEX FELLS

A Thesis Presented

by

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Submitted to the Office of Graduate Studies,
University of Massachusetts Boston,
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2011

Biology Program

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ABSTRACT

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December 2011

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Amphibians are indicators of local ecosystem health – they are weak dispersers in comparison to other vertebrates, have sensitive permeable skins, and typically exhibit a biphasic lifestyle, utilizing both aquatic and terrestrial habitats during different lifestages. By cataloging the amphibian species inhabiting different parts of the Middlesex Fells Reservation, this research is intended to provide one measure of the reservation's ecological health and to examine patterns of amphibian species richness at two spatial scales (in the landscape and at aquatic breeding habitats) that are relevant to amphibian ecology.

The Fells is a mixed-use natural recreational area within a thirty minute drive of millions of Boston area residents. Reservoirs and roads separate sections of the Fells from

one another and provide an opportunity to comparison test how these sections serve as refuges preserving amphibian species diversity.

The study area consists of six forested sections that vary in size, shape and number of: pools, ponds and streams. Area of the six sections studied is the single best predictor of amphibian species richness in a section. In addition, landscape analysis indicates that amphibian richness is high in sections with numerous pools, and with a low average distance between pools. In the habitat-level analysis, linear regression showed that pool area and pool hydroperiod are strong predictors of amphibian species richness. A multiple stepwise regression model including both landscape and habitat variables was the best explainer of amphibian richness at vernal pools.

During two years of field research (2007-2008), I found evidence of breeding for nine amphibian species. Species-specific analyses have shown some marked differences in habitat preferences among amphibian species. Wood frogs and American toads were tolerent of the smallest and most ephemeral breeding pools in the study, whereas spring peepers and spotted salamanders required larger, late-drying breeding pools.

This research will begin a baseline record of amphibian species in the Fells. It is my intent that the patterns of species richness observed at the landscape and habitat level, as well as the habitat requirements I have documented, will assist agencies that wish to preserve amphibian species diversity by making ecologically sound management decisions.

DEDICATION

For my nephew, Dominic Kemmett, good luck in science.

ACKNOWLEDGEMENTS

I wish to thank the following individuals and groups for their assistance in the field, at school and elsewhere: Dr. John Ebersole, Tiffany Luongo, Jade Luongo, my mother - Mary - and family, Bridget Kevane, Jill Taylor, Nick Greaves, Joe Martinez, Maureen Cremin, Renee Eriksen, Joyce Morrissey, Mike Read, Jay Ebersole, Ellen Stanley, Dr. Rob Stevenson, Dr. Alan Christian, Paul Jahnige, the Friends of the Middlesex Fells Reservation and finally the staff of Dickson Brothers.

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CHAPTER 1

INTRODUCTION

What features determine the success of conservation areas as refuges for wildlife? Landscape features such as refuge size, refuge connectivity, border to area ratio, habitat diversity, nearness to other preserves and undeveloped surroundings, and protection from human activity may all act to diversify plant and animal life by promoting persistence of natural populations. Features of habitats in a preserve, such as quality of habitats, connectivity of similar habitats, and lack of aggressive aliens may also enhance diversity by promoting long-term population survival. The actual effects of such features on diversity are of general interest to ecologists, and applied interest to agencies responsible for managing public reservations. Proper management and protection of preserves depends on understanding how this wide range of factors acts on real organisms in real environments.

The Middlesex Fells -- a reservation near Boston, MA that receives heavy recreational use and is also dissected by roads and isolated from other areas of natural habitat by development -- provides an opportunity to examine, at several spatial scales, the consequences of urbanization and habitat fragmentation on amphibians. In addition, the Middlesex Fells Reservation has never been systematically surveyed for amphibians, so this research, although aimed primarily at examining the effects of isolation and fragmentation on persistence of amphibian populations, also provides a first survey that could begin an historical record of amphibians in the Fells.

In recent decades, biologists have found marked declines and extinctions in many amphibian populations (Blaustein et al.1994, Alford et al. 1999, Pounds et al. 2006), and several species in Massachusetts could be in trouble. To comprehend the magnitude of the threat, to know how amphibian diversity has changed and will continue to change over time, it is imperative to have an historical record of amphibian occurrence in accessible and widely used urban and suburban reserves like the Middlesex Fells. Since few data exist concerning amphibians in the Fells, this research will begin to establish a baseline record.

The vernal pool is a habitat of particular interest for amphibian research in general and for this study in particular. The most abundant aquatic habitat in the Fells, vernal pools are ephemeral water bodies that often do not support populations of large predators, and so provide breeding habitat for many species of amphibians and invertebrates. Due to their temporary nature and small size, vernal pools are more sensitive than permanent bodies of water to the building of subdivisions and roads. Negative impacts of urbanization, including local extirpation, affect populations of amphibians that breed exclusively in vernal pools more than those that breed in other habitats (Rubbo and Kiesecker 2005). All amphibian species listed by the state of Massachusetts (http://www.mass.gov/dfwele/dfw/nhesp/nhesp.htm - Massachusetts Natural Heritage website) as “threatened” (“likely to become endangered in the foreseeable future, or declining or rare") or " of special concern" ("have suffered a decline that could threaten the species if allowed to continue unchecked, or occur in such small numbers or with such restricted distribution or specialized habitat requirements that they could easily become threatened") depend to some degree, if not entirely, on vernal pools for breeding. Since vernal pool-breeding amphibians inhabit the surrounding terrestrial environment for the majority of their lives, a major challenge to the conservation of vernal pool amphibians is protecting both the breeding pools and the matrix of terrestrial habitat between pools (Semlitsch 1997, Semlitsch and Bodie 2003, Homan et al. 2004). Human activities outside the immediate area of a vernal pool can threaten the amphibian populations breeding in the pool. This research explores the consequences of habitat fragmentation and isolation caused by roads and other human development on amphibian occurrence (see Study Site section of METHODS for more detail).

In addition to landscape level effects on the occurrence of vernal pool breeding amphibians, often the specific qualities of pools themselves can affect which species utilize them as breeding sites. Pool features, such as pool area, hydroperiod, vegetative cover, and distance to nearby pools are likely to influence the specific host of species present. The presence (or absence) of predators is another such feature. Salamanders, especially newts, prey on the larva of amphibian species. Morin (1981, 1983) found that experimental pools containing predatory salamanders, specifically *Notophthalmus* and *Ambystoma* species, had higher overall diversity of metamorphosed amphibians. How is the amphibian species diversity of a vernal pool affected by the presence of larval *Ambystoma* and adult *Notophthalmus viridescens*? Morin suggests that competitive pressure between competing anuran tadpoles is neutralized when the total number of tadpoles is reduced by salamander predation. In this way, vernal pool predators such as the newt, *Notophthalmus viridescens*, may act as ‘keystone predators’: boosting diversity by preventing competitive exclusions. Newts at a vernal pool may maintain a high level of diversity by allowing more species to persist to metamorphosis and return, due to natal pool fidelity, to breed. Thus, newts may prevent anuran species from going extinct from a given pool.

The Fells is a fairly sizeable suburban conservation area (2,060 acres) entirely surrounded by developed land in the towns of Medford, Malden, Winchester, Stoneham, and Melrose, Massachusetts (Figure 21). Because the Fells is isolated from other natural habitats, it should be considered an area of special ecological concern. Little biological research has been done in the preserve. One notable exception is the work by Drayton and Primack (1996) that compared botanical surveys carried out by the authors in 1993 with surveys conducted in 1894 (around the time the reservation was founded). Their results indicated that in 100 years many native plant species were extirpated from the park. How have the amphibian residents of the Fells fared during the same period? It seems likely that human activity has substantially altered the array of amphibian species in this suburban park.

Hypotheses

Examining the underlying causes of variation in amphibian richness throughout different parts of the Fells reservation is an important goal of this research. This study of amphibians in The Fells is organized into three perspectives, each with particular research hypotheses:

I. Landscape level effects are examined by comparing different sections (contiguous forested areas delineated by roads or other boundaries) of the Fells that are separated from one another by roads and bodies of water, to evaluate four hypotheses.

1. I predict that larger sections host more species than smaller ones. Larger sections will tend to contain more species as a consequence of their size and therefore likelihood of species occurring by chance. In addition, the theory of Island Biogeography states that larger sections may also maintain more species because they support larger populations that are vulnerable to chance local extinction events over time (MacArthur and Wilson 1963 and Brown 1971).

2. I predict that sections of the park with a higher density of pools have more species. Density of vernal pools is related to habitat quality. Sections with higher quality habitat, that is habitat where vernal pools are more densely distributed, should maintain more species.

3. I predict that sections of the park with more pools will have more species. More pools in a section will correlate with a greater range of pool types and habitats that can sustain

more amphibian species. The more pools in a section, the more resilient amphibian metapopulations will be and the more likely pools will receive colonists.

4. Edge coefficient is the measure of the ratio of perimeter to area in a section. I predict that sections with a larger area to perimeter ratio will maintain more total species. Section perimeter represents less than optimal habitat in this research. Roads, deep reservoirs, and developed areas- poor amphibian habitat- line the perimeter of sections. Sections with less edge would contain pools that are better insulated from the ill effects of poor habitat beyond their boundaries.

II. Habitat effects are examined by comparing different vernal pools, and these five hypotheses are specifically addressed:

1. I predict that larger vernal pools will host more breeding species than smaller pools. Vernal pools with larger areas are more attractive to amphibians as breeding sites because they will be more resilient to drought and other environmental perturbations. Larger area pools are larger targets and are therefore more likely to receive colonists of new species.

2. Pools with longer hydroperiods should also host more breeding species. Metamorphosis times vary among species, so pools with longer hydroperiods host more species.

3. I hypothesize that total species-breeding occurrence in vernal pools will be correlated with distance to roads. Roads are unsuitable as amphibian habitat and can be dangerous barriers (Vos and Chardon 1998, Carr and Fahrig 2001). Pools that are farther away from roads should be better insulated from their ill effects and should consequently host more amphibian species.

4. Total species breeding occurrence in vernal pools will be negatively correlated with distance to other vernal pools. Isolated vernal pools are less likely to receive colonists that help maintain healthy populations than pools that occur closer to one another.

5. The presence of newts at a vernal pool will increase overall amphibian species richness. Newts may act as “keystone predators” maintaining high levels of richness by reducing competition between amphibian larvae.

III. Species-level effects are examined through analysis of species-specific preferences for different habitat features, guided by two general hypotheses.

1. Species with short metamorphosis times that allow rapid completion of the larval phase of the life cycle, such as wood frogs and American toads, will be tolerant of small vernal pools with short hydroperiods.

2. Breeding pools for a given species will tend to be clumped spatially. Vernal pools represent individual populations in a wider metapopulation. Each vernal pool in a metapopulation model depends on colonists from other populations. Amphibians are small animals of limited mobility; therefore it is likely that occupied pools will be clustered together.

I tested for relationships between amphibian occurrence and a variety of landscape features. Using GPS with USGS maps allowed me to associate elevation, the area of water bodies and the proximity to developed land with the arrays of species observed at vernal pools. Not only does landscape ecology of this kind have important consequences for conservation but also for gaining insight on the habitat choices made by species. Some amphibians are totally dependent on vernal pools for breeding (obligate vernal pool breeding species- see Table 2), while others are habitat generalists and breed in a variety of permanent, semi-permanent and ephemeral bodies of water (facultative vernal pool breeding species). I intend to establish a clearer understanding of the specific ranges of aquatic habitats that different amphibians in the Fells use as breeding sites. A keen understanding of the habitat requirements of species is of great value to the conservationists and ecologists who wish to preserve them. This research will add to what is known about the breeding pool habitat of amphibian species. Hopefully this work will contribute to better predictive models and habitat management of these pools in the future.

CHAPTER 2

METHODS

Study Site

The Fells reservation was created in 1894 with additions of land being made for decades after. It has been managed by three agencies: the Metropolitan Parks Commission (1893-1919), the Metropolitan District Commission (1919-2004), and the Massachusetts Department of Conservation and Recreation (2004 - present).

The list of human activities in the Fells that may have negatively impacted amphibian habitat is extensive: direct habitat loss due to development, road building, mining, quarrying, farming, timber gathering, ice harvesting from ponds, milling, damning, reservoir building and consequent landscaping (Levin 1990). The Fells is not a pristine natural preserve, activities likely to affect its biota began before its inception and have continued since. The Fells contains several man-made or man-altered water bodies, including six reservoirs (Figure 21). In the Eastern Fells, the largest reservoir (296 acres) was created through dredging and vegetation removal along the banks of Spot Pond. The 8-acre Fells Reservoir and Covered Reservoir are located in the Southeastern section on the border of Malden, Stoneham and Medford. North, Middle, and South Winchester reservoirs, covering a total of 198 acres were created in the Western Fells between 1874 and 1880 by the enlargement of a large wetland known as Turkey Swamp and the diversion of Molly’s Brook (Levin 1990). The Wright’s pond section of the Fells is named for a pond constructed in the 1880’s for use by the ice industry. In addition, water-driven Mills were working along Spot Pond brook during the 1800’s (Levin 1990). Such activities alter aquatic habitats and likely had negative impacts on the amphibian species that utilized them.

The Fells Reserve was conceived from the start as a recreational resource for the people of greater Boston, as well as a natural preserve. Today, joggers, dog walkers, and cyclists maintain a heavy presence in the park alongside birdwatchers and naturalists. The Stone Memorial Zoo, a public pool, and a skating rink are examples of recreational centers built in the reservation. (MDC Trail map 2002 and http://www.fells.org/thefells/history.cfm).

Route 93, a heavily used, multi lane highway, was built in the 1960’s, bisecting the park. Near the Stoneham line in Medford, MA there is a paved underpass and pathway under route 93. This underpass is an unnatural corridor and probably does little to maintain connectivity between amphibians in the Eastern and Western sections of the Fells. Numerous carriage paths constructed in the 1800’s, as well as hiking and cycling paths, crisscross the reservation potentially disrupting habitat contiguity.

Finally, the Fells has been host to numerous plant and animal introductions. During the 1880’s the North Reservoir was stocked with Black Bass (Levin 1990). During fieldwork I encountered Asian Koi fish and red-eared slider turtles from the Southeastern U.S. in ponds. It is likely that fish introduced to water bodies in the Fells have had deleterious impacts on amphibian populations.

Vernal pools are defined in two ways: hydrologically, they are ephemeral; biologically, they are defined by the absence of some species (often fish) that require water year-round, and the presence of some species (e.g., fairy shrimp, wood frogs, and spotted salamanders) that occur nowhere else, -- so the line between vernal pools and ponds can be blurry. For the purpose of this study a vernal pool was defined as an ephemeral body of water that lasted at least one month, had no discernable current, and lacked a permanent fish presence during the course of the study. Ponds were defined as uncovered reservoirs or any permanent body of water with a fish presence throughout the course of the study. Streams were defined as any watercourses over 20 meters long that had a discernable current after May 1st. Many vernal pools could have been described as seasonal, stream-fed swamps, since they were connected to streams (or brooks, as they are called in the Fells) during the early spring when water levels were high. I categorized wet areas connected to streams as vernal pools when no current was observed around the majority of the pool's perimeter. No site within the study area failed to fit clearly into one of these three categories: pond, stream, or vernal pool.

Sections / Landscape Data

Fifty per cent of the 833 hectares that comprise the Fells Reservation were included in the 420-hectare study area. Roads, lakes and reservoirs divide the Middlesex Fells into nine distinct sections that differ in size and other landscape features. For practicality's sake, only six of these sections were surveyed and are included in this study (Figure 21 and Table 1.). The Extreme Eastern section is 0.5 km^2, with 10 vernal pools;

The Lawrence Wood section is 0.9 km^2, with 33 vernal pools and 1 permanent pond; The Southeastern section is 0.9 km^2, with 21 vernal pools and 1 pond; The Southwestern section is 1.2 km^2, with 30 vernal pools and 1 pond; The Virginia Wood section is 0.2 km^2, with 1 vernal pool; The Wright's Park section is 0.5 km^2, with 5 vernal pools and 3 ponds (Figure 22)**.**

Reservoirs and large ponds occur throughout the study area. The most southerly of the three Winchester reservoirs provides the northern boundary of the Southwestern Fells study section. Though much of the reservoir bordering the Southwestern Fells possesses a steeply banking slope with deep water (poor amphibian habitat), corridors of forest habitat occur around the reservoir allowing connectivity between amphibians from the North and South. Whitmore pond is at the westernmost boundary of the Lawrence woods section. The Fells reservoir and a nearby covered reservoir occur to the North of the Southeastern Fells section. The Wright's park section is bounded on the North by the largest body of water in the reservation, Spot Pond and on the South by Wright's Pond.

Research on amphibian metapopulations often focus on variables such as the size and isolation of aquatic breeding sites - the quality of interevening terrestrial habitat (as dispersal cooridors and over-wintering habitat) is overlooked (see review; Marsh and Trenham2001). However, in landscape ecology there is strong evidence that the quality of terrestrial habitat can be important to amphibian occurrence and species richness (Windmiller 1996, Joly et al. 2001, Regosin et al. 2003, Semlitsch and Bodie 2003, Homan et al. 2004, Van Buskirk 2005, Rubbo and Kiesecker 2005, Piha et al. 2007, Pillsbury and Miller 2008). In the study sites of other published research (Joly et al.

2001, Knutson et al. 2004, Rubbo and Kiesecker 2005, Van Buskirk 2005, Piha et al. 2007, Pillsbury and Miller 2008) there has often been a matrix of forest, urban and agricultural patches thus making landscape variables such as percent forest cover especially important. Satellite images and ground truthing over the course of my fieldwork revealed that the vast majority of all the Fells sections were densely forested and of similar terrestrial habitat quality. Section area, perimeter and edge were therefore used to qualitatively differentiate sections in the Fells.

Geographic Features

In the winter and spring of 2007 and 2008, I mapped all pools and ponds on trail maps and obtained coordinates with a hand-held Garmin GPS unit (GPSMAP 60CSX). I collected the following data: perimeter, area, coordinate, location, altitude, and shape of vernal pools and permanent ponds with a handheld Garmin GPS unit. I collected pool and pond site shape, perimeter & area by walking around each pool with the GPS unit in ‘track’ mode. These data were transferred into Garmin Base Camp 2.0.7, 2009 and Mapsource GIS programs, which enabled me to record the straight-line distances between sites of interest. I measured distances between pools or ponds from nearest edge to edge. When pools or ponds were included in analysis in which boundary delineations were not acquired, the coordinate point associated with the vernal pool or pond was used as the terminus of measure.

Hydroperiod

I estimated hydroperiod by documenting the date in which a pool was first observed dry. It was impossible to visit every pool, each day, therefore I assigned pools a hydroperiod score between 1 and 7 to represent when they dried up: (1) dry before May 1; (2) Goes dry in May; (3) Goes dry in June; (4) Goes dry in July; (5) Goes dry in August; (6) Goes dry after September; (7) Permanent pond. These hydroperiod scores are rough estimates of hydroperiod, assigning each vernal pool to a time period for which it can be said with confidence that the pool dries up. I collected hydroperiod data for the 2007 and 2008 seasons. Groundwater levels from April – August were normal or above normal during both years according to published USGS reports (2007, 2008). While variation in hydroperiod during the two years of inquiry is expected, the absence of drought conditions or extreme flooding supports the method as representative of most year-to-year conditions. The hydroperiod data are likely characteristic of most years.

Vegetation

I approximated the percentage of area covered by emergent vegetation (such as cattails or sweet pepper bush) in vernal pools by using a GPS handheld unit to record the area of vegetated patches in vernal pools. Then I divided the net vegetated area by the total pool area. Certain pools were too choked to delineate using this process. In these cases, I approximated the percentage vegetated area by visual estimate.

Amphibian Surveys

I collected evidence of breeding activity of amphibians in vernal pools and ponds between March and September during the years 2007 and 2008. I netted vernal pools and ponds extensively over this period -around their entire perimeter whenever possible- to sample larvae and adults and used eyes and ears to find egg masses and chorusing adults, scheduling fieldwork to maximize the chance of documenting all potential breeding species by including their distinct and different breeding seasons (as in DeGraaf and Rudis 1983). Though I used the same method of dip netting to survey ponds and vernal pools, ponds required a greater investment of time because of their large size. I searched each stream with a dip net, and overturned submerged rocks and debris along stream banks. I also regularly overturned logs throughout each of the study areas as a means of documenting the occurrence of land-breeding red-backed salamander (*Plethodon cinereus*). I searched for evidence of marbled salamanders (*Ambystoma opacum*) by inspecting dried vernal pools in late summer and early fall for mating adults and eggs in addition to dip netting for their larvae in the spring. I inspected the mossy hummocks of vernal pools in an attempt to locate breeding four-toed salamanders (*Hemidactylium scutatum*) and their eggs. All the discovered eggs, larvae, and adults were identified to species; eggs, larvae, and the presence of mating adults (salamanders) or large chorusing aggregations (frogs) were all considered evidence of breeding. American toads were encountered in breeding pools and in terrestrial habitat frequently over the course of the study - but not the similar, sympatric species Fowler’s toad (*Bufo woodhousii fowleri*).

The preponderance of the American toad led to the author's assumption from here on that all toad eggs and larvae were American and not Fowler's toads.

