TOOLKIT FOR AMPHIBIAN HABITAT MONITORING IN ONTARIO

TOOLKIT TO MANAGE KEY HABITAT FOR AMPHIBIANS IN ONTARIO FORESTS

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LAY ABSTRACT

Amphibian declines represent one of the hallmarks of the current biodiversity crisis. While there are many factors responsible for amphibian declines, the most significant threats are habitat loss and degradation. This Ph.D. thesis describes amphibian habitat use in Ontario forests and provides resource managers with tools and techniques to protect habitat. Using satellite imagery, I developed a strategy to map small amphibian breeding wetlands (vernal pools) in forests of eastern Georgian Bay. I also identified the importance of pool drying times and forest canopy cover to the amphibians that breed in these wetlands. In the fragmented forest patches of southern Ontario, I mapped suitable habitat for the endangered Jefferson salamander and identified the importance of large deciduous/mixed forests. Lastly, I assessed the effectiveness of a long-term salamander monitoring program in southern Ontario and demonstrated the use of techniques to optimize the allocation of effort and maximize the accuracy of monitoring results.

GENERAL ABSTRACT

Forest-dwelling amphibians contribute to diverse ecosystem services in Ontario but are threatened by habitat degradation and fragmentation. My thesis investigated key amphibian habitats in Ontario forests with the goal of providing resource managers with tools and techniques to protect and restore amphibian populations. I primarily focussed on amphibians that breed in temporary forested wetlands known as vernal pools, as these wetlands are overlooked in provincial legislature and particularly sensitive to changes in land-use and climate.

First, I investigated the distribution and community structure of vernal pools in forests of eastern Georgian Bay, Lake Huron. I developed an accurate remote sensing technique to map vernal pool habitat using readily available spatial data and found that undocumented vernal pools accounted for over half of the wetlands in the region. I documented the importance of the length of pool inundation (hydroperiod) and canopy openness in determining the composition of vernal pool amphibian assemblages. In particular, pools with short hydroperiods and closed canopies tended to support only early breeders and canopy generalists.

Next, I used two case studies to demonstrate the usefulness of existing amphibian occurrence datasets, specifically for the improvement of habitat mapping and monitoring. For the first case study, I created habitat suitability models using known locations of the endangered Jefferson salamander (*Ambystoma jeffersonianum*). Models yielded good discriminatory ability between presence and pseudo-absence data and confirmed the importance of deciduous/mixed forests as key habitats. Habitat suitability maps revealed potential undocumented habitat in the Greenbelt region of Ontario. For the second case study, I developed optimizations of time and effort for a salamander monitoring program. I verified the need for at least ten years' worth of monitoring data for reliable trend detection and demonstrated that the precision and accuracy of occupancy estimates are dependent on the allocation of effort across monitoring sites and repetitions.

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LIST OF ABBREVIATIONS AND SYMBOLS

AIC_c: Akaike’s Information Criterion

AUC: Area under the curve

CART: Classification and Regression Tree

CI: Confidence interval

CIR: Colour-infrared

COSEWIC: Committee on the Status of Endangered Wildlife in Canada

CPUE: Catch per unit effort

CTA: Classification Tree Analysis

CV: Cross-validation

DEM: Digital elevation model

DTM: Digital terrain model

eDNA: Environmental DNA

EMAN: Environmental Monitoring and Assessment Network

ESA: European Space Agency

FDA: Flexible Discriminant Analysis

GB: Georgian Bay

GBE: Georgian Bay Ecoregion

GBM: Generalized Boosted Model

GLCM: Gray Level Cooccurrence Matrix

GLDV: Gray Level Difference Vector

GLM: Generalized Linear Model

GLMM: Generalized linear mixed models

GPS: Global Positioning System

KNN: K-Nearest Neighbour

LiDAR: Light detection and ranging

LPBR: Long Point World Biosphere Reserve

MAE: Minimum acceptable error predicted area

ME: Maximum Entropy

MEG: Mid-eastern Georgian Bay

NDWI: Normalized Difference Water Index

NIR: Near infrared

NMDS: Non-metric multidimensional scaling

NN: Neural Network

NWI: National Wetland Inventory

OBIA: Object-based image analysis

OMNRF: Ontario Ministry of Natural Resources and Forestry

OOP: Ontario Orthoimagery Project

PERMANOVA: Permutational multi-variate analysis of variance

PVP: Potential vernal pool

QAIC: Quasi-Akaike’s Information Criterion

RA: Random background pseudo-absence

RF: Random Forest

RFE: Recursive Feature Elimination

RGB: Red green blue

SCOOP: South Central Ontario Orthophotography Project

SDM: Species distribution model

SEG: Southeastern Georgian Bay

SIMPER: Similarity percentage routine

SNAP: Sentinel Application Platform

SOLRIS: Southern Ontario Land Resource Information System

SVM: Support Vector Machines

TA: Target species pseudo-absence

TRI: Terrain ruggedness index

VIF: Variance inflation scores

DECLARATION OF ACADEMIC ACHIEVEMENT

The following thesis includes a general introduction chapter, four data chapters, and a general discussion chapter. Chapters 2 and 5 have been peer-reviewed and published in scientific journals. Chapter 3 has been submitted to a scientific journal for peer-review. Chapter 4 is presented as a manuscript but has not yet been submitted for peer review. Completed references for all data chapters are found below. As first author on all chapters, I analysed the data and wrote the manuscripts under the supervision of Dr. Patricia Chow-Fraser. Collection and acquisition of data for this thesis relied on the effort and support of numerous undergraduate and graduate students, research partners, and community members, who are formally recognized in the acknowledgements.

Chapter 2: Luymes N and Chow-Fraser P. (2021) Detection of potential vernal pools on the Canadian Shield (Ontario) using object-based image analysis in combination with machine learning. *Canadian Journal of Remote Sensing*

Chapter 3: Luymes N and Chow-Fraser P. (2021) Determinants of amphibian community composition in vernal pools of south-central Ontario: Considerations for conservation under changing climate and land-use. Submitted to *Canadian Journal of Fisheries and Aquatic Sciences*

Chapter 4: Luymes N and Chow-Fraser P. (2021) Fine-scale habitat suitability modelling for Jefferson salamanders (*Ambystoma jeffersonianum*) using spatially biased species occurrence data in southern Ontario, Canada

Chapter 5: Luymes N and Chow-Fraser P. (2019) Optimizations for time and effort in long-term monitoring: a case study using a multidecadal terrestrial salamander monitoring program. *Environ Monit Assess, 191*(597), 10

CHAPTER 1: GENERAL INTRODUCTION

Amphibians are an extremely sensitive group of species and their worldwide declines outnumber those of any other vertebrate group. Amphibians contribute to diverse ecosystem services including pest control, medical innovations, energy and nutrient cycling, and significance for many cultures around the world (e.g. spirituality, mythology, art, food, and recreation; Hocking and Babbitt, 2014). It is difficult to predict the ecological consequences of continued declines in amphibian biodiversity, but current losses have already led to significant changes in ecosystem functions and services (Whiles et al., 2013). While there are a number of interacting stressors that have led to the current decline in amphibians, the loss, fragmentation, and degradation of amphibian habitat are among the most detrimental factors (Hamer and McDonnell, 2008). To prevent further declines in amphibians and their habitat, especially in the face of new stressors like disease and global climate change, there is an urgent need to determine what is important habitat and to map and to monitor these remaining habitats (Wren et al., 2015).

Forests and associated wetlands make up the most widespread habitat for amphibians in Ontario. Forests provide access to habitat for all life-cycle activities, including breeding, foraging, hibernation, growth, and development. Due to the uneven distribution of urban and agricultural development in Ontario, forests have been differentially impacted by land-use changes. Land-use changes in the boreal forests of northern Ontario are largely non-existent, whereas the deciduous and mixed forests of southern Ontario exist as fragmented patches within urban and agricultural matrices. In contrast, the mixed forests comprising central Ontario are mostly intact but are experiencing development pressures from a booming cottage industry and expanding urban centers. Land-use changes have had negative effects on Ontario's amphibians; all seven of Ontario's listed amphibians are restricted by range to the highly fragmented habitat in southern Ontario.

Almost all of Ontario's amphibians require wetland habitat for breeding and recruitment and some of the most commonly used wetlands are vernal pools: forested wetlands that dry yearly or every few years. The short hydroperiods of vernal pools, which are functionally defined as the length of time that standing water is available in the spring and summer (or beyond), are especially important for amphibians that rely on the absence of predatory fish populations for successful reproduction (e.g. *Ambystoma* salamanders and wood frogs (*Lithobates sylvaticus*)). Amphibians that breed in vernal pools have evolved to have quick development cycles to deal with the short hydroperiods (Rowe and Dunson, 1995; Wellborn, Skelly, and Werner, 1996), whereas those that breed in wetlands with long hydroperiods have evolved strategies to cope with high competition and predation risk (Kats, Petranka, and Sih, 1988; Rubbo and Kiesecker, 2005). Altered drainage pathways and weather patterns can disrupt hydroperiods and can lead to less suitable conditions for amphibians (deMaynadier and Houlahan, 2008). Climate change, in particular, is expected to reduce the long-term average hydroperiod of vernal pools and result in an overall reduction in the total amount of available amphibian habitat (Brooks, 2009).

Despite their sensitivity to climate and land-use changes, vernal pools are often overlooked in legislature due to their small size and relative obscurity within forests (Mahaney and Klemens, 2008). There is ample evidence from other N. American jurisdictions to suggest that vernal pools are vital for the maintenance of local amphibian populations (Calhoun et al., 2003; Hunter, 2008; Preisser, Kefer, and Lawrence, 2016), but less efforts have been made to understand the conservation value of Ontario's vernal pools and their associated amphibian communities.

Studies on amphibian habitat use in Ontario have mainly focussed on breeding wetlands in the southern part of the province. Due to the highly fragmented nature of forests in this region, species richness and abundance tend to be positively associated with the size of forest plots and negatively associated with the presence of roads, agriculture, and urban development (Scott and Houlahan, 1997; Hecnar and M'Closkey, 1998; Findlay, Lenton, and Zhang, 2001; Houlahan and Findlay, 2003; Gagné and Fahrig, 2007). Other habitat characteristics, such as wetland cover and the presence of fish, have also been identified as important drivers of amphibian richness and abundance (Hecnar and M'Closkey, 1997; Hecnar and M'Closkey, 1998; Houlahan and Findlay, 2003), but these relationships have primarily been evaluated across large ranges of wetland types.

More research is needed to identify the species-habitat relationships for small ephemeral wetlands like vernal pools, particularly in central Ontario where land-use changes have been less prevalent, but development pressure is high.

The identification of species-habitat relationships for pool-breeding amphibians at risk, including endangered *Ambystoma* salamanders (*A. jeffersonianum*, *A. texanum*, unisexual *Ambystoma*), is a primary goal of recovery strategies in Ontario (Hossie, 2018; Linton, McCarter, and Fotherby, 2018). Studies have identified a variety of habitat suitability characteristics for each species, including forest composition, wetland permanence, and home range sizes (Faccio, 2003; Peterman, Crawford, and Kuhns, 2013; Van Drunen et al., 2020; Ward and Hossie, 2020). Despite these well-studied relationships, efforts to map the distribution of potential habitat across the province have been relatively non-existent. Knowledge of habitat distribution can help with the identification of undocumented populations and potential locations for relocations and restoration, especially for species with large ranges.