I conducted amphibian surveys on 16 dates between March 29th and September 17^{th} in 2007 and again, on 16 days, in 2008. I visited each of the six sections on an average of eight distinct dates, with a minimum of four visits to the smallest section and a maximum of thirteen visits to the largest section (Table 16). I devoted more time to larger sections because they contained more pools and ponds, therefore requiring more time to achieve a similar sampling effort of pool and pond sites. I estimate my field hours to total 61.5 hours in 2007 and 53 hours in 2008. I conducted a mean of 3.2 visits per pool and pond over the study period. Surveys were done during the day with the exception of two night field visits during 2007 and one during 2008. Most often, I documented the breeding of a given species in a given vernal pool during both study years. However, due to the large number of vernal pools, I was not able to survey each pool during the optimal breeding period for each species during both years, so many were documented during only one year.

Field ecologists often fail to locate amphibians even when they are present (MacKenzie et al. 2002, Skelly et al. 2003). Adult and larval amphibians are often small, cryptic and sparsely distributed throughout aquatic habitats. Every effort was made to give each pool, pond and stream a similar level of sampling effort, but it is likely that some species, at some sites were missed - especially considering the large amount of sites visited. In 35 of the 115 a species was located at a site one year of the study and not the other. It is likely that during one of the years the species was simply missed and not absent – and this low percentage of misses –30%, or 70 out of 230 pools, streams and pond seasons) indicates a low probability of any species being missed altogether in any sites ($p = 0.0926$).

Testing for Negative Interactions Between Species

I used the “checkerboard” method to test for negative interactions between amphibian species at vernal pools. Using the proportions of vernal pools occupied by each species, I then calculated the proportion of vernal pools that would be occupied if pool occurrence was independent for each species. I compared this expected value with the actual proportion of pools occupied by each pair of species to assess whether negative interactions between species were most important or species habitat needs took precedence in the selection of vernal pools. The following species pairs were analyzed using this method: wood frog and green frog, wood frog and American toad, wood frog and spring peeper, wood frog and spotted salamander, green frog and spring peeper, spotted salamander and green frog, spotted salamander and spring peeper, spotted salamander and American toad.

Breeding Site Analysis: Newts as a Habitat Feature

I compared the mean number of anuran species when newts were present with the mean number of anuran species when newts were absent. I confined the analysis to vernal pools with at least one anuran species, since newts inhabited no pools without anurans and pools without anurans were more likely unsuitable because of nonbiological factors such as small size- not the absence of newts.

Analysis: Total Section Stream Length

I used the Garmin base camp program's track function to measure stream length in sections. I divided total section stream length by section area and regressed this value against the dependant variable of number of amphibian species per section.

Analysis: Section Area, Perimeter, Edge and Vernal Pool Dispersion

I measured area and perimeter by using the track function to delineate each section on the program map in Garmin Base Camp. This delineation set the parameters by which both area and distance were calculated in the program. The edge of each section was calculated relative to its area: $Perimeter/\sqrt{Area}$.

I used the Clark and Evans (1954) test for aggregation in a population on vernal pools to test for clumping of pools within sections $R = (mean\ r)/E(r)$ - where mean r was the observed mean distance between a vernal pool and its nearest neighbor, E(r) is the expected or mean value of the average distance between a randomly selected pool and its nearest neighbor if the dispersion is at random, and R is the ratio. If the value of R is between 1 and 2.149, then we can assume a regular dispersion pattern (adapted from Poole 1974). This value was included with other section landscape variables that were analyzed for their effects on species number in sections.

Analysis: Multiple Regression and Least Square Regression

I used least squares regressions to test for relationships between the independent variables of hydroperiod, pool perimeter, vegetative cover, distance to nearest road, distance to nearest pool and pool area against species occurrence for each vernal pool. I also used regression analysis to test for relationships between the variables of section area, density of pools per section, section edge, average distance between pools within a section, and number of pools per section with the total number of species found in each section. I used the Microsoft Excel version 9.0; 2000, Statistica 5.1; 1997 and Systat 11; 2004 programs to perform these analyses.

Stepwise Multiple Regression

To determine whether the number of species found in a section is significantly related to any landscape level variables, I conducted a forward stepwise regression analysis. This method establishes which members of an array of independent variables contribute significantly to a model explaining the variation observed in the dependant variable -- in this case species occurrence in different sections of the Fells. To reduce non-normality and heteroscedasticity, I used the log-transformed independent variables of section area, section perimeter, number of water bodies, number of vernal pools, water bodies per area, pools per area, stream length, stream length per area, clumping and edge. I used the Systat 11 program to run all stepwise regression analyses.

Are Area and Hydroperiod Tandem Variables?

It is important to discern whether area and hydroperiod of pools are discrete, separate variables or are two measures of the same signal. Using Systat 11, I did a forward stepwise logistic regression for each of six species (American toad, red-spotted newt, spotted salamander, wood frog, green frog, and spring peeper) with Log area and hydroperiod of vernal pools as independent variables and presence/absence of each species as the dependant variable. I used this method to determine whether or not area and hydroperiod both accounted for the same amount of variation in species occurrence or one was dominant.

Earliest Projected Time of Transformation

I calculated the earliest projected date of transformation for four species: American toad, wood frog, spring peeper and spotted salamander by referring to my own observations of the peak breeding time for each species in the Fells, as well as egg development period and larval development period as in DeGraaf and Rudis (1983). The mean hydroperiod and mean area of vernal pools occupied by species were separately regressed against the earliest projected date of transformation. The earliest possible date of transformation was used -instead of mean date of transformation- because it best represented a species level of adaption to small, ephemeral vernal pools. These four species were chosen because they all bred in enough vernal pools for meaningful analysis and did not overwinter as larva, which would have biased my methods of observing breeding time, egg laying and within-year larval development.

Effects of Habitat and Section Variables on Species Richness in Vernal Pools

Environmental characteristics at both the local and landscape level affect amphibian species diversity and richness at breeding ponds and pools (Knutson et al. 2004, Van Buskirk 2005, Piha et al 2007, Pillsbury and Miller 2008). Using Systat 11, I conducted a forward stepwise regression with the dependant variable of number of breeding amphibian species at vernal pools and the section-wide independent variables of: log-transformed section area and number of pools per section and the independent habitat variables: log-transformed area of pools and hydroperiod pool score. These variables were chosen because they were either good predictors of overall section species number in the case of the section-wide variables or good predictors of vernal pool species number in the case of the habitat variables.

CHAPTER 3

RESULTS

The Middlesex Fells hosts many of the more common amphibian species native to Massachusetts. I found a total of nine amphibian species during this study (Table 2): wood frog (*Rana sylvatica*), green frog (*Rana clamitans melanota*), bullfrog (*Rana catesbeiana*), spring peeper (*Pseudacris crucifer*), pickerel frog (*Rana palustris*), American toad (*Bufo americanus*), eastern red-backed salamander (*Plethodon cinereus cinereus*), spotted salamander (*Ambystoma maculatum*), and red-spotted newt (*Notophthalmus viridescens viridescens*). With the exception of the ubiquitous, completely terrestrial red-backed salamander, I found evidence of breeding for each of them (larvae for all species and eggs for most species). The red-backed salamander was frequently encountered under debris during all field visits and in all sections. It was the most frequently encountered amphibian in the Fells (personal observation) and certainly the most abundant in agreement with Burton and Likens' (1975) New England-based research. No state-listed rare, threatened, or special concern species were encountered during the two field seasons. Conspicuous by their absence from my surveys were all species of stream salamanders, however, researcher Joe Martinez found Northern two-lined salamanders (*Eurycea b. bislineata*) twice in a stream that runs from the border of the Southwestern Fells section and into the Lawrence Woods and once in a stream in the Southeastern Fells (personal communication). Overall, amphibians bred in 46 of the 99 (46%) vernal pools, and in all six of the ponds found in the Fells study area. The small Extreme Eastern and Virginia Wood sections had the fewest species (four), and the large Southwestern section had the most species (nine).

Section Analysis: Landscape Features and Amphibian Richness

I analyzed the patterns of amphibian species richness in sections of forested landscape in the Fells reservation. Some sections were far more diverse than others (Table 1). I conducted a forward stepwise regression analysis to discern which section features had the greatest effect on species number in sections. I used the log-transformed dependant variable of section species number and the log-transformed independent variables: section area, section perimeter, number of pools, number of water bodies, pools per area (pool density), water bodies per area (water body density), stream length, stream length per area, clumping and edge coefficient. In the best model, only section area ($p = 0.003$) and vernal pool clumping ($p = 0.045$) accounted for the variation in species richness ($R^2 = 0.964$; Table 7). The next best model retained only section area ($p = 0.011$) in the model ($R^2 = 0.831$; Table 6).

Using linear regression analysis, I observed strong relationships between species number and some section wide features. Log section area accounts for most of the variation in log species number (slope = 1.3617; $R^2 = 0.8306$; Fig. 2). Section perimeter has nearly as strong an effect on the number of species in a section (slope = 1.3222; $R^2 =$

0.5113; Fig. 4). The number of pools in a section had a strong, positive relationship with the total species number of a section (slope = 0.1136; R^2 = 0.637; Fig. 5).

Edge coefficient of section had no relationship with the number of species per section. Linear regression analysis resulted in a slope = -1.06 and R^2 = 0.02. Using linear regression analysis, I found that the area of sections had no discernable effect on the percentage of vernal pools occupied by breeding amphibians (slope = 6.88; R^2 = 0.02). The average distance between the vernal pools in a section has a strong, negative relationship with species number. Linear regression analysis resulted in a slope = -0.048 and R^2 = 0.537 (Fig. 1). The density (pools per hectare) of vernal pools in a section also had some positive effect on the total species number of a section. Linear regression analysis resulted in a slope= .120 and R^2 = 0.482 (Fig. 17). The degree of vernal pool clumping in a section had no effect on the number of species in a section (slope = -0.88; R^2 = 0.005). Section stream length / section area had a negligible, negative effect on species number (slope = -0.9687; R^2 = 0.0884).

Breeding Site Analysis: Habitat Features and Amphibian Richness

The sites surveyed in this study varied from small puddles (area as low as 28.7 m^2) to large permanent ponds (maximum area = 27,921 m^2; Table 6). Considering only the vernal pool class of aquatic habitats, there is a very strong positive relationship between area and number of species at a pool. Regressing log number of species versus log pool area resulted in a slope of 0.307 and R^2 = 0.352 (Fig. 6). Log- transformed pool perimeter (meters) had a somewhat weaker, positive relationship (slope = 0.438; R^2 =

0.281) with the overall log species number of vernal pools (Fig. 18), but it seems clear that area and perimeter are related variables. Hydroperiod also had a strong relationship with the number of species at a vernal pool. Linear regression analysis resulted in a slope of 0.577 and $R^2 = 0.288$ (Fig. 7). Hydroperiod and pool area are related variables, since large pools tend to have long hydroperiods.

Using linear regression analysis, I found that the distance from vernal pool to the nearest road had no effect on species number (slope = -9E-05; $R^2 = 0.0004$) and that the percentage vegetative cover of vernal pools had no effect on species number (slope = 0.007; $R^2 = 0.01$), the distance of vernal pools to the nearest water body had no effect on species number (slope = 0.001; $R^2 = 0.0012$) and the distance from the vernal pools to the nearest vernal pool had no effect on species number (slope = 0.003; $R^2 = 0.007$).

Newts inhabited only 3 of the 99 vernal pools surveyed. Considering only vernal pools that had some breeding activity, the average number of amphibian species per pool was higher for pools with newts (mean = 5.33) than for pools without newts (mean = 1.73), and pools without either newts or spotted salamanders had the fewest amphibian species of all (mean = 1.32) (Figure 8 and 9). Unfortunately, the small number of newt pools limits the strength of any analysis of the effects of salamanders on overall amphibian richness.

The vernal pool features of pool area and hydroperiod, proved to be strong predictors of the total number of species. However, this level of analysis yielded comparatively weaker relationships when compared with those of the section level perspective.

Effects of Habitat and Section Variables on Species Richness in Vernal Pools

Forward stepwise regression with the dependant variable of number of breeding amphibian species at vernal pools and the independent variables of: log-transformed section area, number of pools in section, hydroperiod score of vernal pool, and log-transformed area of vernal pool. The best model retained all four independent variables in the following order: section log area ($p = 0$), section number of pools ($p = 0.002$), log area of vernal pool ($p = 0.021$) and hydroperiod of vernal pool ($p = 0$) with $R^2 = 0.599$ (Table. 8). The section level factors are more important than habitat factors in the regression model.

Species Preferences and Requirements

With regard to the vernal pool habitat variables in this study, each amphibian species exhibited its own unique range of preferences and tolerances in choice of breeding habitat (Tables 3, 5 and 15). Pool area and hydroperiod are clearly related variables, since the amount of time needed for a pool to dry up depends on how much water is in it. Using the forward stepwise logistic regression made it evident that for certain species both variables are significant while for others one was eliminated from the model -- leaving only one significant variable. These results show that while pool hydroperiod and pool area may be related variables they are not inseparable in their importance to the habitat selection of breeding amphibians. Spotted salamanders and spring peepers both retained log area and hydroperiod as significant variables in their pool occurrence models. Log area was retained in the models for green frogs (p = 0.003 ; McFadden's Rho-squared = 0.235 - Table 9) and wood frogs while hydroperiod was eliminated- or in the case of wood frogs only marginally significant at p = 0.13; McFadden's Rho-squared = 0.230 (Table 10). For newts and toads, hydroperiod is the only variable retained in the models – with only marginally significant p-values (0.068 for newt, 0.071 for toad- Table 11), and the newt result is especially unreliable since only three newt pools were found. Stepwise regression was not done for pickerel frog and bullfrog occurrence because pickerel frogs were in only two vernal pools and bullfrogs – a pond species -- were not encountered in vernal pools at all. Species occurrence at ponds was not analyzed because too few ponds were encountered in the study area (N=6).

Differences in Hydroperiod

The range in hydroperiod of breeding pools varied according to species (Table 5). Wood frogs, American toads and Green frogs exploited the most ephemeral pools. Wood frogs bred in some pools that dried before May 1st, and American Toads and Green frogs both bred in some pools that dried before July 1st. Wood frogs and American toads had similarly low mean hydroperiods and did not appear in permanent ponds. Interestingly, wood frogs and American toads both made use of some vernal pools in the latest-drying class (those that dried in September). The average wood frog pool dried up before July 4th and the average American toad pool dried up before July 5th. Forward stepwise regression retained only pool log area as an important variable (*p* = 0.071; McFadden's Rho-squared = 0.070) affecting American toad occurrence at pools - area was eliminated from the rather weak model. Species that typically breed in permanent or semi-permanent ponds displayed, not surprisingly, a preference for pools with longer hydroperiods or in the case of the Bullfrog only ponds (N= 4).

Green frogs, which appear in permanent ponds, had a higher mean dry-by date of July 27th. All other pool breeding species bred in pools that (on average) dried after August 1st. Wood frogs bred in pools with the widest range of hydroperiods- those that dried before May 1st to those that dried after September 1st. Hydroperiod was retained in the wood frog forward stepwise regression model with only marginal significance (p = 0.13). Spring peepers utilized pools that dried up before July 1st to those that dried up after September 1st. Spring peepers were also found in 2 ponds. Forward stepwise regression retained hydroperiod as an important variable for spring peeper with (p = 0.022; McFadden's Rho-squared = 0.559- Table. 12). Spotted salamanders and newts were only found breeding in pools with longer hydroperiods. Both species were only found in pools that dried up after August 1st. This finding, that spotted salamanders prefer late-drying vernal pools, is in agreement with Windmiller (1996). Forward stepwise regression retained hydroperiod as an important variable for both species with p-values = 0.034 for spotted salamanders (McFadden's Rho-squared = 0.645; Table. 13) and 0.068 for newts. The newt model eliminated pool area as an important variable. This stepwise regression was likely unrevealing because of the paucity of newt pools (N=3).

Differences in Pool Area Tolerance

The species encountered in the Fells exhibited a wide range of preferences and tolerances regarding the area of breeding pools (Table 3). Wood frogs were able to utilize tiny pools, with a lower limit of 141 M^2 -- the smallest pool utilized by any breeding amphibians in this study (Table. 3, Figure 10)! Wood frogs retained area as an important explanatory variable of occurrence at vernal pools ($p = 0.035$). Bull, pickerel and green frogs all made use of the largest pond surveyed for area at 27,921 M^2. Green and pickerel frogs bred in the widest range of pools and ponds in terms of area. When only vernal pools were considered, forward stepwise regression retained only pool log area as an important variable ($p = 0.003$) affecting green frog occurrence- hydroperiod was eliminated from the model. Green and pickerel frogs were able to utilize both vernal pools and at least one permanent pond as a breeding site. Bullfrogs were the only species that bred exclusively in permanent ponds. Spotted salamanders bred in pools ranging in area from 465 m^2 to 6,024 m^2 with a mean of 2,365 m^2 (Table 3, Figure 10). Forward stepwise regression retained log vernal pool area as an important model variable ($p = 0.054$). Spring peepers bred in vernal pools ranging from 361 m^2 to 6,024 m^2 with a mean of 2,055 m^2 (Table 3, Figure10). Forward stepwise regression retained log vernal pool area as an important model variable ($p = 0.042$).

Pool Area and Hydroperiod Effects on Species Occurrence Graphs

To further test the effect of vernal pool hydroperiod and vernal pool area on the breeding presence of each of five pool breeding species, I performed five logistic regressions using the presence or absence of each species as the dependant variable (categorical) and vernal pool hydroperiod (categorical - each hydroperiod score indicated specific date by which a pool had dried up but not the exact date in which it first dried up) and log-transformed vernal pool area (continuous) as independent variables. The three dimensional graphs (Figures 12-16) show a strong positive effect of hydroperiod and area together on the presence of spotted salamanders and spring peepers. Area has a strong effect on green frog and wood frog occurrence. Hydroperiod has a strong effect on American toad and some effect on wood frog occurrence. The graphs show the importance of larger, later-drying pools for the breeding occurrence of all species and provide a useful graphical illustration for the above stepwise logistic regressions. Newts and pickerel frogs were not included in these regressions because sample sizes are too small.

Spotted Salamander Distribution

The 10 Spotted salamander breeding pools were located in 3 sections: Virginia Wood, Southeastern Fells, and Southwestern Fells. Using the smallest breeding pool's area and the lowest documented hydroperiod as lower limits of tolerance for potentially suitable spotted salamander breeding sites, I found 6 vernal pools in the Fells for which hydroperiod and area should be tolerable for spotted salamanders yet they were absent. All 6 sites were located in the Lawrence woods section, though there are probably more of such pools in the Lawrence woods and throughout the other 5 study sections for which accurate area or hydroperiod measures were never taken thus calling their suitability as spotted salamander sites into question. Though, I found no evidence for spotted salamanders in the Lawrence Woods, Researcher Joe Martinez did report a find of one spotted salamander egg mass in a vernal pool there (personal communication).

Earliest Projected Time of Transformation

The earliest date of transformation was calculated to be near June 19th for wood frogs, near June 3rd for American toads, near July 20th for spring peepers and near July 7th for spotted salamanders (based on my observations of breeding times with period-to-hatching and larval period as in DeGraaf and Rudis 1983). Most of the observed variation in the mean hydroperiod of vernal pools used by these species was explained by the earliest projected date of transformation (slope = 0.029; $R^2 = 0.730$) (Figure 19). The relationship was even stronger for the mean area of vernal pools used by these species (slope = 35.441; $R^2 = 0.839$) (Figure 20).

Interactions Between Species

The actual number of vernal pools containing both species of any of the analyzed pairs was always higher than the number expected if occurrences were randomly distributed among pools for each species of a pair. This result was similar to Piha et al. (2007) in indicating that any negative interactions between species are outweighed by similarities in their habitat needs for the following species pairs: wood frog and green frog, wood frog and American toad, wood frog and spring peeper, wood frog and spotted salamander, green frog and spring peeper, spotted salamander and green frog, spotted salamander and spring peeper, spotted salamander and American toad.

CHAPTER 4

DISCUSSION

The Middlesex Fells supports many amphibian species common to Massachusetts. In contiguous forested sections in the Fells, large size, a large number of vernal pools and a low average distance between pools were all important factors promoting amphibian species richness. In vernal pool habitat, pool area and pool hydroperiod were the most important factors contributing to species richness. The amphibian species encountered in the Fells exhibited different tolerences for aquatic breeding habitats varying in area and hydroperiod. Among spring-breeding amphibians, species with short metemorphosis periods - such as: wood frogs and American toads - utilized smaller, more ephemeral pools than species with longer metamorphosis times such as: spotted salamaders and spring peepers. The discussion section will present topics in the following order: sections and species richness, vernal pool habitat and species richness, species-specific requirements and conclusions.

Section Size

Size (area and perimeter) of contiguous Fells sections had a strong, positive effect on the number of amphibian species occurring in them. More species are found on larger sections of open space than smaller ones for at least three reasons. First, larger sections act as larger sampling quadrats that effectively capture more species than similar, smaller sections. In addition, the larger sampling units (in this case, larger sections) may support more species because the larger populations they support are less likely to go extinct due to either stochastic or deterministic processes. Finally, larger sections are larger targets for dispersing amphibian colonists. Area of section is more strongly related to species number than section perimeter, because it is a more accurate measure of actual section size. This conclusion presents a challenge to diversity- minded land management as it is constantly at odds with encroaching development and habitat fragmentation. Quality of habitats (Homan 2004) and the presence of connective habitat corridors between populations (Vos and Chardon 1998, Carr and Fahrig 2001, Semlitsch and Bodie 2003) are important factors affecting amphibian species richness and occurrence. However, the paramount importance of refuge size cannot be ignored -- especially for amphibians and other taxa of limited dispersal abilities (Semlitsch 1997, Smith and Green 2005). In the Fells, I found a positive relationship between the number of vernal pools per section and the overall amphibian species richness of the section. More pools in a section generally indicate a larger section and it follows that the above explanations for the positive relationship between section size and section species number apply to the relationship between number of pools and number of species as well.