Increases in the availability of large species occurrence datasets, remotely sensed data, and advanced statistical and GIS techniques have brought about new analysis options for conservation biology. For amphibians in Ontario, these represent inexpensive opportunities to study spatial and temporal trends of species occurrence, abundance, and habitat. These datasets are rarely collected with hypothesis testing in mind (Bystriakova et al., 2012), so care must be taken to ensure that sampling biases are accounted for during the analysis and evaluation processes. Field surveys can be expensive and time-intensive, so analyses of existing datasets can be particularly useful for urgent conservation issues, such as those dealing with species at risk.

Thesis Objectives

This thesis introduces novel tools and techniques to assess the distribution of amphibian habitat and monitor species assemblages in Ontario forests. Amphibians in Ontario have complex habitat requirements and are particularly sensitive to habitat loss and degradation (Houlahan and Findlay, 2003). For the successful establishment and maintenance of protected areas, information regarding the characteristics and distribution of important amphibian habitat is essential. The tools, techniques, and species-habitat relationships presented in this thesis are ultimately intended to guide conservation efforts for forest-dwelling amphibians in Ontario, especially in the face of land-use alterations and global climate change.

The eastern shore of Georgian Bay, Lake Huron, offers a unique environment for amphibian habitat. The large continuous forests and rough topography of the Canadian Shield support a dense network of wetlands. Given the recent increase in cottage and residential development in eastern Georgian Bay, especially near the town of Honey Harbour and the City of Parry Sound, it is urgent that locations of all potential breeding wetlands be included in official plans so they can be protected from future development. Unfortunately, because of their small size and ephemeral nature, vernal pools are difficult to map with conventional technology (Carpenter et al., 2011) and do not exist in provincial or municipal databases. To address these deficiencies, Chapter 2 presents a novel mapping strategy to accurately detect vernal pools using GIS and remote-sensing techniques. The development of this strategy is described within the context of eastern Georgian Bay but can be applied to other areas in Ontario that are lacking information on vernal pool distributions.

Little is known about the types of amphibian assemblages that exist in the eastern Georgian Bay region, especially for species that use forested vernal pools as breeding habitat. In Chapter 3, we develop an efficient survey technique to measure and compare relative proportions of amphibian larvae in vernal pools. We apply this technique to vernal pools mapped in Chapter 2 to assess relationships between habitat characteristics and the composition of amphibian communities. Based on studies of amphibians in other parts of the N. America (e.g. Vermont and Massachusetts), we hypothesize that species-specific adaptations to particular hydroperiods and vegetation communities drive the composition of amphibian communities in vernal pools in eastern Georgian Bay (Semlitsch et al., 2015; Werner et al., 2007; Schiesari, 2006; Skelly, Freidenburg and Kiesecker, 2002). The habitat suitability relationships identified in this chapter will be useful for assessing the conservation value of undocumented vernal pool habitat in eastern Georgian Bay.

In the fragmented forests of southern Ontario, the identification of suitable habitat for at-risk amphibians is an urgent conservation goal, but the time-consuming processes involved in conducting research on private and public land in this region limit the applicability of large-scale field surveys. The increasing availability of large species occupancy datasets from government, community, and research collections present an alternative opportunity to evaluate species-habitat relationships. In Chapter 4, we apply species distribution models to a long-term occurrence dataset of the endangered Jefferson salamander (*Ambystoma jeffersonianum*) to map suitable habitat in southern Ontario. We hypothesize that Jefferson salamanders primarily inhabit large deciduous forest patches with complex terrain because these forests support preferred breeding wetlands and upland overwintering sites (Petranka, 1998; Linton, McCarter, and Fotherby, 2018). Habitat suitability maps from this study can be used to inform efforts to identify undocumented populations of Jefferson salamanders and to help in the delineation of protected areas.

Once populations of amphibians are identified, the success of or need for conservation strategies can be assessed through the use of monitoring programs. In Chapter 5, we use an existing monitoring dataset of a terrestrial amphibian, the redback salamander (*Plethodon cinereus*), to discuss how to optimize time and effort to produce meaningful results in long-term amphibian monitoring programs. We also discuss how long-term monitoring programs can be structured to address short-term research questions, such as those related to habitat preferences. The techniques used for this chapter are meant to be widely applicable and can serve as a technique for the development of new amphibian monitoring programs and the improvement of existing ones.

References

Brooks RT. (2009). Potential impacts of global climate change on the hydrology and ecology of ephemeral freshwater systems of the forests of the northeastern United States. *Climatic Change*, *95*, 469–483.

Bystriakova N, Peregrym M, Erkens RHJ, Bezsmertna O, and Harald S. (2012). Sampling bias in geographic and environmental space and its effect on the predictive power of species distribution models. *Systematics and Biodiversity*, *10*(3), 305–315.

Calhoun AJK, Walls TE, Stockwell SS, and McCollough M. (2003). Evaluating vernal pools as a basis for conservation strategies: a Maine case study. *Wetlands*, *23*(1), 70–81.

Carpenter L, Stone J, and Griffin CR. (2011). Accuracy of Aerial Photography for Locating Seasonal (Vernal) Pools in Massachusetts. *Wetlands*, *31*(3), 573–581.

deMaynadier PG and Houlahan JE. (2008). Conserving vernal pool amphibians in managed forests. In *Science and conservation of vernal pools in Northeastern North America* (pp. 253–280).

Faccio SD. (2003). Postbreeding emigration and habitat use by Jefferson and spotted salamanders in Vermont. *Journal of Herpetology*, *37*(3), 479–489.

Findlay CS, Lenton J, and Zheng L. (2001). Land-use correlates of anuran community richness and composition in Southeastern Ontario wetlands. *Ecoscience*, *8*(3), 336–343.

Gagné SA and Fahrig L. (2007). Effect of landscape context on anuran communities in breeding ponds in the National Capital Region, Canada. *Landscape Ecology*, *22*(2), 205–215.