The percentage of pools occupied in a section was unrelated to section area, indicating that the same size unit of habitat in a large section is not qualitatively better habitat than that in a small section, or that a pool is no more likely to be occupied if it is in a large section than a smaller one. This supports the conclusion that larger sections simply capture more species by acting as larger sampling quadrats and not because of a positive feedback relationship between section size and habitat quality. This unexpected result is difficult to reconcile with the distributions of a few species (see below).

Section Pool Distribution

The average distance between the vernal pools in a section is negatively correlated with the number of amphibian species breeding in sections of the Fells. This result agrees with other studies that have found a negative relationship between wetland isolation and species richness or occurrence (Joly et al. 2001, Lehtinen and Galatowitsch 2001). Amphibian species richness of sections is better predicted by the average distance between pools (negative) than the number of pools per area (positive) – probably because inter-pool distance better describes the hazards faced by individual amphibians that would colonize new sites.

Streams

The lack of stream salamanders encountered in the studied sections of the Fells diminishes the direct contribution of streams as breeding sites to overall amphibian species richness in the reservation. The total section stream length relative to section area did not have a significant effect on section amphibian species number. However, it is certain that streams are very important focal points of amphibian breeding activity in similar reserves elsewhere – and possibly in parts of the Fells beyond the study area. It is likely that in such areas stream length would play a measurable role in the local richness of amphibians. Streams are essential to amphibians, even those species that do not breed in them – as an integral part of the water table (many vernal pools were created by the swelling of adjacent brooks) and as habitat outside of the breeding season (Carr and Fahrig 2001).

Vernal Pools

My research shows that large vernal pools are likely to be occupied by a large array of breeding amphibian species. Lehtinen and Galatowitsch (2001) found low species richness at small restored wetlands, and Windmiller (1996) found spotted salamander occurrence was positively correlated with vernal pool size. The impact of pool size on species richness can be explained by a number of factors. First, the area of a pool is related to the volume of water it contains and, consequently, how late in the year it will dry. As hydroperiod increases, the larval periods and breeding seasons of more species are accommodated, thus increasing richness.

The equilibrium theory of Island Biogeography (MacArthur and Wilson 1963) predicts that larger habitat patches should be more speciose than smaller ones (species-area relationship) due to reduced extinction rates on larger islands. However, we cannot use a pools-as-islands approach in this case because of the life histories of vernal pool amphibians. For the pool-breeding amphibians in my research, a substantial part of their life history takes place in the woodlands surrounding their breeding pools. The habitat island conception is not appropriate for these species because the “sea” surrounding vernal pool “islands” is not inhospitable – rather it is essential for completion of their life cycles. Due to their biphasic life cycle, amphibians breeding in vernal pools do not fit neatly into an island biogeography paradigm. On the other hand, the entire Fells reservation, as a habitat island within the suburban “sea”, may be described more accurately by an island biogeography approach.

Some biogeographical concepts are relevant to vernal pool ecology - first larger vernal pools probably act as larger targets for dispersing colonists (Lehtinen and Galatowitsch 2001), potentially explaining some of the variation in species richness in response to pool size. Finally the populations, or subpopulations, of amphibian species will be less likely to undergo local extinction due to stochastic population fluctuations and environmental perturbations such as drought in large pools. Amphibians population at individual pools can be extripated by local outbreaks of disease, drought and severe predation pressure. This dynamic relationship between amphibian species and the environment can be best probed and elucidated by creating a historical record of the species present at pools over time.

As expected, hydroperiod has a strong, positive relationship with the number of breeding species at vernal pools. The larval periods of a greater proportion of species are accomodated by late drying pools, compared with pools of short hydroperiod, resulting in higher species numbers. Though the large, late-drying vernal pools are particularly species-rich, ponds which are even larger and that do not dry may not be more speciose habitats because of the prescense of predatory fish. Water bodies of a permanent hydroperiod (ponds) likely exhibit a reduction or leveling off of species number as fish predation precludes the prescense of facultative vernal pool breeding species -such as wood frogs and spotted salamanders - and pond species such as bullfrogs take their place.

Some habitat features had surprisingly minor effects on species richness at vernal pools. It was expected that the distance to the nearest pool would have a negative and distance to the nearest road would have a positive effect on species richness as documented in other research (Vos and Chardon 1998, Joly et al. 2001, Lehtinen and Galastowitsch 2001, Carr and Fahrig 2001) but the analysis showed no significant relationships for either variable, perhaps because the scale of measurements in this study were not sensitive to these effects or that the study areas in other research were less pool dense. Species richness in vernal pools was not significantly affected by percentage of the pool covered in emergent vegetation. The lack of demonstrable effect of vegetation on species richness may be attributed to the differing habitat requirements of breeding amphibians – spring peepers (whose vernal pools had a mean percentage of surface vegetative cover = 45%, N = 19, confidence level (95.0 %) = 11.17 – Table 15) frequently utilize emergent vegetation as calling sites (personal observation) whereas wood frogs prefer open water for courtship (mean pool vegetative cover = 35%, N = 33, confidence level (95.0 %) = 6.02 – Table 15).

Integrative Analysis

An integrative analysis of both landscape (section) and habitat (pool) variables resulted in a model that explained amphibian diversity in vernal pools more successfully than models that used only landscape or only habitat variables. This integrative model included a set of local and landscape variables: section area, number of pools in section, pool area and pool hydroperiod. This finding is consistent with a variety of studies that have found both landscape and habitat features to affect species richness at amphibian breeding sites (Knutson et al. 2004, Van Buskirk 2005, Piha et al 2007, Pillsbury and Miller 2008), it was therefore necessary to utilize both section and habitat data to explain richness at vernal pools. Conservation agencies can benefit from the consideration of both local and landscape variables to assess vernal pools for their potential species richness and value as amphibian habitat.

According to Rubbo and Kiesecker (2005), vernal pool habitat is the most readily eradicated in the face of encroaching urbanization and consequently the species which rely solely on this habitat for breeding and development are the most vulnerable. Vernal pools are more easily drained than permanent ponds, tend to be smaller in size, are devoid of fish and are largely incompatable with conventional landscaping aesthetics. Popular education is essential for increasing public support and awareness for vernal pool protection and the preservation of these unique habitats' biodiversity.

Species Habitat Requirements

Where a species-specific perspective is concerned, the characteristics of individual vernal pools can rule out or support the possibility of species occurrence at a pool that has not or cannot be surveyed for amphibians. In the Fells, spotted salamander breeding is unlikely to take place in small pools with short hydroperiods. In agreement with Windmiller (1996), I documented a mean area of 2365 m^2, with a median of 1574

m^2, and a range of 465-6024m^2 (n = 8) for spotted salamander breeding pools. In all pools where spotted salamanders were documented (n = 10), drying occurred after August 1st with mean drying occuring in mid August. All pools where spotted salamander breeding occurred were fish-free.

While most Fells amphibians need a breeding site with suitably natural surrounding terrestrial habitat, bullfrogs – which spend 1-2 years in the aquatic larval stage (DeGraaf and Rudis 1983)-- may need only a permanent pond to thrive. In the Middlesex Fells, bullfrogs were found in four ponds. Bullfrogs were found in every section except the Virginia Woods and the Extreme Eastern Fells- the two section without pond habitat (Figure 29) .

Adult green frogs were observed in vernal pools, streams and ponds and evidence for their breeding was found in vernal pools and ponds. Green frogs disperse and forage in streams (Carr and Fahrig 2001), so it is likely that the adults and juveniles encountered in streams may have been exhibiting these behaviors. Green frogs bred in vernal pools that dried as early as June with a mean drying time of near July 27th. In Spring 2007 a few vernal pools were occupied by large green frog larva that had apparently overwintered indicating that these pools had not dried completely the previous year. Breeding and complete drying occurred in these study sites in 2007 and 2008. Green frogs were found in every section. Pickerel frogs were encountered most often in terrestrial habitat during the course of the study, but larva were found in two vernal pools (one in the Lawrence Woods and another in the Southwestern Fells- Figure 30) and one pond (in the Wright’s Park section) as evidence of breeding. Spring peepers tended to breed in late-drying and large vernal pools as well as ponds. Spring peeper choruses were often localized in and around patches of wetland vegetation- cattails, *Phragmites australis* and sweet pepperbush. Spring peepers were found in every section. Spring peeper, green frog, pickerel frog and bullfrog were all found breeding in one or more ponds with predacious fish. Beyond the study area, newts and American toads utilize ponds with fish during their aquatic life stages and can be considered habitat generalists, however this was not observed in the Fells. In the Northeastern U.S., wood frogs and spotted salamanders are associated entirely with vernal pools and surrounding forest habitats. These two species are excellent indicator species of vernal pool habitat in eastern Massachusetts.

Wood frogs inhabited a wide range of vernal pools, but were notable for breeding in the smallest and most ephemeral. The peak breeding period of this species is early April in the Fells with a time-to-hatching of 10-30 days and a larval period of 6-15 weeks (DeGraaf and Rudis 1983), resulting in a projected transformation time of mid-June to mid-August for the majority of tadpoles. American toads also occupied small, ephemeral pools. The peak breeding period of this species is late April in the Fells, with a a time-to hatching of 3-12 days and a larval period of 5-10 weeks (DeGraaf and Rudis 1983) resulting in a projected transformation time of early June to mid-August for the majority of tadpoles. The early spring breeding and variable larval periods in these species are likely adaptations to the explotation of small pools of brief hydroperiod. Spotted salamanders inhabited a range of vernal pools, but were notable for breeding in the large, late-drying pools. The peak breeding period of this species is early April in the

Fells with a time-to-hatching of 31-54 days and a larval period of 61-110 days (DeGraaf and Rudis 1983), resulting in a projected transformation time of early July-to late-August for the majority of larva. Spring peepers also occupied late drying pools. The peak breeding period of this species is mid April in the Fells, with a a time-to hatching of 6-12 days and a larval period of 90-100 days (DeGraaf and Rudis 1983) resulting in a projected transformation time of mid July to mid-August for the majority of tadpoles. These species are adaptated to the explotation of late-drying vernal pools for breeding and in the case of spring peepers also shallow areas of ponds.

The strong, positive relationship between the projected date of earliest transformation and both the hydroperiod and areas of vernal pools of four species can likely be demonstrted across a wide range of wetland breeding amphibians. Earliest projected date of transformation is a measure of the maximum metamorphic capabilities of each species in drought years or when utilizing rapidly drying pools at the lower limit of species tolerence. In avoiding sites below a lower limit of size and hydroperiod, these species exhibit an adaptation to reduce their likelihood of reproductive failure by selecting breeding sites where their offspring will transform before pool-drying occurs. Natal pool fidelity is one mechanism that explains the selection of good breeding sites, but what guides dispersing individuals? In anurans orienting toward a conspecific chorus may be one such mechanism (Oldham 1967, Resatarits and Wilbur 1989, Resatarits and Wilbur 1991)- but what about salamanders?

I found no evidence of negative interactions between species pairs by inspecting occupancy rates at vernal pools. The observed rates for coexistence of species pairs was -

in every case- higher than expected, likely indicating that habitat characteristics are the predominant factor in shaping vernal pool assemblages. It is of great interest that newts and spotted salamanders inhabit vernal pools with substantially higher numbers of amphibian species than pools without these salamanders (newt mean = 5.33, N = 3, confidence level (95.0%) = 3.79; spotted salamander mean = 4, N = 10, confidence level (95.0%) = 1.01; spring peeper mean = 3.32, N = 19, confidence level (95.0%) = 0.70; toad mean = 2.5, N = 14, confidence level (95.0%) = 1.15; and wood frog mean = 2.09, N = 33, confidence level (95.0%) = 0.56- Table 14). Adult spotted salamanders spend only short spans of time in vernal pools (breeding, but not feeding in this habitat) and it is unlikely that spotted salamander larvae achieve a large enough size to impact larval anuran survival, but newts spend extended periods of time in pools as adults, and are famously voracious predators of larval anurans. It therefore seems likely that newts, are acting as keystone predators in pools enhancing amphibian diversity. In my research, with no experimental manipulation and a paucity of newt pools, it is not clear whether newts are a causal factor in maintaining species richness or are simply attracted to more productive pools. Morin (1983 and 1986), Wilbur et al. (1983) and Morin et al. (1983) and (Knutson et al. (2004) have shown that the prescense of predatory salamanders (newts and *Ambystoma* with large larvae) is associated with high species diversity in larval anuran communities and consequently total amphibian diversity in experimental aquatic habitats.

Even though I found no historical records for stream salamanders (such as: Northern two-lined salamanders and Northern dusky salamanders {*Desmognathus fuscus*

fuscus}) or gray treefrogs (*Hyla versicolor*) in the Fells, I was surprised to find that they were absent from the Fells. I observed seemingly suitable habitat for these species, so their absence suggests that some amphibian species may have been extirpated early in the history of the reservation. Activities such as timber gathering, agriculture, water diversion, and road building (see Study Site section of METHODS for details) could have contributed to sensitive species being extirpated from the Fells. Damning and diverting water for agriculture and mills may have had especially deleterious effects on stream salamanders. The koi and other introduced fish I have observed in the reservoirs and ponds, which are linked to many of the streams in the Fells, may have subjected stream salamanders to intense predation that would not have affected vernal pool species.

Northern sections of the reserve were not surveyed during the study period and could contain additional amphibian species such as the stream salamanders or gray treefrogs. However, there is no indication that these unstudied areas contain better-quality amphibian habitat or are more insulated from the pressures of urbanization than the rest of the Fells. All the same, given the patchy distributions of state-threatened species, it is possible that some of these species that were not encountered in the study area may exist in other parts of the Fells. Further research is needed in these unstudied areas, to determine whether undocumented populations of imperiled amphibians -- marbled salamanders (*Ambystoma opacum* - a state threatened species), blue-spotted salamanders (*Ambystoma laterale* - a state species of special concern), four-toed salamanders (*Hemidactylium scutatum* - a state species of special concern) and spadefoot toads

(*Scaphiopus holbrookii holbrookii* - a state threatened species) -- may persist in these parts of the Fells.

Metapopulations / Breeding Site Distribution

Spotted salamanders were found in three sections: the smallest, Virginia Wood; and the two largest Southeastern Fells and Southwestern Fells (Figure 26). In these sections during the breeding season, every pool of appropriate area and hydroperiod was occupied by spotted salamanders (logistical difficulties precluded the collection of hydroperiod and/or area data of a few pools in these three sections, but none of these pools were utilized by spotted salamanders). By contrast, not one of six vernal pools with appropriate area and hydroperiod in the Lawrence Woods section had spotted salamanders. The absence of spotted salamanders in these six suitable pools -- and the entire Lawrence Woods section -- could indicate some historical land use incompatible with spotted salamander populations (as observed in other amphibians; Piha et al. 2007).

In the Fells study area, newts occurred only in three vernal pools of the largest section- the Southwestern Fells (Figure 28). These newts may need the largest sections to persist, because the documented metapopulation structure of the red-spotted newt requires a matrix of source-sink pools (Gill 1978) and Fells roads may reduce connectivity between habitats (Vos and Chardon 1998, Carr and Fahrig 2001). Newts are far more abundant in other areas in New England where large permanent ponds often provide the only breeding habitat and adult over-wintering sites (personal observation) in an area. However, the small, ephemeral nature of the vernal pools used by newts in my research bear similarity to those described in Gill (1978), so newts in the Fells likely exhibit a similar metapopulation structure.

What accounts for the more infrequent occurrence of spotted salamanders and newt breeding sites? Perhaps these species are adapted to an interconnected matrix of suitable breeding sites and are therefore limited to suitably, large parts of the Fells where such a matrix is not disrupted. The apparent clumped distribution of salamander and newt-breeding sites may also be a consequence of their inferior colonizing abilities and more demanding habitat requirements. Hanski (1999) described four conditions for a metapopulation structure: 1) habitat patches support local breeding populations, 2) no single population is large enough to ensure long-term survival, 3) patches are not too isolated to ensure colonisation, and 4) local dynamics are sufficiently asynchronous to make simultaneous extinction of all local populations unlikely. In this research I only tested conditions 1 and 3- 2 is discussed in greater depth below and 4 is assumed.

Anurans tend to be better dispersers than caudates (Semlitsch and Bodie 2003, Smith and Green 2005). In Minnesota, Lehtinen and Galatowitsch (2001) found that blue-spotted salamanders and red-spotted newts -- though present in nearby natural wetlands -- were absent from restored wetlands where American toads and a variety of other anurans had established themselves. However, in the case of newts, Gill (1978) found evidence that terrestrial red-spotted newt efts are excellent long-distance colonizers, able to move more than a kilometer from their natal ponds. In contrast, spotted salamander adults were documented dispersing from the breeding pool on average only 64.2-192.0 (see review Semlitsch 1997), while the maximum recorded movement for an individual was 756.0 m

(Smith and Green 2005). The distances between all but one spotted salamander breeding pool (the pool in the Virginia Wood section) fall well below the maximum recorded dispersal ability of the species (Smith and Green 2005). If the Virginia Wood pool is particularly isolated, it is of interest that spotted salamanders are abundant at this site (personal observation) and perhaps the particular characteristics which allow it to support a robust population allow it to be excluded from typical metapopulation dynamics by not meeting Hanski's second condition for a metapopulation structure.

I have encountered one other completely isolated spotted salamander population breeding in a lone vernal pool in the Mt. Auburn Cemetery Cambridge, MA.. Egg mass surveys of this pool seem to indicate a larger population than those found in most Fells pools containing spotted salamanders (Joe Martinez personal communication, personal observation). The three red-spotted newt pools were all less than 400 m apart-- much closer than Gill's (1978) documented maximum dispersal distance of over 1 km for this species. The distances between all breeding sites (vernal pools and ponds) of wood frogs, American toads and green frogs fall well below the maximum recorded dispersal distances of these species (2530 m for wood frogs, 6437.38 m for American toads and 4800 m for green frogs) (Smith and Green 2005), so colonists of these species should disperse readily among vernal pools and ponds within Fells sections - as18% of juvenile wood frogs have been shown to do (Berven and Grudzien 1990). Species that disperse to colonize new pools maintain genetic connectivity and can reestablish a subpopulation where extirpation has occurred.

My observations on species breeding site distributions may be due to historical artifacts coupled with varying degrees of natal pool fidelity and a tolerence of a wider range of hydroperiods in many of the study anurans -- and not poorer colonization abilities of salamanders. More research is needed concerning the relative natal pool fidelity and dispersal capabilities of the array of amphibian species native to Massachusetts. Finally, metapopulation dynamics may be operating when the subpopulations involved are small and vulnerable to local extinction, but the evidence above suggests that, in some cases, exceptional populations may only require a single high-quality breeding pool or pond.

Ecological research has revealed a consistently negative effect of urbanization on amphibian species richness and occurrence (Windmiller 1996, Rubbo and Kiesecker 2005, Pillsbury and Miller 2008). To preserve amphibians in reservations, managers must strive to reduce negative impacts on amphibian habitat, while researchers must continue to identify what amphibian species require in a landscape to thrive, and which species are most sensitive to human development. I hope that this work will help to address questions pertaining to land management and amphibian species preservation in general – especially in the case of the Middlesex Fells. Follow-up surveys in the coming years will test for changes in amphibian distribution and occurrence within the reservation. Such large-scale surveys of vernal pools are a means of measuring the health in the local ecosystem.

This research shows the extent to which biodiversity can persist, even without special attention or documentation, in the suburban environment. A popular site for day hikers, amateur naturalists, and mountain bikers, the Fells is within a short drive of millions of residents in the greater Boston metropolitan area, yet it is woefully under-studied and under-funded. It is hoped that this work will do some small part to increase public attention for and appreciation of this suburban reservation and its amphibian residents.

APPENDIX

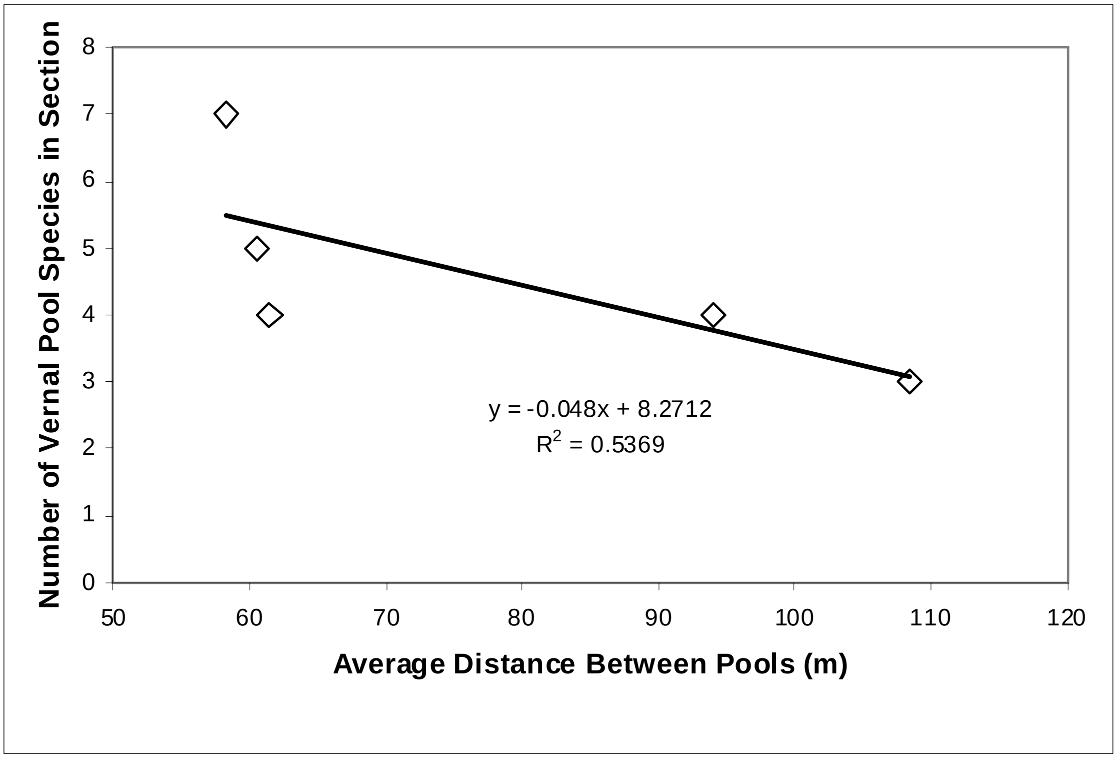

Figure 1. Number of vernal pool-breeding amphibians, as a function of average distance between pools (m) in five sections. The Virginia Wood section is excluded because it contained only one pool.

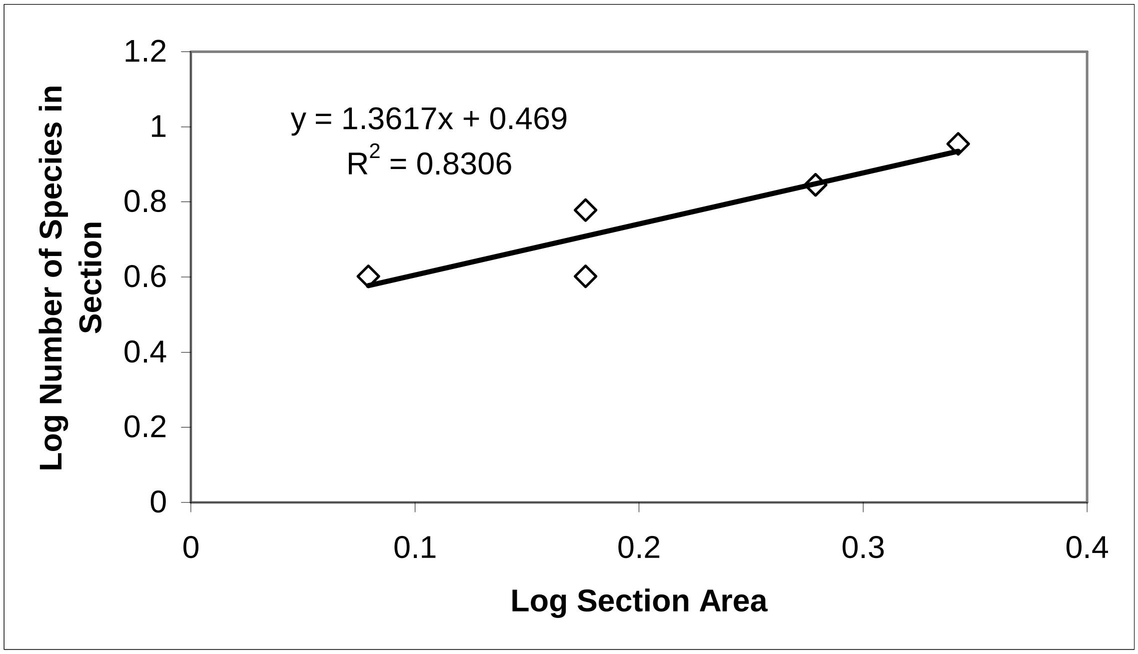

Figure 2. Number of amphibian species found to breed in six sections of the Middlesex Fells Reservation, as a function of Log section area.

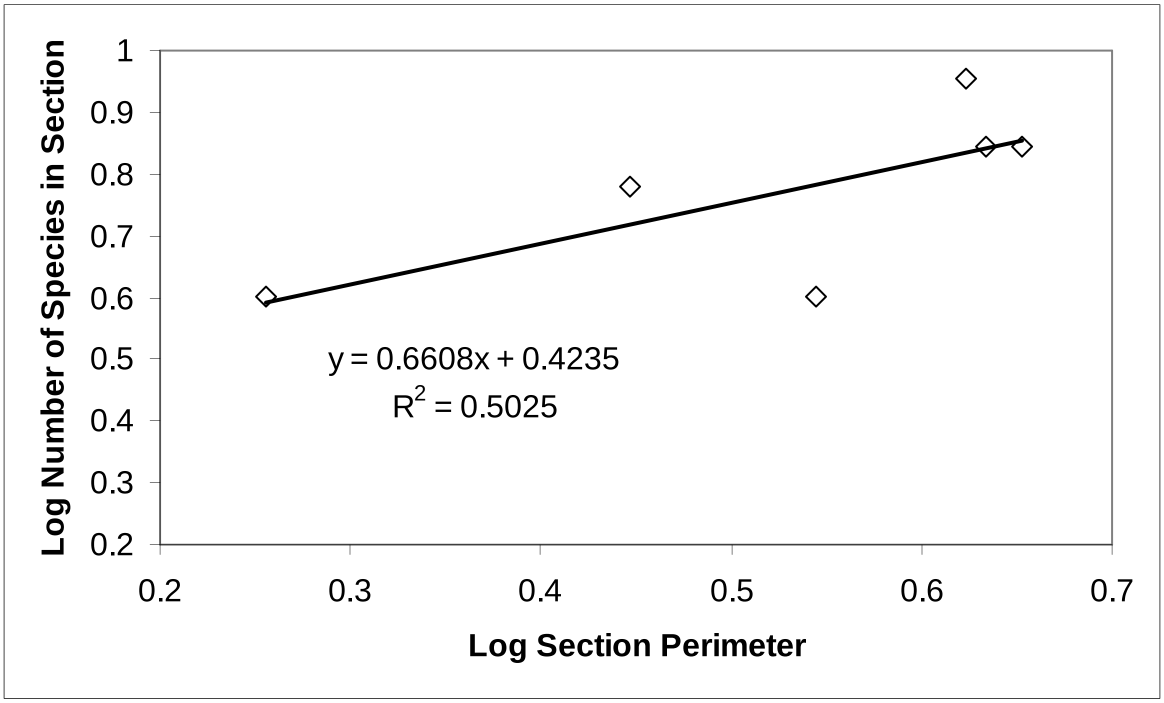

Figure 3. Number of log-transformed species found to breed in six sections of the Middlesex Fells Reservation, as a function of log-transformed section perimeter (km).

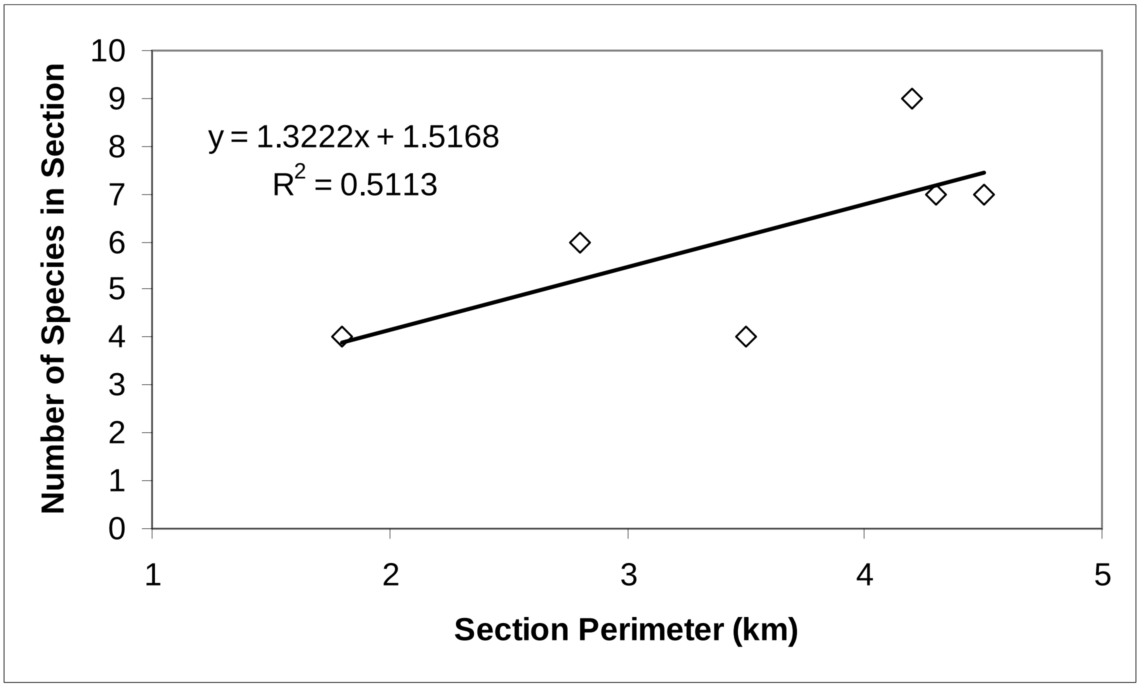

Figure 4. Number of species found to breed in six sections of the Middlesex Fells Reservation, as a function of section perimeter (km).

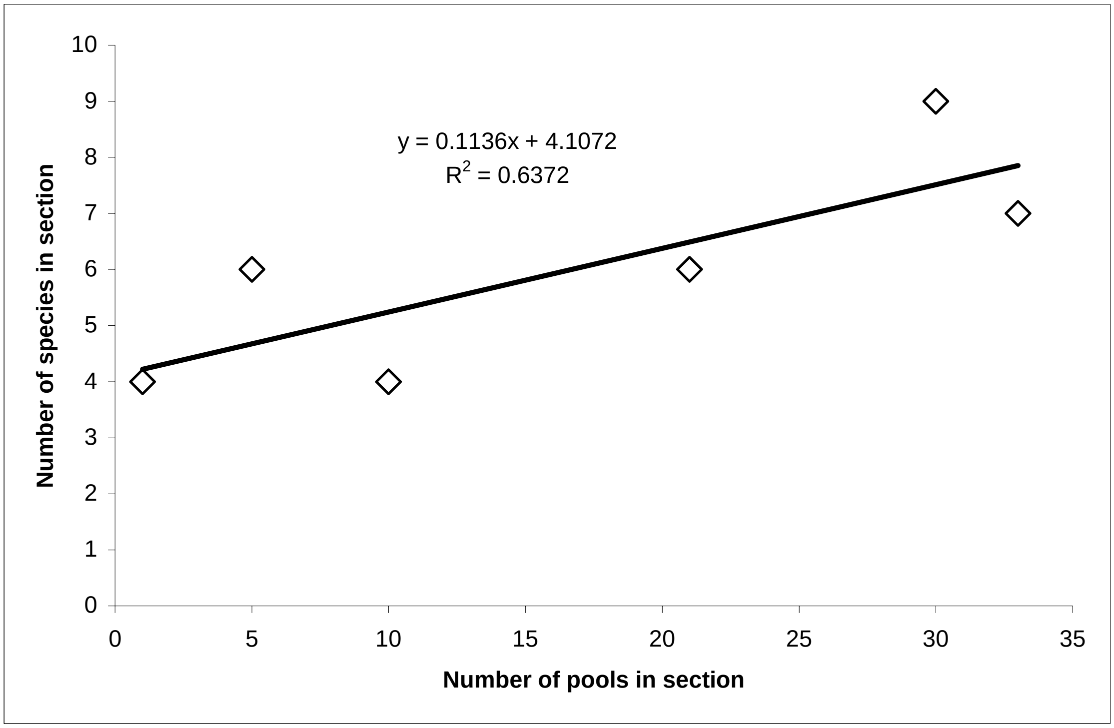

Figure 5. Number of amphibian species in six sections of the Middlesex Fells Reservation, as a function of the number of vernal pools per section.

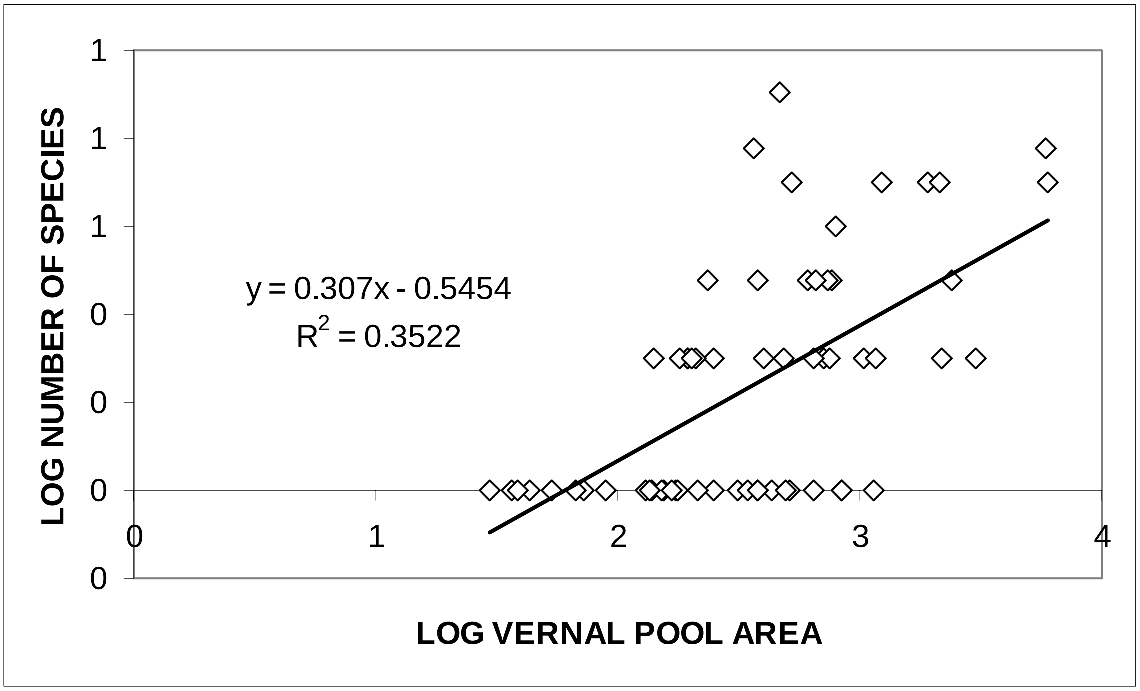

Figure 6. Log-transformed number of amphibian species, as a function of the log-transformed area of vernal pools.

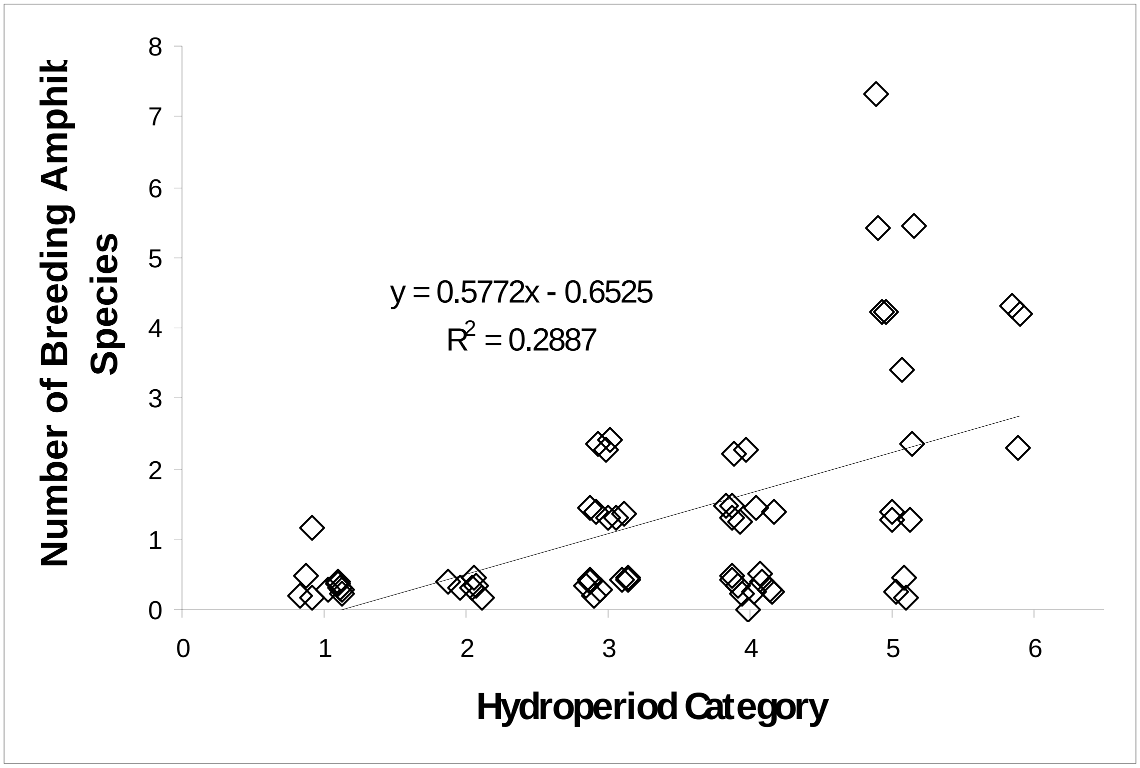

Figure 7. Number of amphibian species breeding in vernal pools, as a function of pool hydroperiod score.

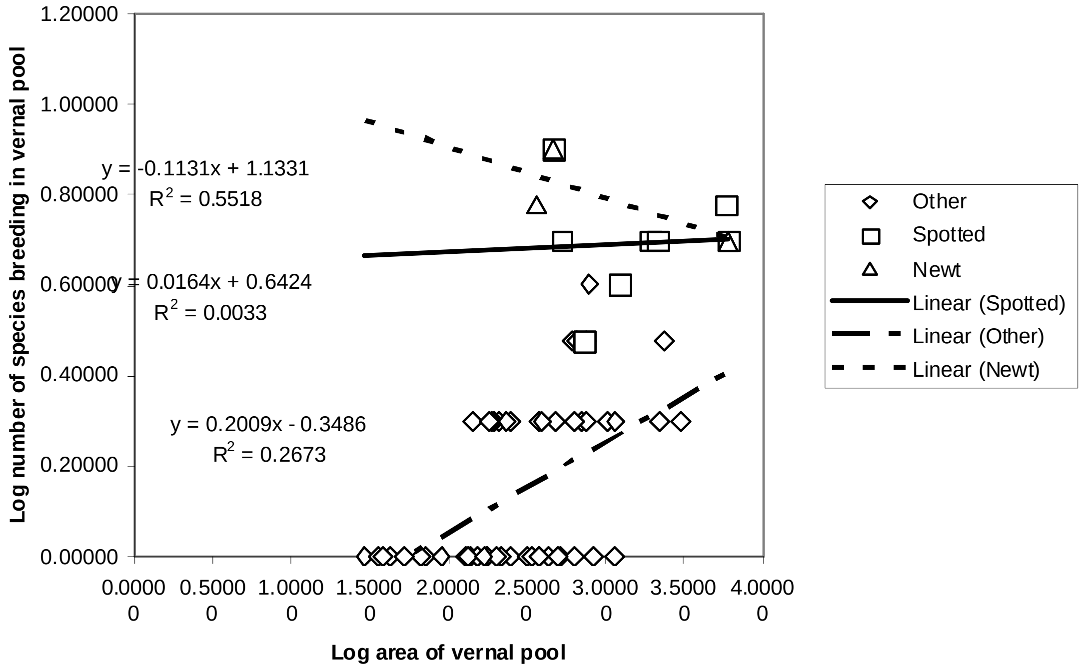

Figure 8. Log number of amphibian species breeding in vernal pools, as a function of the log area of vernal pools for: spotted salamander pools, newt pools, and other pools (those containing no newts or salamanders).

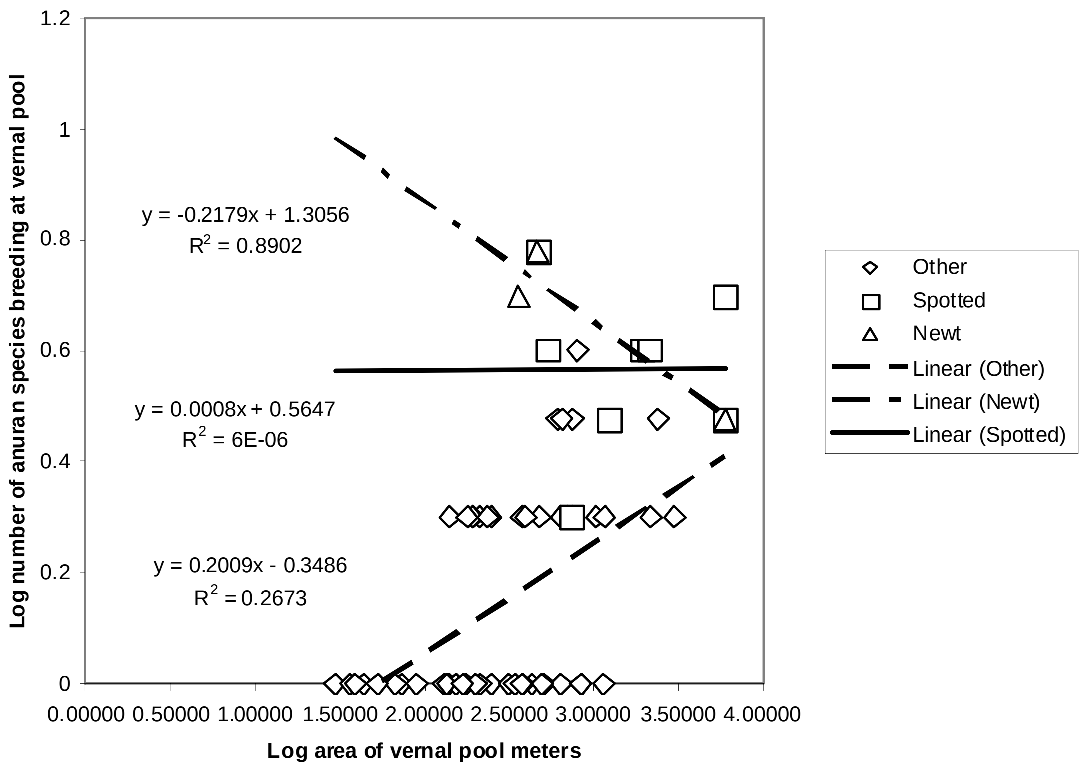

Figure 9. Log number of anuran species breeding in vernal pools, as a function of the log area of vernal pools for: spotted salamander pools, newt pools, and other pools (those containing no newts or salamanders).