Hamer AJ and McDonnell MJ. (2008). Amphibian ecology and conservation in the urbanising world: A review. *Biological Conservation*, *141*(10), 2432–2449.

Hecnar SJ and M'Closkey RT. (1997). The effects of predatory fish on amphibian species richness and distribution. *Biological Conservation*, *79*(2–3), 123–131.

Hecnar SJ and M'Closkey RT. (1998). Species richness patterns of amphibians in southwestern Ontario ponds. *Journal of Biogeography*, *25*(4), 763–772.

Hocking DJ, Babbitt KJ, and Hocking DJ. (2014). Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology*, *9*(1), 1–17.

Hossie TJ. (2018). *Recovery Strategy for Small-mouthed Salamander (Ambystoma texanum) and Unisexual Ambystoma Small-mouthed Salamander dependent population (Ambystoma laterale - texanum) in Ontario*. Ontario Recovery Strategy Series. Ontario Ministry of Natural Resources and Forestry.

https://www.ontario.ca/page/small-mouthed-salamander-and-small-mouthed-dependent-unisexual-ambystoma-recovery-strategy

Houlahan JE and Findlay CS. (2003). The effects of adjacent land use on wetland amphibian species richness and community composition. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*(9), 1078–1094.

Hunter Jr. M. (2008). Valuing and Conserving Vernal Pools as Small-Scale Ecosystems. In *Science and Conservation of Vernal Pools in Northeastern North America* (pp. 1–10).

Kats LB, Petranka JW, and Sih A. (1988). Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology*, *69*(6), 1865–1870.

Linton J, McCarter J, and Fotherby H. (2018). *Jefferson Salamander (Ambystoma jeffersonianum) and Unisexual Ambystoma Jefferson Salamander dependent population (Ambystoma laterale – (2) jeffersonianum) in Ontario*. Ontario Recovery Strategy Series. Ontario Ministry of Natural Resources and Forestry. https://www.ontario.ca/page/jefferson-salamander-recovery-strategy

Mahaney W and Klemens M. (2008). Vernal Pool Conservation Policy: The Federal, State, and Local Context. In *Science and Conservation of Vernal Pools in Northeastern North America* (pp. 193–212).

Peterman WE, Crawford JA, and Kuhns AR. (2013). Using species distribution and occupancy modeling to guide survey efforts and assess species status. *Journal for Nature Conservation*, *21*(2), 114–121.

Petranka J. (1998). *Salamanders of the United States and Canada*. Smithsonian Institution Press.

Preisser E, Kefer J, and Lawrence J. (2016). Vernal pool conservation in Connecticut: An assessment and recommendations. *Environmental Management*, *26*(5), 503–513.

Rowe CL and Dunson WA. (1995). Impacts of hydroperiod on growth and survival of larval amphibians in temporary ponds of Central Pennsylvania, USA. *Oecologia*, *102*(4), 397–403.

Rubbo MJ and Kiesecker JM. (2005). Amphibian breeding distribution in an urbanized landscape. *Conservation Biology*, *19*(2), 504–511.

Schiesari L. (2006). Pond canopy cover: A resource gradient for anuran larvae. *Freshwater Biology*, *51*(3), 412–423.

Scott C and Houlahan J. (1997). Anthropogenic Correlates of Species Richness in Southeastern Ontario Wetlands. *Conservation Biology*, *11*(4), 1000–1009.

Semlitsch RD, Peterman WE, Anderson TL, Drake DL, and Ousterhout BH. (2015). Intermediate pond sizes contain the highest density, richness, and diversity of pond-breeding amphibians. *PLoS ONE*, *10*(4), 20.

Skelly DK, Freidenburg LK, and Kiesecker JM. (2002). Forest canopy and the performance of larval amphibians. *Ecology*, *83*(4), 983–992.

Van Drunen SG, Linton JE, Bogart JP, McCarter J, Fotherby H, Sandilands A, and Norris DR. (2020). Estimating critical habitat based on year-round movements of the endangered Jefferson salamander (*Ambystoma jeffersonianum*) and their unisexual dependents. *Canadian Journal of Zoology*, *98*(2), 117–126.

Ward M and Hossie TJ. (2020). Do existing constructed ponds on Pelee Island, Ontario match the habitat requirements of endangered *Ambystoma* larvae? *Wetlands*, *40*(6), 2097–2108.

Wellborn GA, Skelly DK, and Werner EE. (1996). Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, *27*(1), 337–363.

Werner EE, Skelly DK, Relyea RA, and Yurewicz KL. (2007). Amphibian species richness across environmental gradients. *Oikos*, *116*(10), 1697–1712.

Whiles MR, Hall RO, Dodds WK, Verburg P, Huryn AD, Pringle CM, Lips KR, Kilham SS, Colón-Gaud C, Rugenski AT, Peterson S, and Connelly S. (2013). Disease-driven amphibian declines alter ecosystem processes in a tropical stream. *Ecosystems*, *16*(1), 146–157.

Wren S, Angulo A, Meredith H, Kielgast J, Dos Santos M, and Bishop P. (2015). *Amphibian Conservation Action Plan. April 2015. IUCN SSC Amphibian Specialist Group*. https://www.iucn-amphibians.org/resources/acap/

CHAPTER 2: DETECTION OF POTENTIAL VERNAL POOLS ON THE CANADIAN SHIELD (ONTARIO) USING OBJECT-BASED IMAGE ANALYSIS IN COMBINATION WITH MACHINE LEARNING

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Abstract

Vernal pools are small, temporary, forested wetlands of ecological importance with a high sensitivity to changing climate and land-use patterns. These ecosystems are under considerable development pressure in southeastern Georgian Bay, where mapping techniques are required to inform wise land-use decisions. Our mapping approach combines common machine learning techniques (random forest, support vector machines) with object-based image analysis. Using multispectral image segmentation on high-resolution orthoimagery, we first created objects and assigned classes based on field collected data. We then supplied machine learning algorithms with data from freely available sources (Ontario orthoimagery and Sentinel 2) and tested accuracy on a reserved dataset. We achieved producer's accuracies of 85% and 79% and user's accuracies of 78% and 84% for random forest and support vector machines models, respectively. Difficulty differentiating between small, dark shadows and small, obscured pools accounted for many of the omission and commission errors. Our automated approach of vernal pool classification provides a relatively accurate, consistent, and fast mapping strategy compared to manual photointerpretation. Our models can be applied on a regional basis to help verify the locations of pools in an area of Ontario that is in critical need of more detailed ecological information.