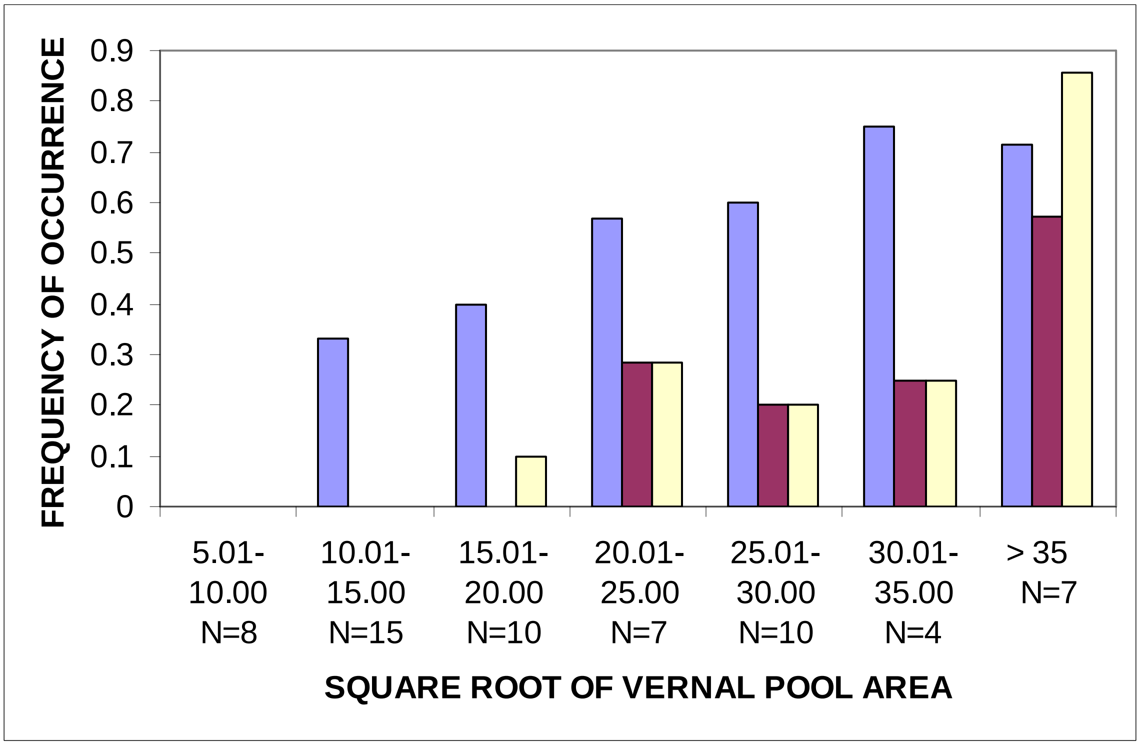

Figure 10. The frequency of species occurrence as a function of classes representing the square root of vernal pool area for three species: wood frog (blue), spring peeper (yellow) and spotted salamander (red).

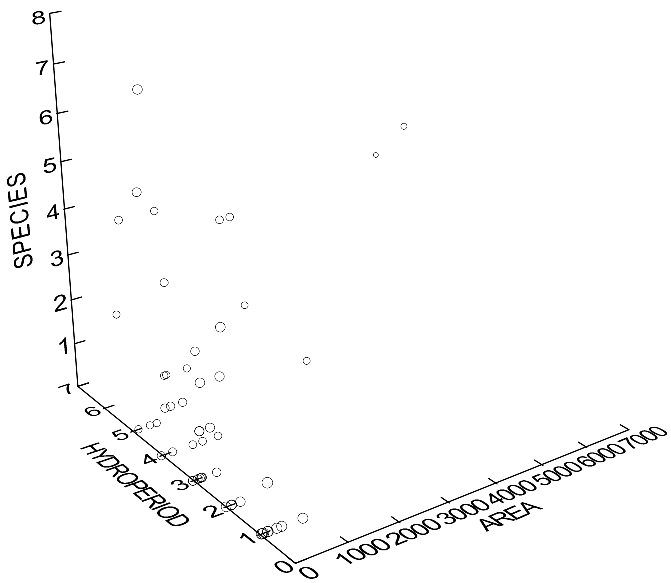

Figure 11. Bi-variate scatterplot of the effects of area and hydroperiod on species number at vernal pools.

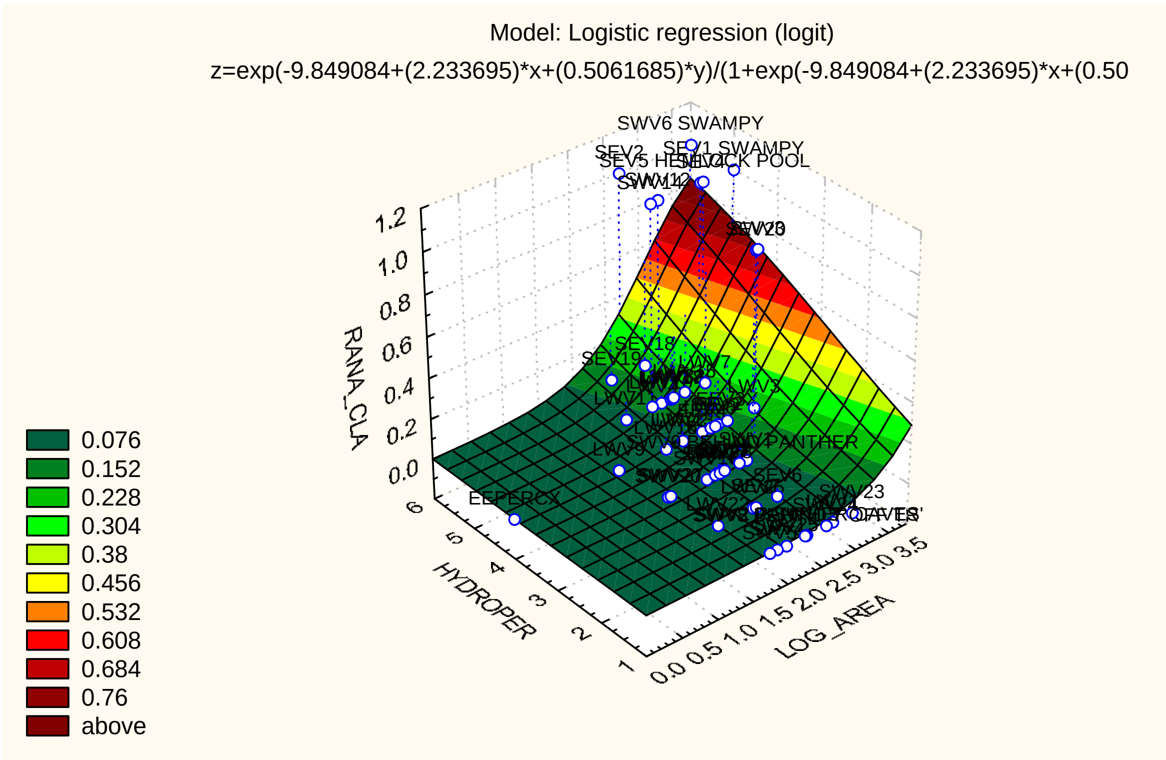

Figure 12. Surface plot showing the relationship between green frog occurrence, log-transformed vernal pool area and hydroperiod score.

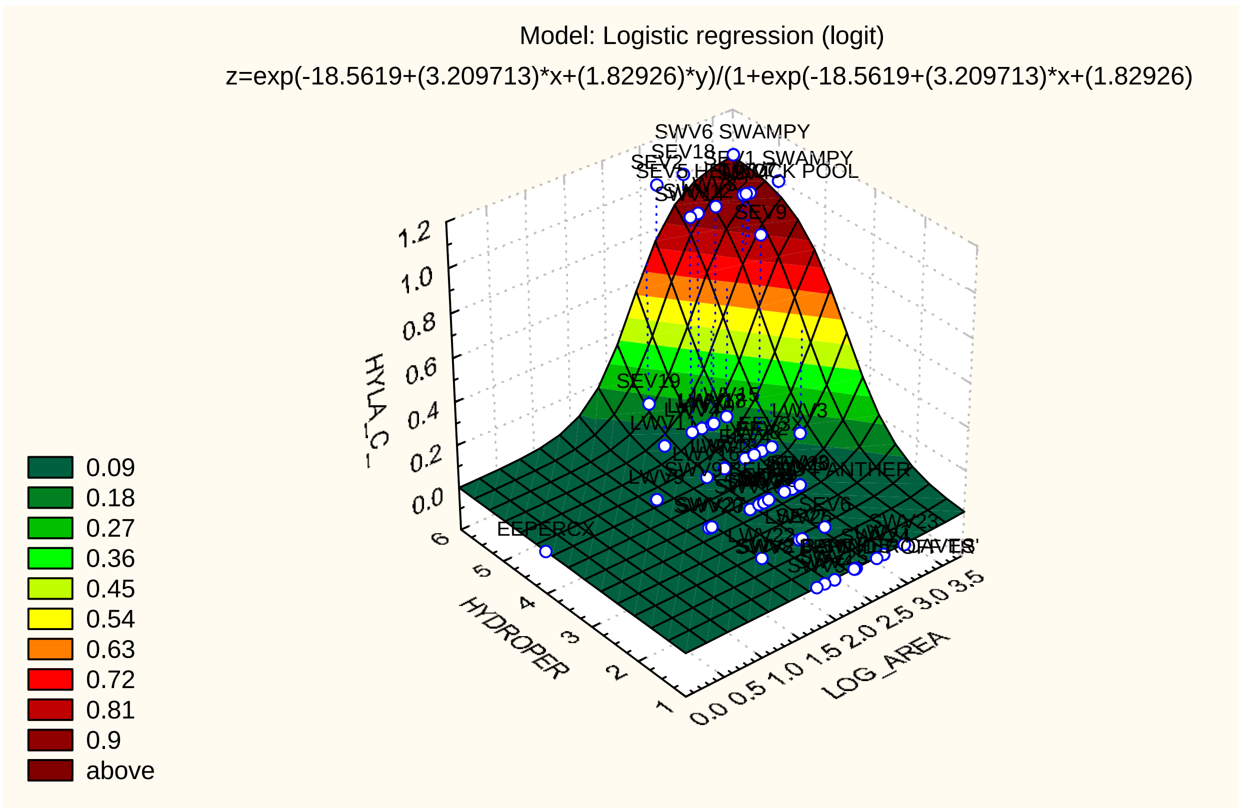

Figure 13. Surface plot showing the relationship between spring peeper occurrence, log-transformed vernal pool area and hydroperiod score.

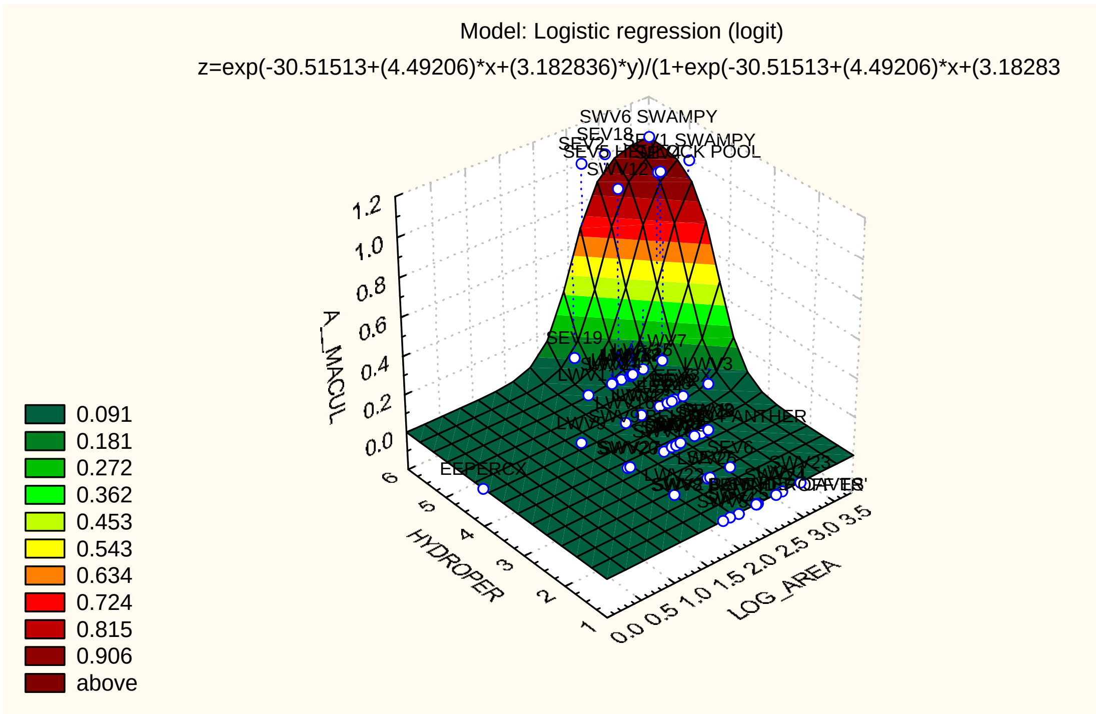

Figure 14 . Surface plot showing the relationship between spotted salamander occurrence, log-transformed vernal pool area and hydroperiod score.

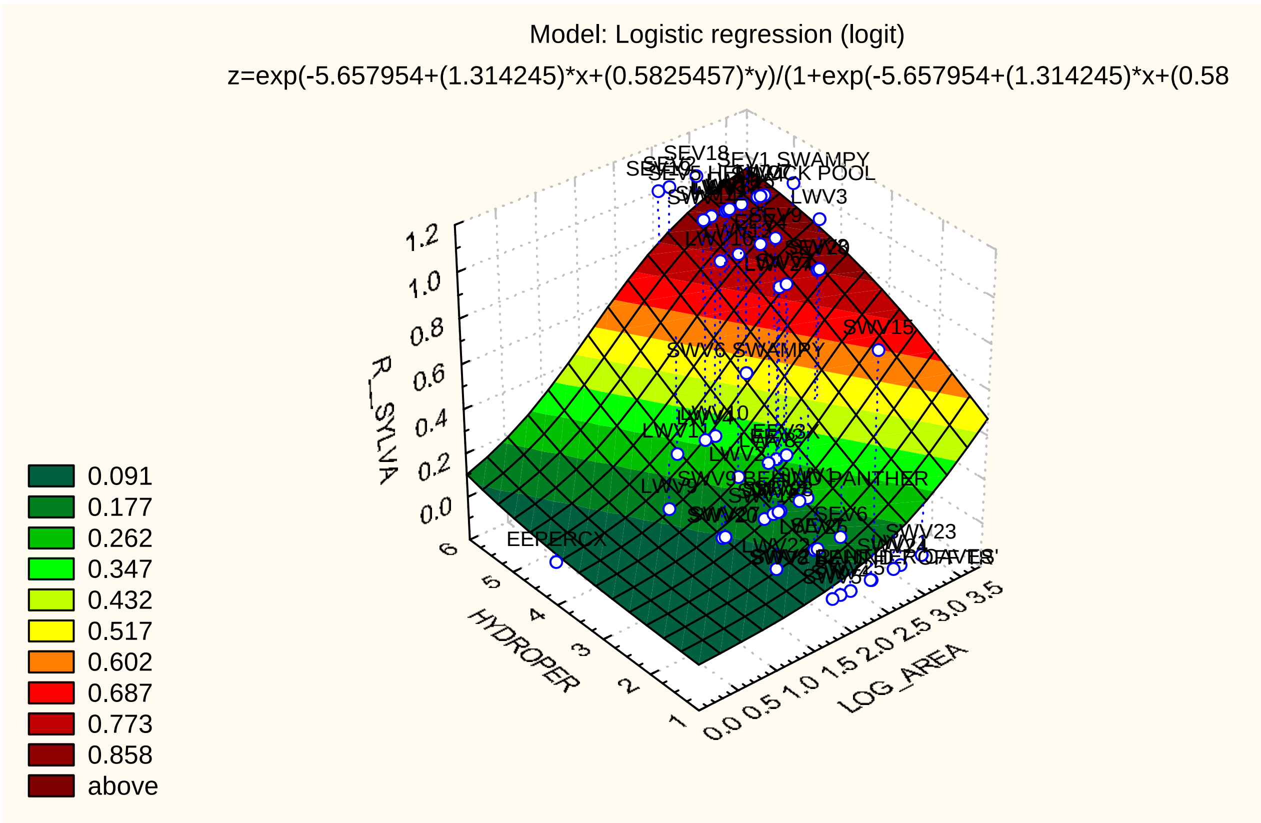

Figure 15. Surface plot showing the relationship between wood frog occurrence, log-transformed vernal pool area and hydroperiod score.

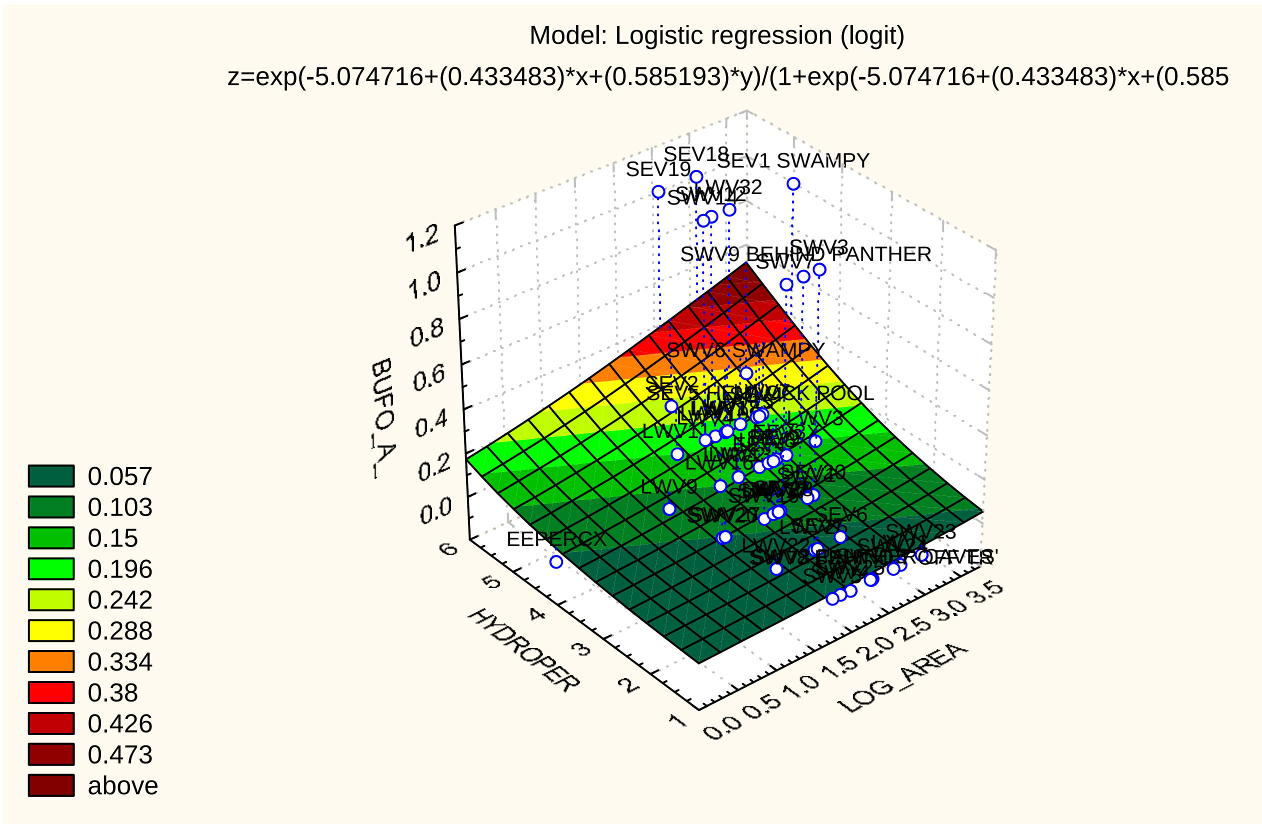

Figure 16. Surface plot showing the relationship between American toad occurrence, log-transformed vernal pool area and hydroperiod score.

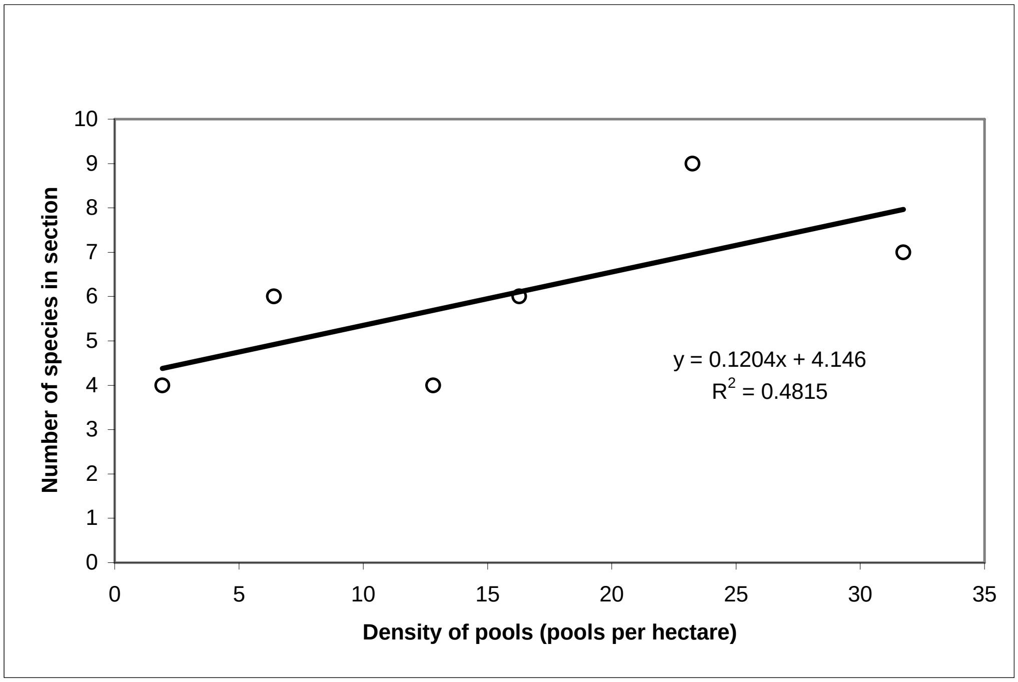

Figure 17. Number of amphibian species breeding in sections, as a function of the number of vernal pools per hectare in each section.

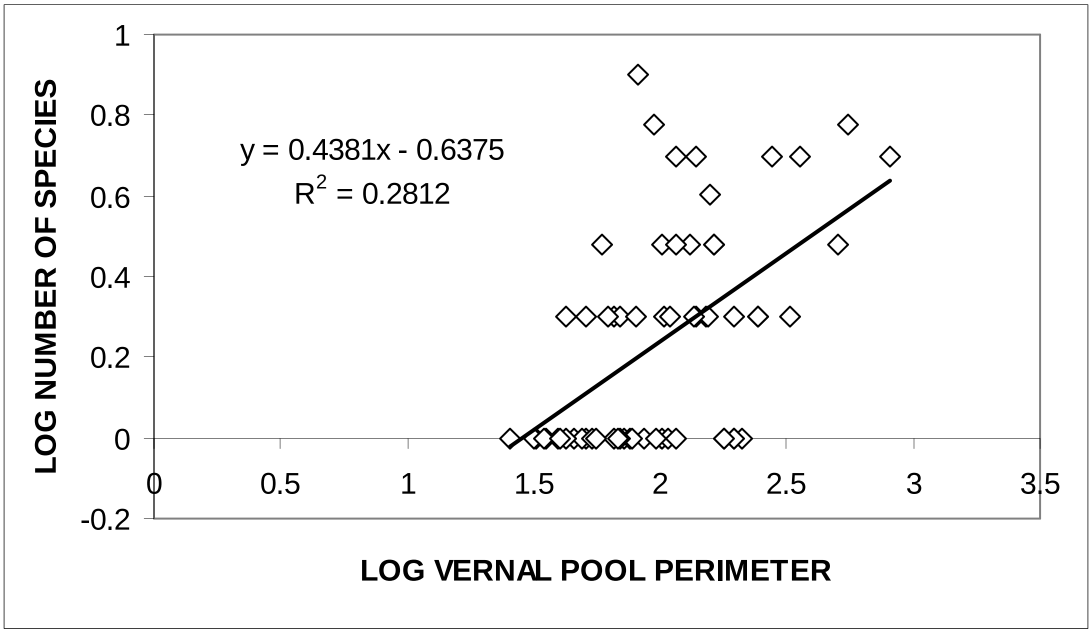

Figure 18. Log-transformed number of amphibian species breeding in vernal pools, as a function log-transformed pool perimeter (m).

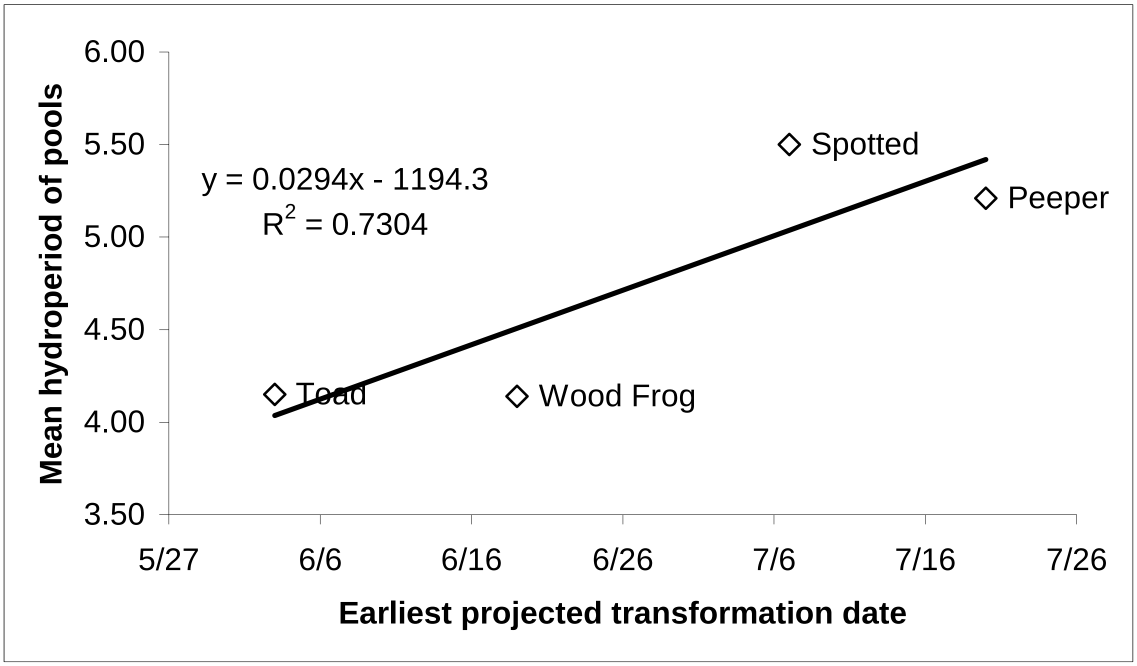

Figure 19. Mean hydroperiod of vernal pools as a function of the earliest projected transformation date of: American toad, wood frog, spotted salamander and spring peeper.

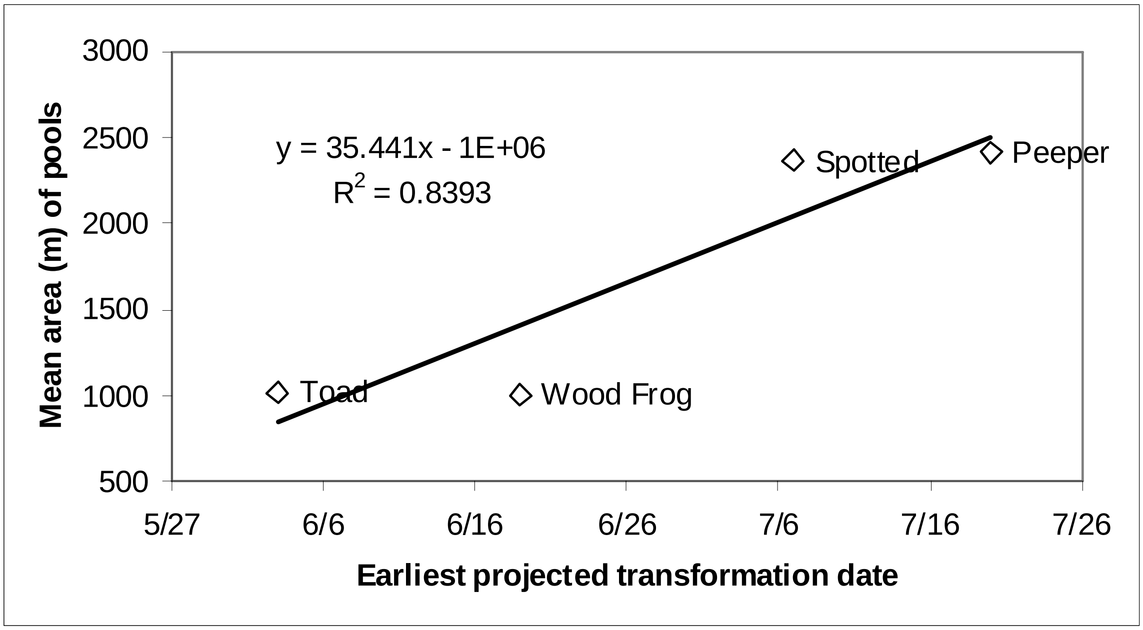

Figure 20. Mean area (m) of vernal pools as a function of the earliest projected transformation date of: American toad, wood frog, spotted salamander and spring peeper.

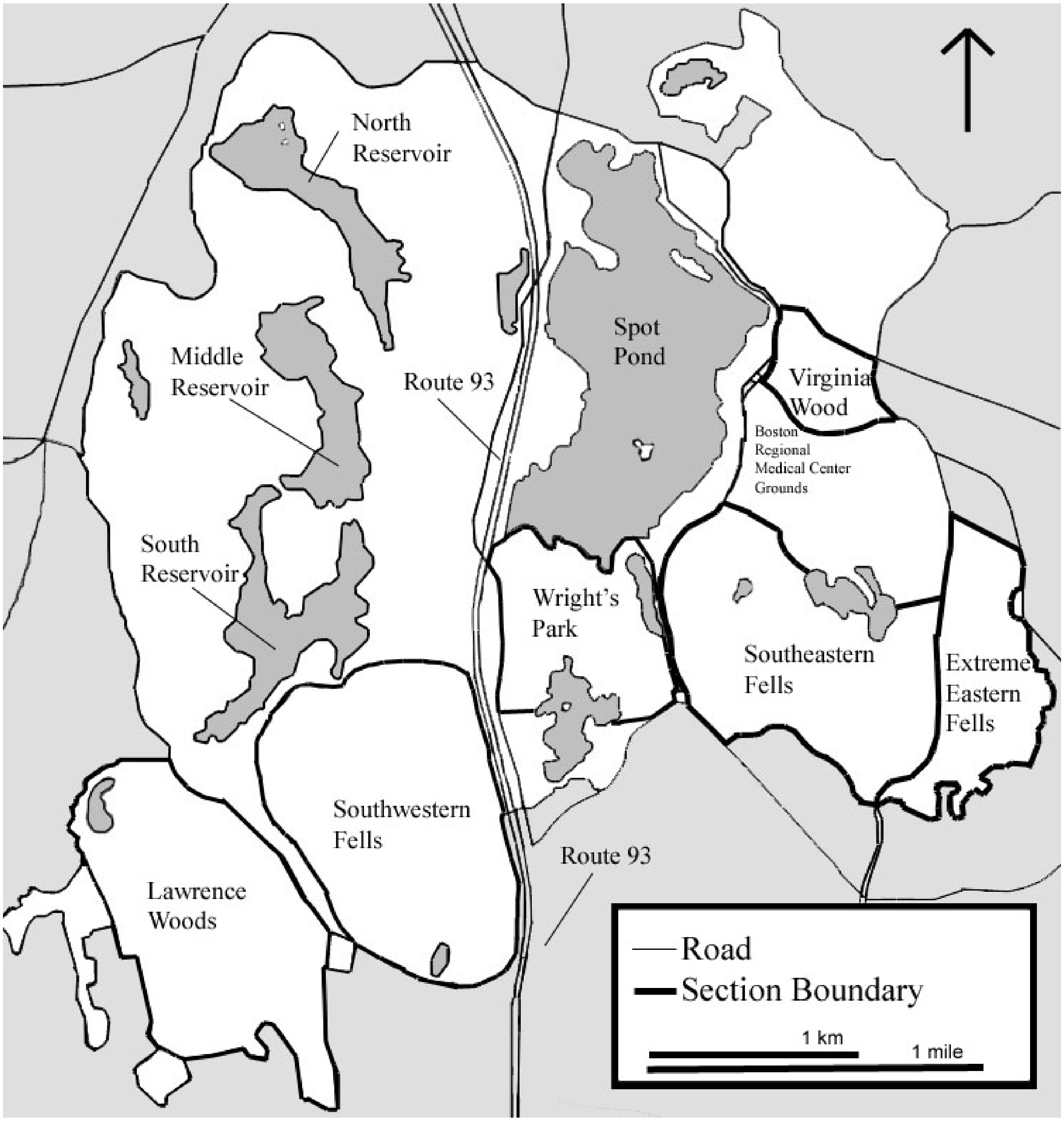

Figure 21. The Middlesex Fells Reservation with study sections.

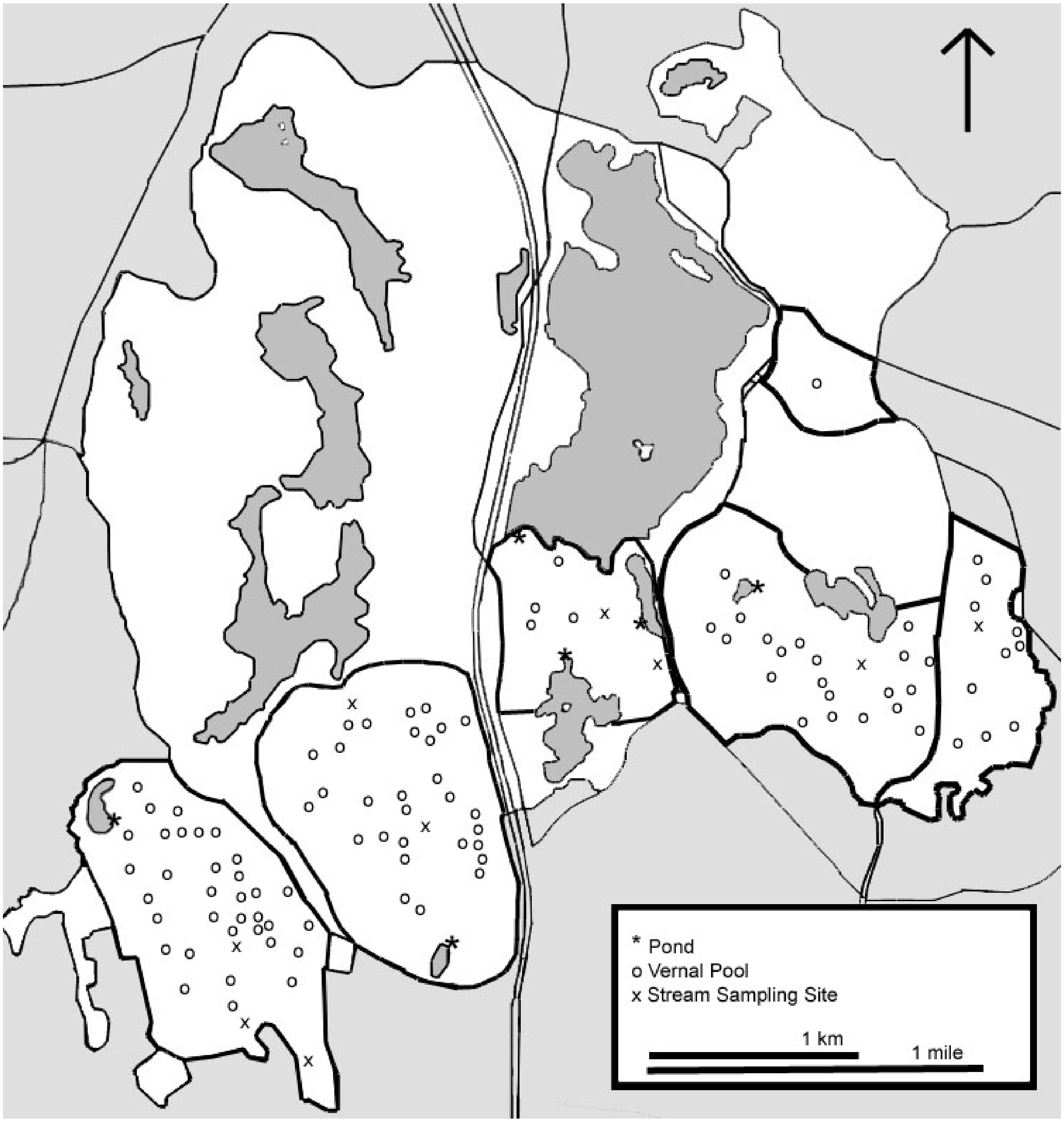

Figure 22. The Middlesex Fells Reservation with ponds, vernal pools and stream sampling sites marked.

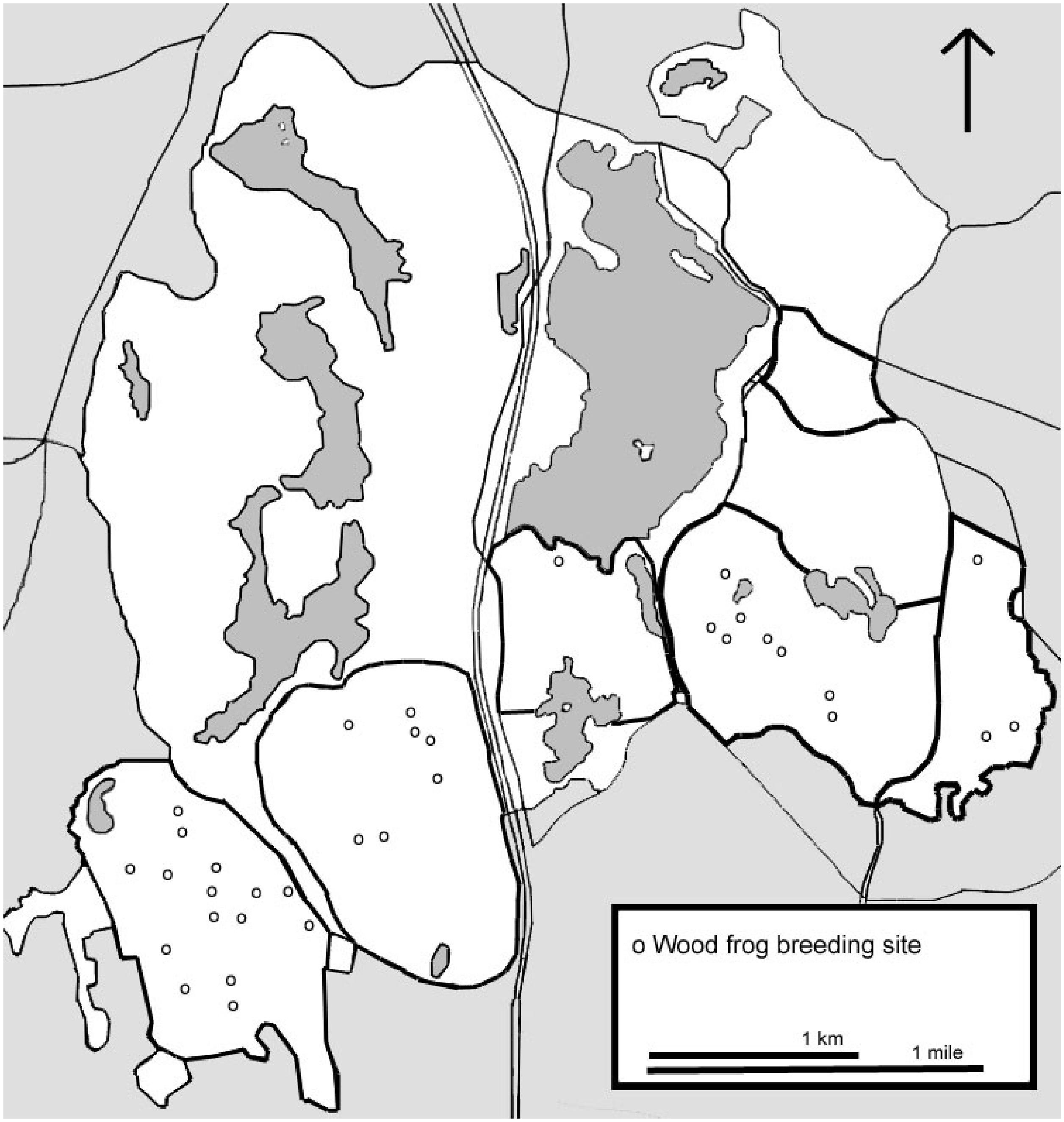

Figure 23. The Middlesex Fells Reservation with documented wood frog breeding sites marked.