Introduction

Temporary wetlands act as critical breeding habitats for amphibians across the globe and are increasingly being recognized for their disproportionate benefits to local biodiversity (Calhoun et al. 2017). In the Pleistocene glaciated part of northeastern North America, temporary wetlands commonly occur as small seasonally available, forested wetlands known as vernal pools. Vernal pools fill with water during the spring or fall and dry in the summer or in drought years. Their ephemeral nature precludes the establishment of permanent fish populations, allowing amphibian larvae and other aquatic species to avoid intense predation. Obligate vernal pool species, including Ambystomatid salamanders (*Ambystoma sp.*) and wood frogs (*Lithobates sylvaticus*), have optimal breeding success and recruitment in these fish-free habitats (Babbit et al. 2003; Semlitsch et al. 2015). These obligate amphibians, and the many facultatively breeding amphibians that frequent vernal pools, are of conservation interest due to their sensitivity to environmental stressors (Semlitsch and Brodie 1998), importance in forest energy and nutrient cycling (Leibowitz 2003), and the fact that some are considered species at risk.

Vernal pools are necessary for the maintenance of local amphibian populations (Leibowitz 2003), but their small size and ephemeral nature often means they are overlooked in wetland legislation (Evans et al. 2017). In Ontario, most vernal pools are ineligible for protection under the Ontario Wetland Evaluation System's current guidelines for provincially significant wetlands (Ontario Ministry of Natural Resources and Forestry 2014). Consequently, they are unlikely to factor into land-use decisions and can face habitat degradation and loss of landscape connectivity as a result (Calhoun et al. 2017). The anticipated effects of climate change, including shorter and less frequent hydroperiods (Brooks 2009), further highlight the need for improved management of vernal pools in the province.

Knowledge of the location and distribution of vernal pools is essential to their protection; however, the canopy-obstructed nature of these wetlands complicates mapping efforts. Techniques for large-scale wetland mapping, including the National Wetland Inventory (NWI), are often unfeasible for vernal pools because the pools are either too small to be reasonably detected or are obscured by overhead forest canopy cover (Baldwin and deMaynadier 2009). Several geopolitical entities within the northeastern United States have developed regulatory protections for vernal pools, which has led to state-wide efforts to map and document vernal pools (e.g. Brooks et al. 1998; Faccio et al. 2013; Jansujwicz et al. 2013; Lathrop et al. 2005). The most common technique used to map vernal pools has been photointerpretation of high-resolution aerial imagery. While easy to implement, photointerpretation often results in highly variable accuracy and can be hindered by interpreter bias and skill (Carpenter et al. 2011).

Recent advances in remote-sensing technology and classification techniques have led to novel strategies for wetland mapping. Object-based image analysis (OBIA), in particular, has emerged in response to the increasing availability of high-resolution remote sensing data. OBIA involves grouping pixels together based on spectral similarities to form image objects, which can then be analysed using spatial statistical models. While traditional pixel-based image analysis methods focus solely on the spectral characteristics of individual pixels, OBIA allows for the integration of object shape, texture, and neighbourhood characteristics in addition to a greater range of spectral properties. OBIA has proven to be especially useful for the classification of high-resolution imagery, where features on the ground (e.g. wetlands, buildings, trees) tend to be represented better by image objects rather than individual pixels (Blaschke 2010). Wetland classification studies using OBIA have had success both with medium-resolution and high-resolution data (Amani et al. 2017; Dronova 2015; Grenier et al. 2008; Rampi et al. 2014; Tian et al. 2016), and comparisons with pixel-based approaches have shown that OBIA consistently produces more accurate results (Amani et al. 2017; Dronovo 2015; Fu et al. 2016; Harken and Sugumaran 2005). Since the detection of small wetlands like vernal pools necessitates the use of high-resolution imagery, we consider OBIA a promising approach to vernal pool mapping.

For the information extracted from OBIA to be used for image classification, the data are often subjected to different machine learning classifiers, including k-nearest neighbour (KNN), classification and regression trees (CART), random forests (RF), support vector machines (SVM), and neural networks (NN). These classifiers are able to handle many types of input data types without making assumptions on the data distributions, making them well suited for the diverse arrays of features extracted in OBIA. Machine learning classifiers have also been shown to consistently outperform conventional classifiers, such as maximum likelihood, and are becoming easier to implement as more image-processing programs integrate machine learning algorithms into their software (Maxwell et al. 2018). Two of the most common machine learning algorithms used in remote sensing applications are RF and SVM. RF is an ensemble learning technique that uses a large number of decision trees to "vote" on class predictions. Each decision tree is constructed using a random subset of the data and predictor variables, resulting in a low correlation between the individual trees and reducing the chance of overfitting the data. SVM is a supervised machine learning classifier that uses multidimensional hyperplanes to maximize the width of decision margins between classes. It uses kernels to map features to higher dimensional space where linear separation is more effective. RF and SVM classifiers do not make formal distributional assumptions but require that the sampled data be representative. Since both classifiers are unable to extrapolate, they are limited in their predictive capabilities and have difficulty classifying data that are not averages of data encountered in model training. Machine learning classifiers can also be computationally intensive but advances in computing capabilities have made this less of an issue, resulting in more widespread use. Studies involving the classification of wetland landcover have had success with RF (Fu et al. 2017; Millard and Richardson 2013; Tian et al. 2016) and SVM classifiers (Amani et al. 2017; Chatziantoniou et al. 2017), and a study involving synthetic aperture radar data was able to map vernal pool locations using RF classifiers (Bourgeau-Chavez et al. 2016).