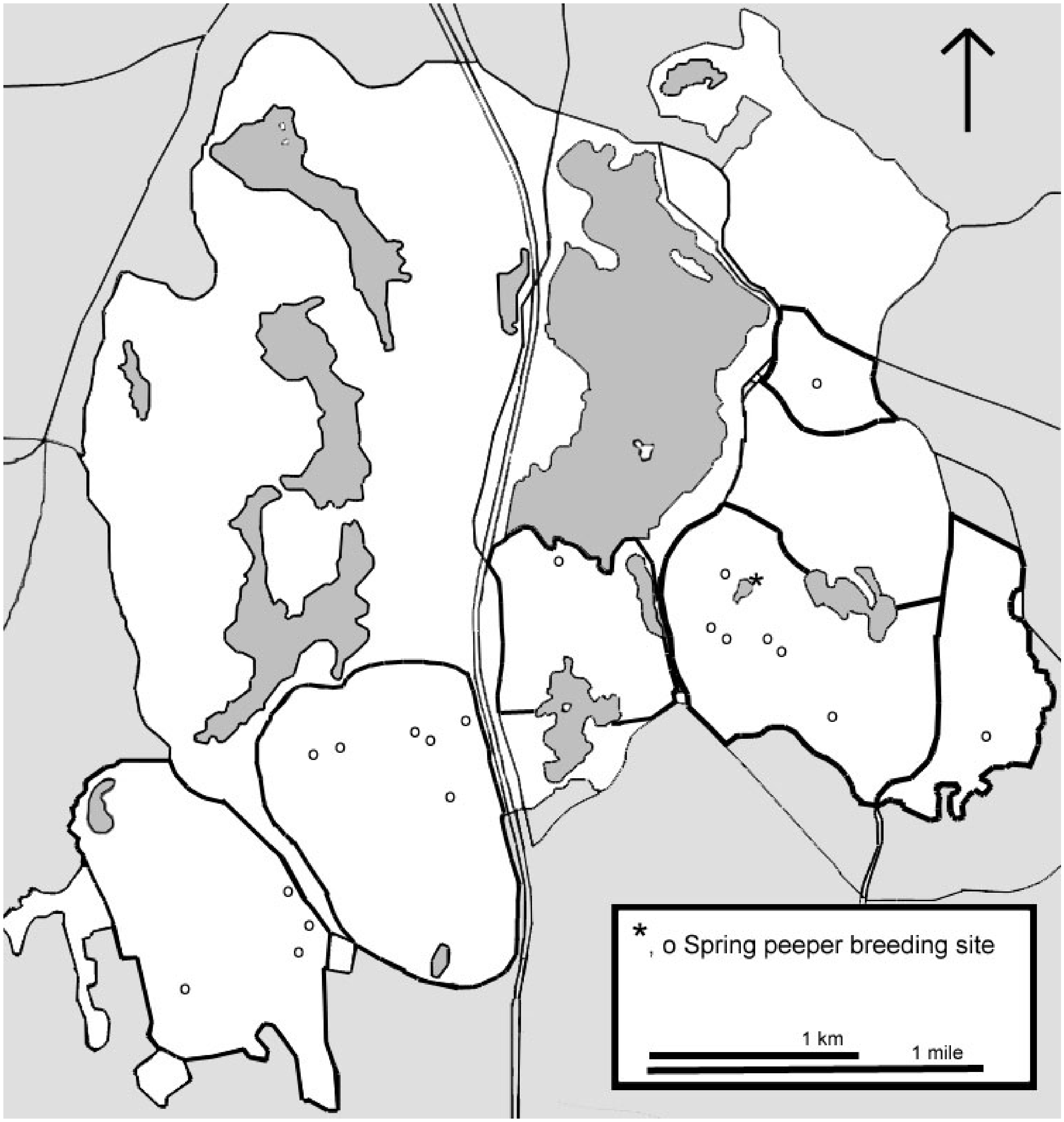

Figure 24. The Middlesex Fells Reservation with documented spring peeper breeding sites marked.

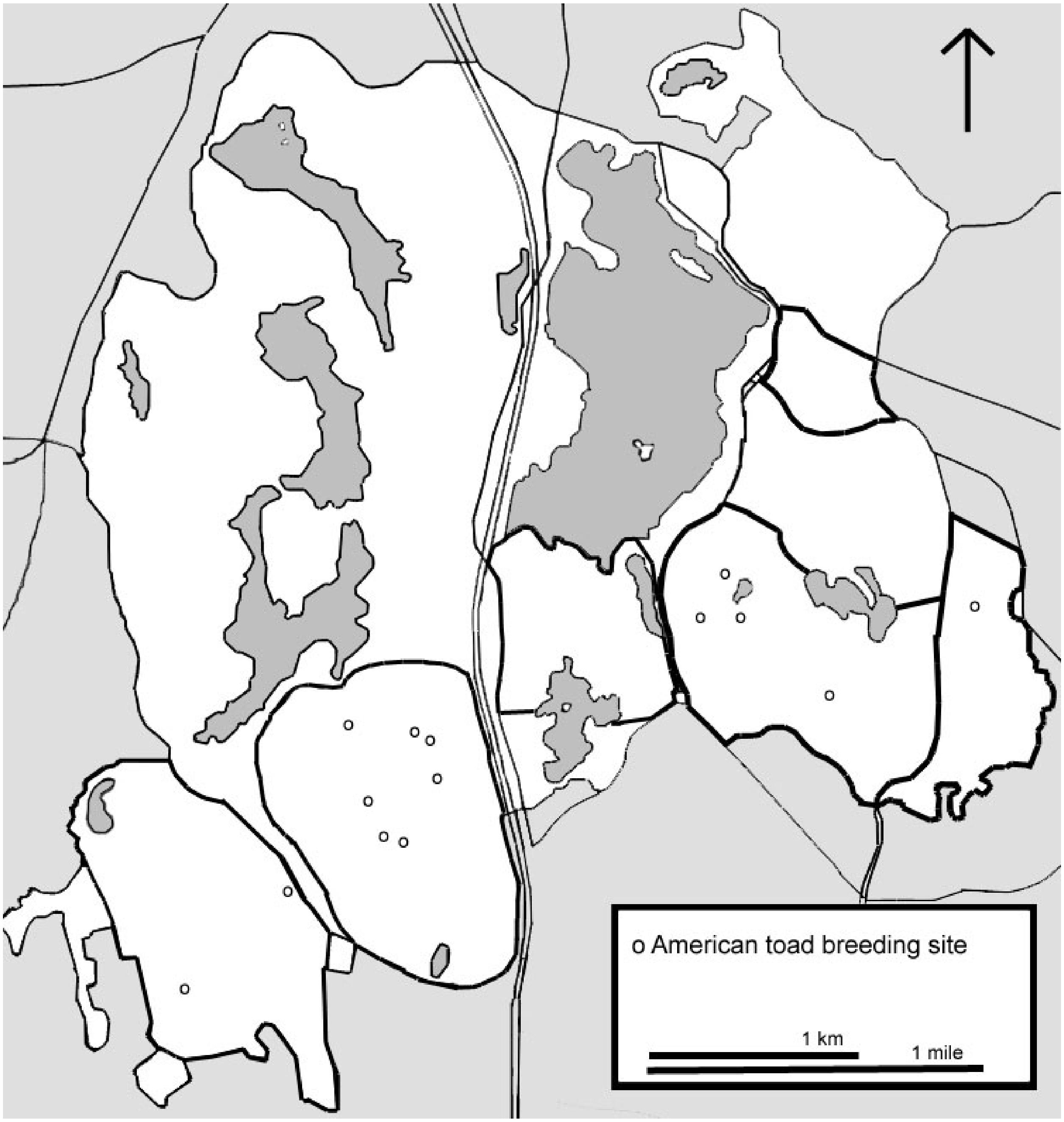

Figure 25. The Middlesex Fells Reservation with documented American toad breeding sites marked.

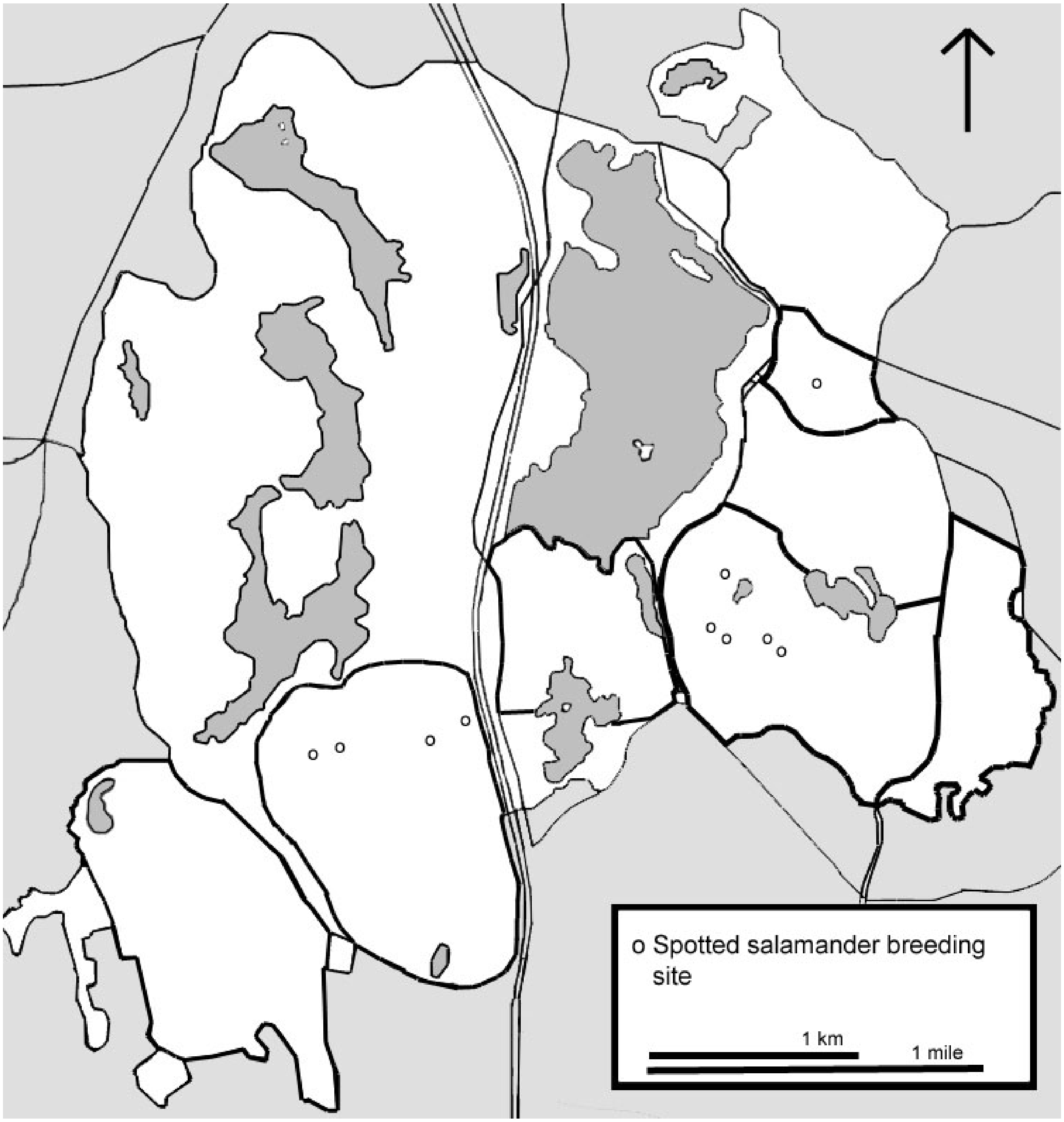

Figure 26. The Middlesex Fells Reservation with documented spotted salamander breeding sites marked.

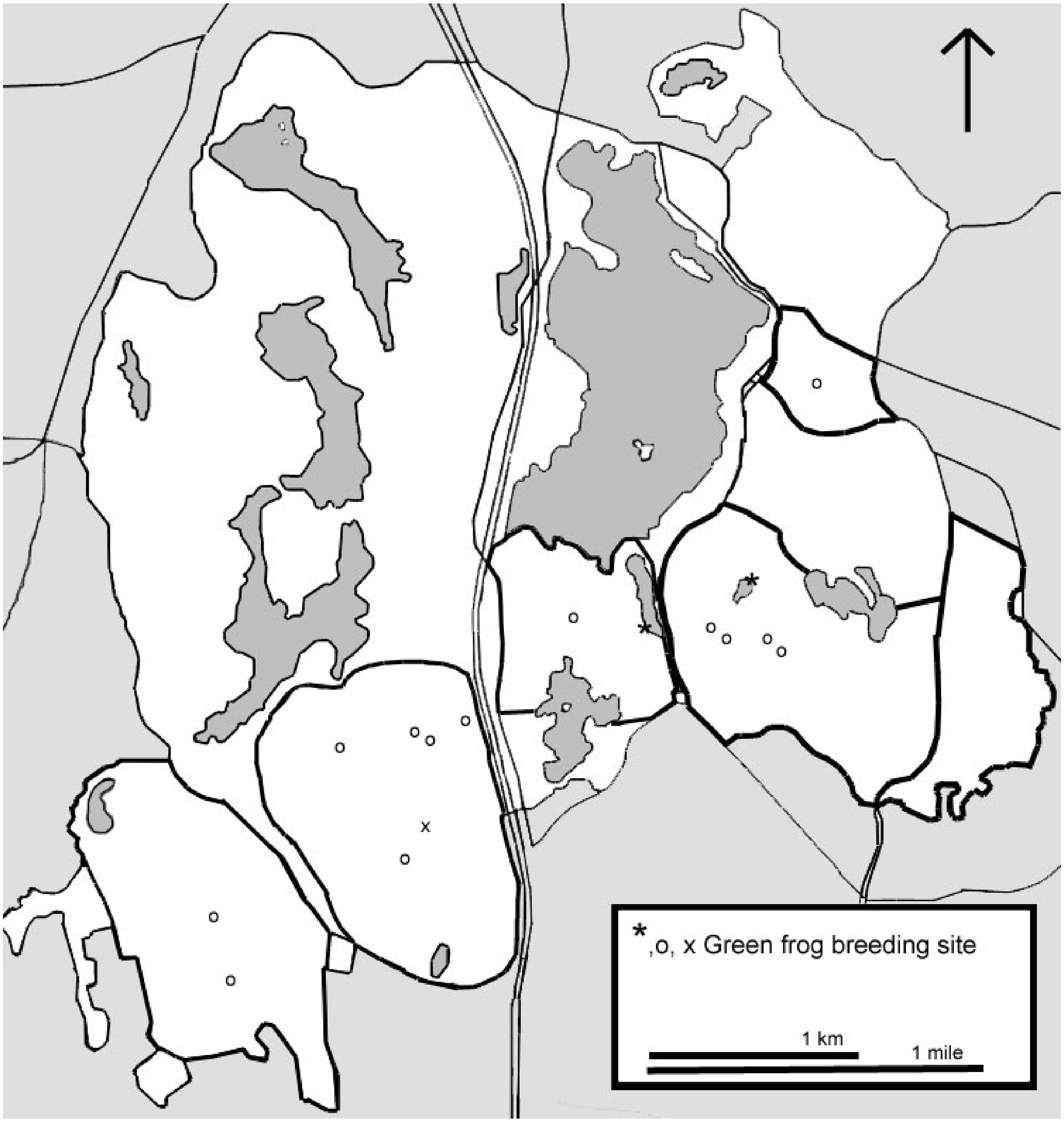

Figure 27. The Middlesex Fells Reservation with documented green frog breeding sites marked.

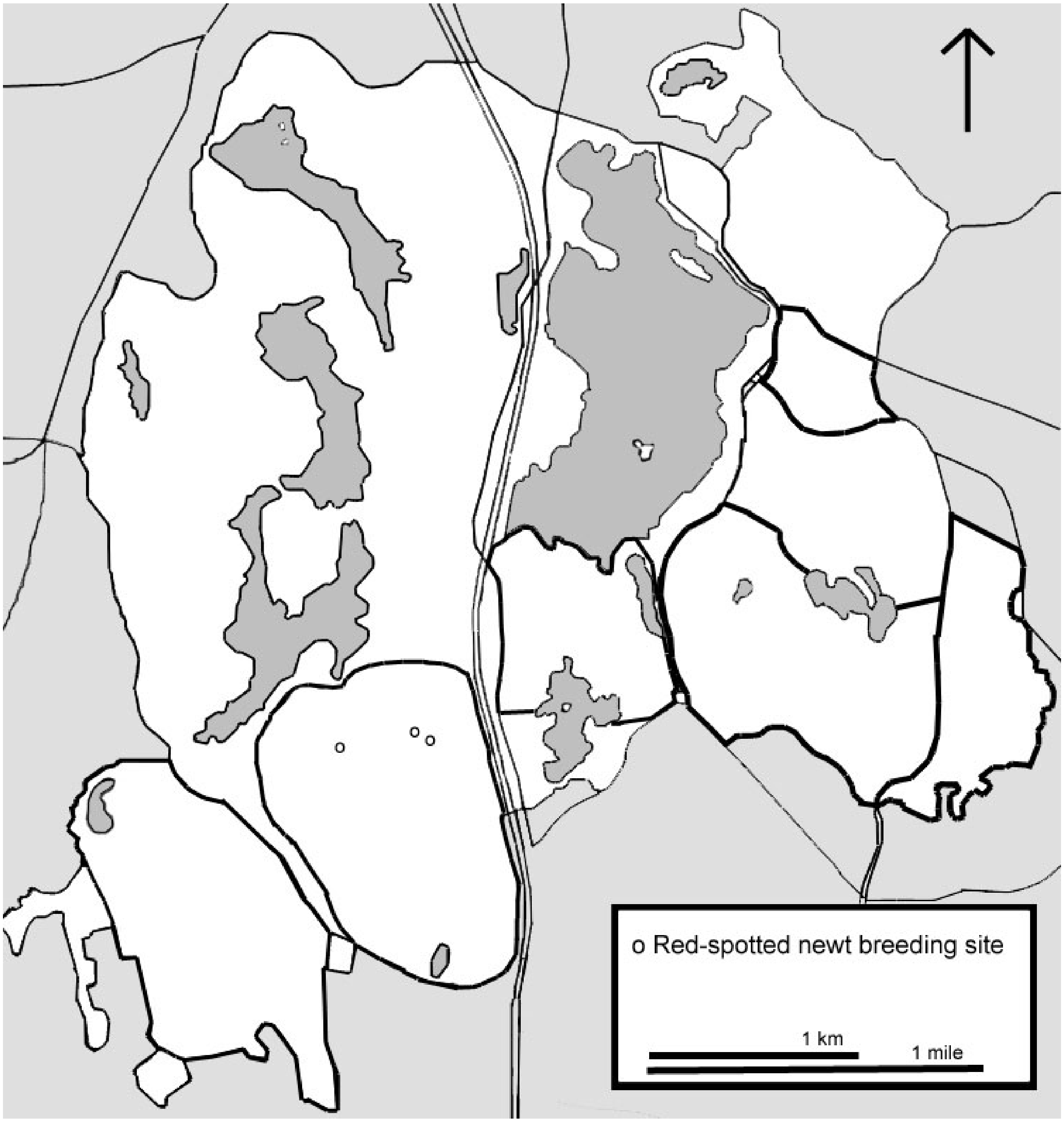

Figure 28. The Middlesex Fells Reservation with documented red-spotted newt breeding sites marked.

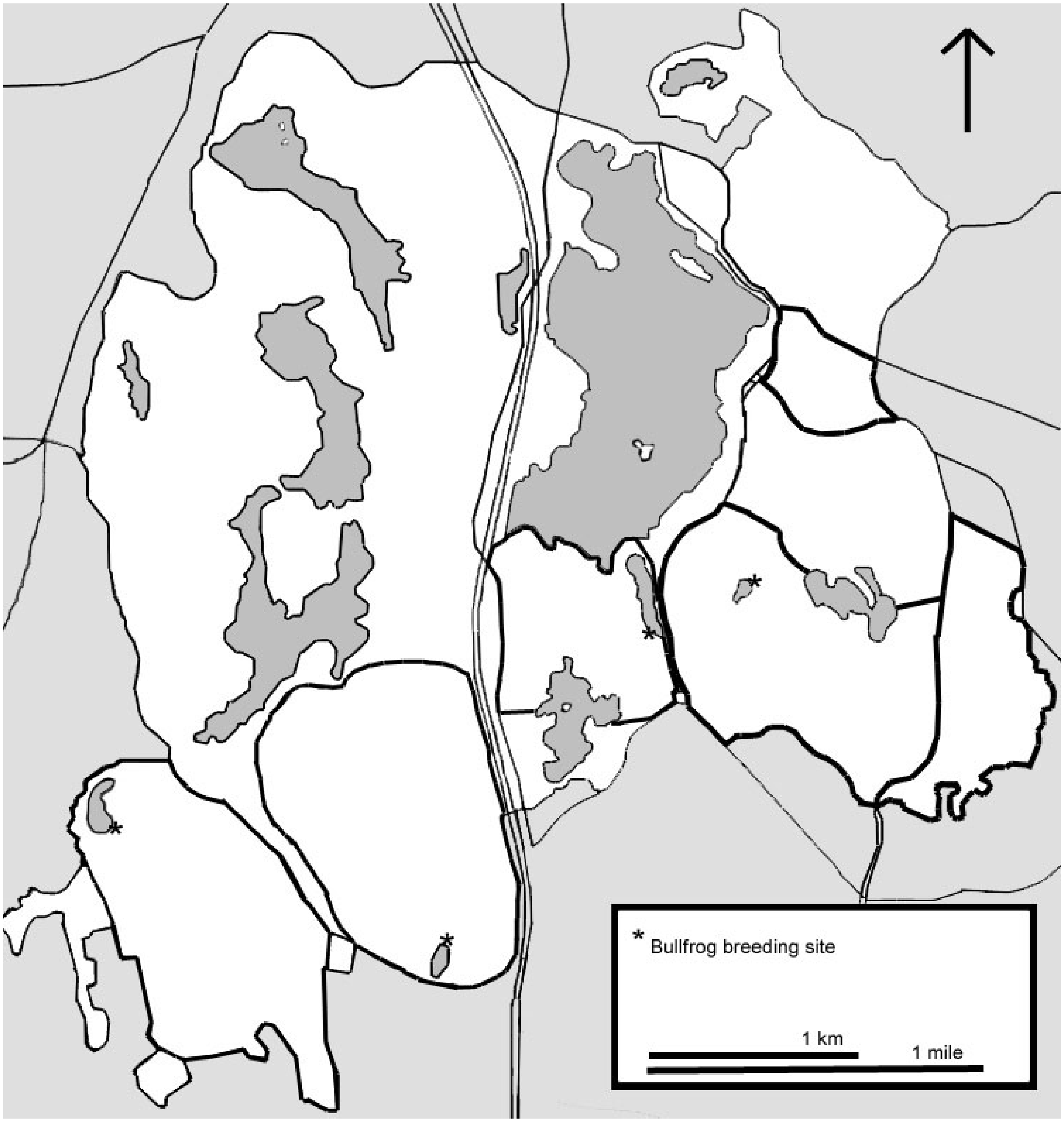

Figure 29. The Middlesex Fells Reservation with documented bullfrog breeding sites marked.