Many of the previously developed approaches to vernal pool mapping have involved the use of light detection and ranging data (LiDAR; Julian et al. 2009; Leonard et al. 2012; Riley et al. 2017; Wu et al. 2014). LiDAR systems allow the penetration of forest canopies and can be used to identify landscape depressions and the presence of water (Lang and McCarty 2009), both of which are crucial for vernal pool detection. Despite the clear benefits of LiDAR data to vernal pool mapping, many remote areas do not have access to the technology because of its high cost.

In this study, we are interested in mapping vernal-pool habitat in a remote area of south-central Ontario, where LiDAR data are not available, and where no large-scale vernal-pool mapping efforts have been attempted. Due to its pristine nature and proximity to Georgian Bay, this region is highly coveted by cottagers and, despite its mostly undisturbed state, is experiencing high development pressure. Therefore, there is an urgency in mapping critical habitat for amphibians and turtles, such as vernal pools, and incorporating such information into official plans of the affected municipalities. Given these constraints, our goal was to develop an automated classification approach using freely available image products, and recent advances in image analysis technology. This is the first regional mapping effort of vernal-pool habitat in a mostly forested portion of Ontario, which is rapidly becoming altered by cottage and urban development. These maps will identify areas that should be targeted for intensive field surveys and ground truthing. Since similar remote sensing datasets are available for other unmapped regions of Ontario, we recommend using this approach to create a provincial database of potential vernal pools to guide effective land-use planning and wildlife conservation.

Methods

Field Methods

Our region of interest for this study was the forested southeastern shore of Georgian Bay, Lake Huron, Ontario. This region is part of the Canadian Shield ecozone of Ontario and consists of a mixed coniferous-deciduous forest interwoven by numerous outcrops of exposed igneous bedrock (Crins et al. 2009). The climate is temperate and humid with an average annual precipitation of 950 mm and an average summer rainfall of 250 mm. The mosaic of exposed bedrock creates numerous depressions in the landscape for waterbodies to form, including lakes, thicket swamps, fens, ponds, and vernal pools. This region includes three vernal pool obligate amphibians (*Ambystoma laterale, A.*

maculatum, Lithobates sylvaticus), as well as several species at risk that derive food, water, and shelter from vernal pools (e.g. *Clemmys guttata*, *Emydoidea blandingii*).

To collect our reference dataset of vernal pools, we selected six study sites (ranging in size from 25 to 175 ha; GB1, GB2, GB3, GB4, GB5, and GB6; Figure 2.1) located within 20 km of the shoreline of eastern Georgian Bay. These sites were selected based on three main criteria: 1) proximity to all-season roads for safe access by the survey team, 2) location on publicly accessible Crown Land, and 3) inclusion in the region of interest. Each study site was divided into one to seven 25-ha plots, depending on the overall site size. Based on available time and resources, we were able to conduct intensive field surveys in a total of 23 plots within the 6 study sites. All plots were surveyed during spring in 2016, 2018, or 2019.

In advance of the field sampling, we imported shapefiles of water bodies and wetlands prepared by the Ontario Ministry of Natural Resources and Forestry (OMNRF) into ESRI Arc Collector application on an Apple^{TM} iPad. We also imported the location of the 23 plots and created transects spaced at 100-m intervals in each plot (see Figure 2.2). Transects in each plot were oriented to minimize encounters with barriers (e.g. large lakes, roads, ledges) and inter-transect spacing was determined based on estimated field of view during leaf-off conditions. We uploaded all transects to handheld GPS devices and walked at a moderate pace along the transects as shown in Figure 2.2. This allowed us to see all water bodies that were located on both sides of the transects. If the field of view was obstructed by hilly terrain, we walked up these inclines to ensure no wetlands were obscured. Whenever we encountered an undocumented wetland, we traced the outline of the wetland by foot and used Arc Collector application to record the information. Large wetlands and water bodies that had been documented by the OMNRF were not traced, but we noted any major areas that had been omitted. We continued in this way until the boundaries of all wetlands and water bodies in the forested regions of each plot were mapped.

Datasets

Reference potential vernal pools (PVPs)

To be consistent with the literature (e.g. Brooks et al. 1998; Calhoun et al. 2003; Lathrop et al. 2005), we define vernal pools as temporary to semi-permanent bodies of water that serve as primary breeding habitat for obligate amphibians. Functionally, these can be defined as confined surface depressions with no permanent inflow or outflow. Based on the results of a concurrent study, we knew that at least some of the undocumented wetlands encountered during the surveys supported vernal-pool obligates; however, given the limited time we had to conduct larval surveys during the breeding season, we could not confirm the presence of obligate amphibian breeders in every wetland we encountered. Therefore, in this study, we have designated all undocumented wetlands as potential vernal pools (PVPs).

We imported the locations and rough boundaries of PVPs identified during the field surveys into ArcGIS Pro 2.5.0 and created a reference dataset by refining the boundaries of each PVP using 20-cm resolution leaf-off colour-infrared (CIR) orthoimagery from the South Central Ontario Orthophotography Project (SCOOP; see Table 2.1). Based on visual interpretation of the SCOOP imagery at each PVP location, we also split each PVP into classes of open water and covered water (water covered by trees or other vegetation). Lastly, we digitized the remaining land cover in the SCOOP imagery into classes of impervious surfaces (e.g. bedrock, roads), shadows, or forest.

Image data sources

The primary sources of data used as input for our machine learning classifiers were the SCOOP products and European Space Agency's (ESA) Sentinel 2 imagery (see Table 2.1). We also used two layers of wetlands and other water bodies created by the OMNRF to screen out water bodies that had had been previously classified.

SCOOP: SCOOP has been funded through multiple government agencies (federal, provincial, municipal) to provide seamless aerial imagery of south-central Ontario at 5-year intervals (2013, 2018, etc.) and is freely available to all stakeholders and applicable research institutions. The 2013 and 2018 SCOOP products included 20-cm resolution leaf-off CIR orthoimagery and a 2-m resolution, stereoscopically derived Digital Terrain Model (DTM). While we were unable to correct for true reflectance using the available orthoimagery metadata, each dataset was derived from overlapping stereo images to be consistent in tone and appearance. Close inspection of each dataset revealed no discernible differences in the spectral signatures of major land classes within the study region. In addition, the imagery was acquired within a four-day rainless period for the study region, meaning changes in vegetation and surface wetness within datasets were likely minimal. Using tools in ArcGIS, we derived a Normalized Difference Water Index (NDWI) and slope data from the SCOOP products. We also derived a depression likelihood map using the Stochastic Depression Analysis tool in Whitebox Geospatial Analytical Tools version 3.3 (Lindsay 2016). Stochastic depression analysis accounts for uncertainties in DTMs when evaluating the likelihood that a particular geographic area exists as a depression in the landscape (Lindsay 2005). In their approach to map vernal pools in Massachusetts, Wu et al. (2014) found success using stochastic depression analysis to account for uncertainty in LiDAR derived digital elevation models. The SCOOP imagery bands and the SCOOP-derived products were used as inputs for the machine learning classifiers. The SCOOP imagery bands were also used during the segmentation procedure to create image objects.

Sentinel 2: We downloaded Sentinel 2 Level 1C products from the U.S. Geological Survey’s (USGS) EarthExplorer portal in mid-spring for dates with low cloud cover that coincided with the field surveys (April 27, 2016; May 7, 2018; May 5, 2019; see Table 2.1). Since most of the snow had melted by late April, we have assumed that vernal pools in these satellite images were maximally inundated. We preprocessed the image in ESA's Sentinel Application Platform version 7.0 (SNAP) using the Sen2Cor processor version 280 to create Level 2A terrain-corrected, bottom-of-atmosphere reflectance products. The Scene Classification map created as part of Sen2Cor processing was used to mask out clouds and cloud shadows from the corrected images. While Sentinel 2 has bands with resolutions of 10 m, 20 m, and 60 m, we were interested in the 10 m bands (Blue, Green, Red, and NIR) for the purposes of detecting small forested vernal pools. The corrected bands were averaged across years to create a single multi-band image and the NIR band was used to screen for pixels likely to be inundated based on a threshold of <0.195 reflectance units. We determined this threshold by averaging reflectance units of pixels along the edges of known wet forest locations. The averaged Sentinel 2 bands were also used as inputs for the machine learning classifiers.

Model development for classification of PVPs

Image Segmentation

Our object-based machine learning approach to classify vernal pools was broken down into two stages (Figure 2.3). The first stage involved segmenting our high-resolution SCOOP imagery using Trimble Geospatial's object-based image analysis platform, eCognition version 9.2. Areas of interest were first extracted for each plot with Sentinel 2 thresholding and by masking previously classified water bodies. Then we performed multiresolution segmentation on these areas of interest using the two sets of imagery bands to create image objects. We assigned a weight of 1 to each of the RGB bands and 2 to the NIR bands because the NIR band exhibits high contrast between water and non-water features. The multi-resolution segmentation algorithm has three parameters that control the shape and position of image objects: scale, shape, and compactness. The scale parameter controls the amount of spectral variation within image objects, which relates to object size. The shape parameter controls the degree to which object shape and colour factor into segmentation. The compactness parameter controls the weighting between the compactness and smoothness of an object's shape. We selected a range of realistic values for each parameter (Scale: 10 to 1000; Shape and Compactness: 0.1 to 0.9) and tested each combination on three test study plots by visually inspecting how well the resultant objects overlapped with the class boundaries on the reference dataset. Using this process, we determined an optimal parameter combination of 100, 0.25, and 0.25 for scale, shape, and compactness, respectively.

To reduce the number of image objects representing non-water bodies, we applied a spectral difference algorithm on the objects created through segmentation. The spectral difference algorithm merges neighbouring objects that have a difference in spectral means below a given threshold. We weighted the NIR bands three times higher than the other bands to make it difficult for water bodies to be merged with non-water bodies. We used a qualitative approach to assign a threshold for the spectral difference algorithm. We started with a threshold of 2 and raised it until the algorithm started to merge water bodies with non-water bodies. Using this approach, we selected a threshold of 8 digital number units.

Image objects were exported in shapefile format (.shp) with feature attributes derived from the SCOOP products, the Sentinel 2 bands, the OMNRF waterbodies, and the shapes of the objects (Table 2.2). In addition to deriving means and standard deviations of our datasets, we derived Haralick texture features from the Gray Level Cooccurrence Matrix (GLCM) and the Gray Level Difference Vector (GLDV) of the mean NIR bands using omnidirectional pixel-pair sampling. These metrics describe the texture of each image object based on the NIR bands and are useful in other wetland-based classification studies (Chatziantoniou et al. 2017; Ma et al. 2015).

Model Building

The second stage of our classification approach involved the evaluation of machine learning classifiers, Random Forest (RF) and Support Vector Machines (SVM), for mapping PVPs using the object features extracted from image segmentation (Table 2.2). Our modelling framework was broken down into five steps: 1) assigning class labels to image objects, 2) creating training and validation datasets, 3) selecting features, 4) training models, and 5) post-classification adjustments. Class labels assignment and post-classification adjustments were completed in ArcGIS Pro, while the rest of the modelling framework was performed in R, version 3.6.2.