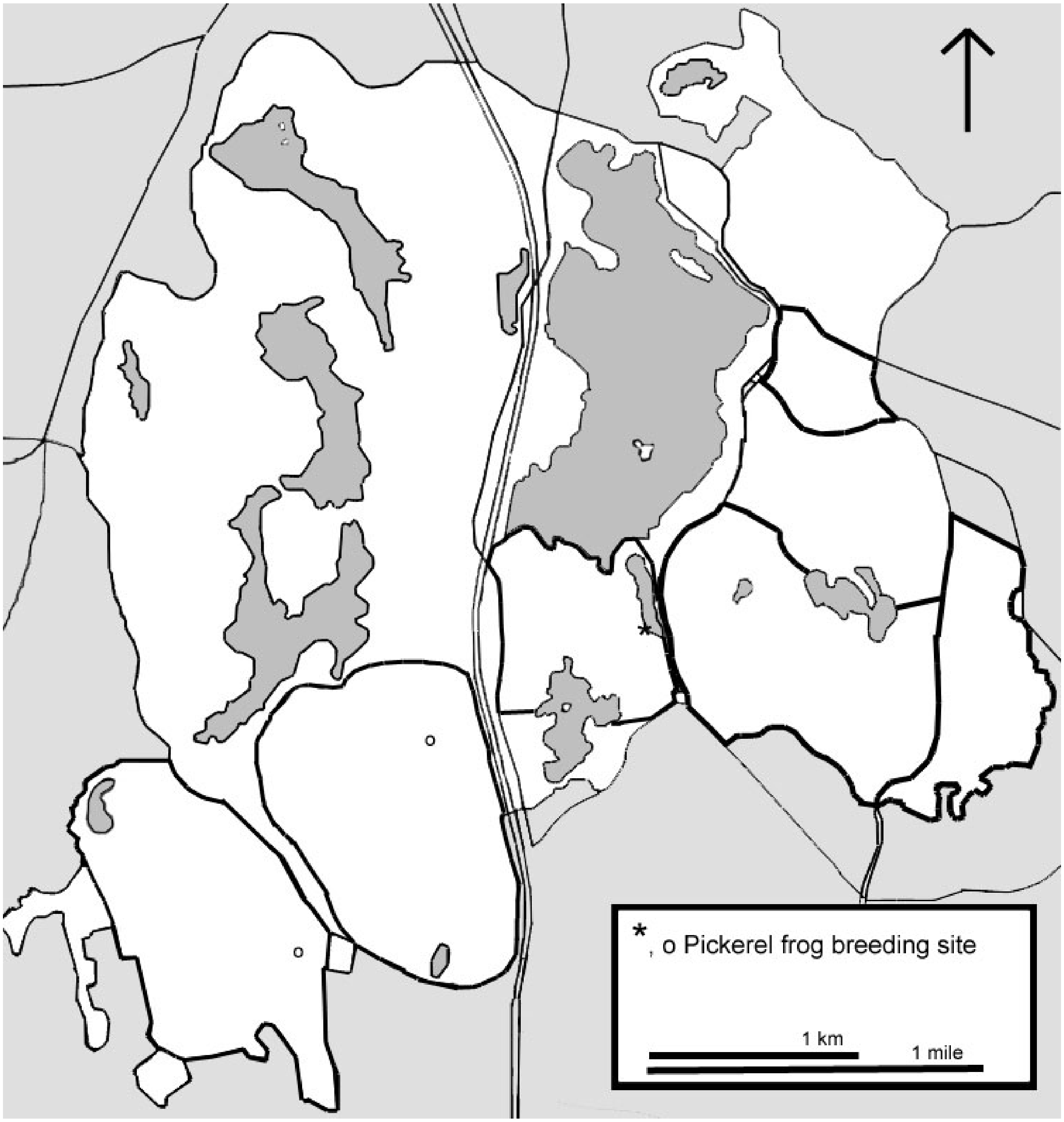

Figure 30. The Middlesex Fells Reservation with documented pickerel frog breeding sites marked.

Table 1. Summary of section characteristics such as: size, number of pools, number of ponds and diversity of amphibians.

Section	area km^2	perimeter km	# water bodies	# pools	#ponds	species	% pools occupied by breeding amphibians
Virginia Wood	0.2	1.8	1	1	0	4	100%
Southeastern	0.9	4.5	23	21	1	6	52%
Wright's	0.5	2.8	8	5	3	6	60%
Extreme Eastern	0.5	3.5	10	10	0	4	30%
Lawrence Woods	0.9	4.3	34	33	1	7	48%
Southwestern	1.2	4.2	31	29	1	9	41%

Table 2. Amphibian species encountered in the Middlesex Fells Reservation.

Species	common name	breeding/ larval habitat	occurrence
Caudata:			
Ambystoma maculatum	Spotted salamander	vernal pools	10 sites
Notophthalmus v. viridescens	Red-spotted newt	ponds, vernal pools, streams	3 sites
Plethodon cinerus	Red-backed salamander	terrestrial	Throughout (terrestrial breeder)
Anura:			
Bufo a. americanus	Eastern American toad	vernal pools/ shallow ponds	14 sites
Pseudacris crucifer	Northern spring peeper	vernal pools/ shallow ponds	19 sites
Rana catesbeiana	Bullfrog	ponds	4 sites
Rana clamitans melanota	Green frog	vernal pools/ ponds/ streams	27 sites
Rana sylvatica	Wood frog	vernal pools	33 sites
Rana palustris	Pickerel frog	ponds /pools/ streams	3 sites

Table 3. Summary statistics for the vernal pool area (m) of six species.

Area of pools for:	wood frog	Am. toad	newt	spotted salamander	spring peeper	green frog
Mean	1004.8	1019.464	2283.2	2365.313	2413.877	5220.35
Standard Error	226.7525	495.5719	1870.49	813.6711	632.4956	2650.3
Median	645.7	464.9	464.9	1574	1925.3	2027.7
Mode	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
Standard Deviation	1199.861	1643.626	3239.785	2301.41	2280.495	8380.985
Sample Variance	1439667	2701506	10496204	5296486	5200658	70240915
Kurtosis	9.998674	10.0941	#DIV/0!	-0.40089	-0.25068	7.513486
Skewness	2.890128	3.136874	1.730047	1.156422	1.126064	2.639458
Range	5756.5	5696.4	5662.7	5558.8	6355.9	27685.4
Minimum	141	201.1	361	464.9	361	235.6
Maximum	5897.5	5897.5	6023.7	6023.7	6716.9	27921
Sum	28134.4	11214.1	6849.6	18922.5	31380.4	52203.5
Count	28	11	3	8	13	10
Largest(1)	5897.5	5897.5	6023.7	6023.7	6716.9	27921
Smallest(1)	141	201.1	361	464.9	361	235.6
Confidence Level(95.0%)	465.2577	1104.203	8048.071	1924.027	1378.089	5995.396

Table 4. Summary statistics for the distances (m) between pools for six species.

Distance to nearest site containing same species for :	spotted salamander	spring peeper	newt	green frog	wood frog	toad
Mean	185.4306	198.8603	104.2447	191.3032	101.1093	262.9509
Standard Error	88.63593	58.94803	28.34467	70.1814	19.86889	69.5668
Median	118.65	72	75.9	99	71.4	140.25
Mode	160.934	321.868	75.9	160.934	42	482.802
Standard Deviation	280.2914	256.9485	49.0944	253.0426	114.1381	260.2951
Sample Variance	78563.28	66022.54	2410.26	64030.57	13027.5	67753.56
Kurtosis	8.838968	3.523783	#DIV/0!	8.275158	16.54755	3.051617
Skewness	2.904955	1.921685	1.732051	2.732697	3.751695	1.713942
Range	950.604	950.604	85.034	941.604	628.736	911.604
Minimum	15	15	75.9	24	15	54
Maximum	965.604	965.604	160.934	965.604	643.736	965.604
Sum	1854.306	3778.346	312.734	2486.942	3336.606	3681.312
Count	10	19	3	13	33	14
Largest(1)	965.604	965.604	160.934	965.604	643.736	965.604
Smallest(1)	15	15	75.9	24	15	54
Confidence Level(95.0%)	200.5086	123.8453	121.9573	152.9121	40.47157	150.2899

Table 5. Summary statistics for the vernal pool hydroperiod scores of six species.

Hydroperiod pool statistics for:	wood frog	American toad	green frog	spring peeper	newt	spotted salamander
Mean	4.148148	4.153846	4.875	5.214286	5.333333	5.5
Standard Error	0.254328	0.336767	0.30104	0.186894	0.333333	0.166667
Median	4	4	5	5	5	5.5
Mode	5	3	5	5	5	5
Standard Deviation	1.321529	1.214232	1.204159	0.699293	0.57735	0.527046
Sample Variance	1.746439	1.474359	1.45	0.489011	0.333333	0.277778
Kurtosis	0.551297	-1.64895	-0.62615	-0.63291	#DIV/0!	-2.57143
Skewness	-0.83274	0.318359	-0.25364	-0.32135	1.732051	0
Range	5	3	4	2	1	1
Minimum	1	3	3	4	5	5
Maximum	6	6	7	6	6	6
Sum	112	54	78	73	16	55
Count	27	13	16	14	3	10
Largest(1)	6	6	7	6	6	6
Smallest(1)	1	3	3	4	5	5
Confidence Level(95.0%)	0.52278	0.733753	0.641652	0.40376	1.434219	0.377026

Table 6. Stepwise forward regression analysis of section amphibian species number on log - transformed section variables.

Model 1. $R^2 = 0.831$, $R = 0.911$								
	Variable	Coefficient	Std. Error	Std. Coef	Tol.	df	F	'P'
In								
	Constant							
	Area	1.362	0.307	0.911	1.0000	1	19.618	0.011
Out		Part. Corr.						
	Perimeter	-0.711			0.1709	1	3.073	0.178
	Edge	-0.804			0.9989	1	5.491	0.101
	Number of Water Bodies	-0.440			0.1205	1	0.721	0.458
	Number of Pools	-0.700			0.1150	1	2.883	0.188
	Number of Pools / Area	-0.150			0.9843	1	0.069	0.810
	Clumping	-0.877			0.9264	1	11.057	0.045
	Stream Length	0.084			0.4742	1	0.021	0.893
	Stream Length / Area	0.166			0.8258	1	0.085	0.790
	Water bodies / Area	0.079			0.9603	1	0.019	0.900

Table 7. Stepwise forward regression analysis of section amphibian species number on log-transformed section variables.

Model 2. $R^2 = 0.964$, R= 0.982								
	Variable	Coefficient	Std. Error	Std. Coef	Tol.	df	F	'P'
In								
	Constant							
	Area	1.515	0.17	1.014	0.9264	1	79.102	0.003
	Clumping	-0.877	0.136	-0.379	0.9264	1	11.057	0.045
Out		Part. Corr.						
	Perimeter	-0.601			0.1214	1	1.134	0.399
	Edge	-0.685			0.603	1	1.766	0.315
	Number of Water Bodies	-0.115			0.0969	1	0.027	0.885
	Number of Pools	-0.195			0.0556	1	0.079	0.805
	Number of Pools / Area	0.326			0.8776	1	0.237	0.674
	Stream Length	0.763			0.4353	1	2.792	0.237
	Stream Length / Area	0.731			0.797	1	2.289	0.269
	Water bodies / Area	0.45			0.9406	1	0.508	0.55

Table 8. Stepwise forward regression analysis of pool amphibian species number on section and habitat variables.

R = 0.774 R-Square = 0.599									
		Effect	Coefficient	Std Error	Std Coef	Tol.	Df	F	'P'
In									
	1	Constant							
	2	SEC LOG AREA	21.344	4.647	0.58	0.51331	1	21.092	0
	3	SEC NUM POOLS	-0.096	0.029	-0.386	0.59648	1	10.845	0.002
	4	LOG AREA	0.65	0.271	0.244	0.78949	1	5.731	0.021
	5	HYDROPERIOD	0.693	0.12	0.638	0.66835	1	33.204	0
Out		Part. Corr.							
	None								

Table 9. Stepwise forward logistic regression analysis of pool area and pool hydroperiod on green frog occurrence.

Step 1					
Log Likelihood: -21.874					
Parameter	Estimate	S.E.	t-ratio	p-value	
1 CONSTANT	8.681	2.649	3.277	0.001	
2 LOGAREA	-2.749	0.934	-2.942	0.003	
Score tests on effects not in model					
		Score	Chi-Sq		
Effect		Statistic	Signif	df	
3 HYDROPERIOD		0.707	0.400	1.000	
Log Likelihood: -21.874					
Parameter	Estimate	S.E.	t-ratio	p-value	
1 CONSTANT	8.681	2.649	3.277	0.001	
2 LOGAREA	-2.749	0.934	-2.942	0.003	
		95.0 % bounds			
Parameter	Odds Ratio	Upper	Lower		
2 LOGAREA	0.064	0.400	0.010		
Log Likelihood of constants only model = LL(0) = -28.604					
2*[LL(N)-LL(0)] = 13.460 with 1 df Chi-sq p-value = 0.000					
McFadden's Rho-Squared = 0.235					

Table 10. Stepwise forward logistic regression analysis of pool area and pool hydroperiod on wood frog occurrence.

Step 1					
Log Likelihood: -29.907					
Parameter	Estimate	S.E.	t-ratio	p-value	
1 CONSTANT	6.231	2.038	3.057	0.002	
2 LOGAREA	-2.386	0.784	-3.043	0.002	
Score tests on effects not in model					
	Score	Chi-Sq			
Effect	Statistic	Signif	df		
3 HYDROPERIOD	2.392	0.122	1.000		
Log Likelihood: -28.702					
Parameter	Estimate	S.E.	t-ratio	p-value	
1 CONSTANT	5.973	1.975	3.023	0.002	
2 LOGAREA	-1.768	0.837	-2.113	0.035	
3 HYDROPERIOD	-0.377	0.250	-1.508	0.131	
		95.0 % bounds			
Parameter	Odds Ratio	Upper	Lower		
2 LOGAREA	0.171	0.880	0.033		
3 HYDROPERIOD	0.686	1.119	0.420		
Log Likelihood of constants only model = LL(0) = -37.282					
2*[LL(N)-LL(0)] = 17.159 with 2 df Chi-sq p-value = 0.000					
McFadden's Rho-Squared = 0.230					

Table 11. Stepwise forward logistic regression analysis of pool area and pool hydroperiod on American toad occurrence.

Step 1					
Log Likelihood: -25.389					
Parameter		Estimate	S.E.	t-ratio	p-value
1 CONSTANT		3.179	1.135	2.802	0.005
2 HYDROPERIOD		-0.476	0.263	-1.808	0.071
Score tests on effects not in model					
		Score	Chi-Sq		
Effect		Statistic	Signif	df	
3 LOGAREA		0.531	0.466	1.000	
Log Likelihood: -25.389					
Parameter		Estimate	S.E.	t-ratio	p-value
1 CONSTANT		3.179	1.135	2.802	0.005
2 HYDROPERIOD		-0.476	0.263	-1.808	0.071
			95.0 % bounds		
Parameter		Odds Ratio	Upper	Lower	
2 HYDROPERIOD		0.621	1.041	0.371	
Log Likelihood of constants only model = LL(0) = -27.297					
2*[LL(N)-LL(0)] = 3.816 with 1 df Chi-sq p-value = 0.051					
McFadden's Rho-Squared = 0.070					

Table 12. Stepwise forward logistic regression analysis of pool area and pool hydroperiod on spring peeper occurrence.

Step 1					
Log Likelihood: -15.124					
Parameter	Estimate	S.E.	t-ratio	p-value	
1 CONSTANT	10.486	3.380	3.103	0.002	
2 HYDROPERIOD	-2.077	0.698	-2.978	0.003	
Score tests on effects not in model					
	Score	Chi-Sq			
Effect	Statistic	Signif	df		
3 LOGAREA	3.863	0.049	1.000		
Log Likelihood: -12.034					
Parameter	Estimate	S.E.	t-ratio	p-value	
1 CONSTANT	18.562	6.512	2.850	0.004	
2 HYDROPERIOD	-1.829	0.798	-2.294	0.022	
3 LOGAREA	-3.210	1.582	-2.029	0.042	
		95.0 % bounds			
Parameter	Odds Ratio	Upper	Lower		
2 HYDROPERIOD	0.161	0.766	0.034		
3 LOGAREA	0.040	0.897	0.002		
Log Likelihood of constants only model = LL(0) = -27.297					
2*[LL(N)-LL(0)] = 30.525 with 2 df Chi-sq p-value = 0.000					
McFadden's Rho-Squared = 0.559					

Table 13. Stepwise forward logistic regression analysis of pool area and pool hydroperiod on spotted salamander occurrence.

Step 1					
Log Likelihood: -10.728					
Parameter		Estimate	S.E.	t-ratio	p-value
1 CONSTANT		14.203	5.346	2.657	0.008
2 HYDROPERIOD		-2.632	1.048	-2.511	0.012
Score tests on effects not in model					
		Score	Chi-Sq		
Effect		Statistic	Signif	df	
3 LOGAREA		4.630	0.031	1.000	
Log Likelihood: -7.395					
Parameter		Estimate	S.E.	t-ratio	p-value
1 CONSTANT		30.515	12.934	2.359	0.018
2 HYDROPERIOD		-3.183	1.499	-2.123	0.034
3 LOGAREA		-4.492	2.329	-1.928	0.054
			95.0 % bounds		
Parameter		Odds Ratio	Upper	Lower	
2 HYDROPERIOD		0.041	0.783	0.002	
3 LOGAREA		0.011	1.076	0.000	
Log Likelihood of constants only model = LL(0) = -20.827					
2*[LL(N)-LL(0)] = 26.863 with 2 df Chi-sq p-value = 0.000					
McFadden's Rho-Squared = 0.645					

Table 14. Summary statistics for the total amphibian species richness in sites containing each of six species.

Species richness of sites containing:	newt	spotted salamander	spring peeper	toad	green frog	wood frog
Mean	5.333333333	4	3.31579	2.5	2.2222	2.090909
Standard Error	0.881917104	0.447214	0.3338	0.53195	0.3633	0.276489
Median	5	4	3	2	2	1
Mode	#N/A	3	3	1	1	1
Standard Deviation	1.527525232	1.414214	1.45498	1.99036	1.8879	1.58831
Sample Variance	2.333333333	2	2.11696	3.96154	3.5641	2.522727
Kurtosis	#DIV/0!	1.22619	0.80536	0.47032	-0.0673	1.730666
Skewness	0.93521953	0.883883	0.8326	1.02435	0.765	1.535577
Range	3	5	6	7	7	6
Minimum	4	2	1	0	0	1
Maximum	7	7	7	7	7	7
Sum	16	40	63	35	60	69
Count	3	10	19	14	27	33
Largest(1)	7	7	7	7	7	7
Smallest(1)	4	2	1	0	0	1
Confidence Level(95.0%)	3.794583033	1.011667	0.70128	1.1492	0.7468	0.56319

Table 15. Summary statistics for percentage of vegetative cover in sites containing each of six species.

Percentage of vegetative cover in pools containing:	wood frog	spring peeper	spotted salamander	American toad	green frog	red-spotted newt
Mean	35.45455	45.26316	46	27.14286	42.30769	36.66667
Standard Error	2.954545	5.318423	7.18022	3.841441	6.420505	16.66667
Median	30	40	50	25	50	20
Mode	20	20	60	20	60	20
Standard Deviation	16.97257	23.18247	22.70585	14.37336	23.14946	28.86751
Sample Variance	288.0682	537.4269	515.5556	206.5934	535.8974	833.3333
Kurtosis	-0.68443	-1.73774	-1.69433	0.509321	-1.25585	#DIV/0!
Skewness	0.692651	0.171876	0.051255	0.766045	-0.37852	1.732051
Range	60	60	60	50	70	50
Minimum	10	20	20	10	0	20
Maximum	70	80	80	60	70	70
Sum	1170	860	460	380	550	110
Count	33	19	10	14	13	3
Largest(1)	70	80	80	60	70	70
Smallest(1)	10	20	20	10	0	20
Confidence Level(95.0%)	6.018212	11.17359	16.24279	8.29893	13.98908	71.71088

Table 16. Summary of field effort.

Section	Number of visits		Estimated hours		Area km²	Number of sites	Hours per site
	2007	2008	2007	2008			
Extreme Eastern	3	2	7.5	5	0.5	11	0.75
Lawrence Wood	5	5	12.5	12.5	0.9	37	0.45
Southeastern	7	5	17.5	12.5	0.9	23	0.86
Southwestern	7	6	17.5	15	1.2	33	0.65
Virginia Wood	3	1	1.5	0.5	0.2	1	1.32
Wright's Park	5	3	12.5	7.5	0.5	10	1.32

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