For the first step, we started by overlaying the classified reference dataset shapefile on the image object shapefile to calculate class percentages for each object. We then labelled each object with their majority class. In order to ensure strong class representation in our training data, we subset the image objects using a threshold of >60% overlap with the majority class.

To separate our image objects into training and validation datasets, we iterated through random assignments of study plots to either the training or validation datasets (60% to training and 40% to validation) until the class distributions for the training and validation datasets did not differ by more than 10% from the class distributions in the complete dataset. We also made sure that the plots used to refine the segmentation parameters were part of the training dataset. While this approach may lead to autocorrelation between the training and validation datasets, we decided it was necessary to ensure sufficient representation of the PVP objects for each dataset.

The feature selection step of our model framework was used to select a subset of relevant features for model training because some features can be either redundant or irrelevant to classification and can lead to poor accuracy. Feature selection techniques help mitigate these problems in addition to problems associated with overfitting and unacceptably long computation time. This is especially important for OBIA where segmentation procedures lead to significantly more features than pixel-based methods. To select the feature subset for model training, we applied Recursive Feature Elimination (RFE) to the training dataset using the caret package in R (Kuhn 2019). RFE is a backward feature elimination technique and works by fitting successive models and removing the weakest features until a specified number of features are left. We used random forest models to run RFE and the mean decrease in accuracy to determine feature importance. This measure determines feature importance by finding the difference in prediction error between models with and without each feature. To select an optimal subset size, we used K-fold cross-validation (CV) for a range of subset sizes (8, 16, 24, 32, 40, and 57 features). CV reduces problems associated with overfitting by splitting the training data into K groups and running K RFE models, such that for each model, one of the K groups is reserved as a test set and the remaining K-1 groups are used to train the model. We chose 10 folds for our study, as this value has been recommended in the literature (Kuhn and Johnson 2013). Average model performance across the 10 repeated models can then be compared for the different subset sizes to determine the optimal subset of features.

Once we decided on a subset of features, we used the train function in the caret package to optimize the machine learning classifiers for our training dataset. Each machine learning classifier has one or more parameters that can be optimized for model performance. For RF, the optimization parameters the number of features that are randomly selected for splitting at each node in the classification tree (mtry), and the number of individual classification trees to run for the model (ntree). For SVM, the optimization parameters depend on the type of kernel used. We used the radial basis function as the kernel for our classifier as it has yielded strong results in other remote sensing applications (Kavzoglu and Colkesen 2009). SVM with a radial basis function has two optimization parameters: sigma, which describes the influence of individual support vectors; and cost, which controls the penalty for misclassified points. Both parameters impact the trade-off between model simplicity and misclassification. For each classifier, we selected a range of possible values for each parameter and used repeated CV to estimate performance metrics for each unique parameterization. Repeated CV accounts for potential variability in model performance metrics across different splits of the data by averaging over multiple CV procedures. We used 10 folds for each CV and took an average after the CV procedure was repeated 10 times. Because our classes were highly imbalanced (there were 10-100 times more objects in the forest class than in other classes), we also incorporated down-sampling into the model training procedure. The main problem with class imbalances is that the majority class is the main driver of model fit, meaning infrequent classes can be underrepresented in the final model. Down-sampling mitigates this by randomly selecting a subset of each class such that every class has the same number of observations. Down-sampling was applied after each of the N x K sub-sampling procedures.

Since our goal was to inventory all PVP locations, we focused on the open-water class rather than all classes to assess the performance of each model parameterization. We used an F-Score as our performance metric, which is a weighted average of precision and recall, and is calculated according to the following equations:

$$precision = \frac{\#\, true\ positives}{\#\, true\ positives + \#\, false\ positives}$$

$$recall = \frac{\#\, true\ positives}{\#\, true\ positives + \#\, false\ negatives}$$

$$F_\beta = (1 + \beta^2)\,\frac{precision \times recall}{(\beta^2 \times precision) + recall}$$

Where β is the weight of the F-Score. When $\beta>1$, recall is weighted higher than precision, and when $\beta<1$, precision is weighted higher than recall. For this study, we were more interested in minimizing false negatives over false positives because false positives are easier to rectify in the field. As such, we chose $\beta=2$ to favour false negatives. The model parameterization with the highest F-Score was used as the final model for each classifier.

Once each machine learning model was trained, we exported the objects with their predicted classes to ArcGIS Pro and performed a final set of post-classification adjustments based on spatial relationships. First, we merged neighbouring objects with the same class together to create seamless objects. To account for boundary inaccuracies of previously classified large waterbodies, we applied a 10-m buffer around these and labelled all open-water or covered-water objects overlapping this buffer as **“OMNRFwater**”. We assigned any impervious surface objects that overlapped with an OMNRF road shapefile to a “**road”** class. To minimize commission errors resulting from shadows on road being misclassified as open water due to similar spectral properties, we assigned any open-water objects sharing a border with a road to the “**shadow**” class. We considered all open-water objects and any covered-water objects that bordered open-water objects as part of the “**PVP**” class. Given that all the PVPs we surveyed in the field were separated by greater than 20 m distance from each other, we merged PVPs occurring within 20 m into a single PVP object. All other covered-water objects that did not share a border with open-water objects were assigned to the “**forest**” class.

Accuracy Analysis

We applied the modelling framework to the validation dataset and completed the same post-classification adjustments used on the training dataset to produce a classified dataset for unbiased accuracy analysis. Since our primary interest was the location of vernal pools, we used an object-based metric of success defined by if the location of PVPs in our classified dataset overlapped those in our reference dataset. As such, true positives were classified as PVPs that overlapped with reference PVPs, false positives were classified PVPs that did not exist as PVPs in the reference dataset, and false negatives were reference PVPs that were not mapped in the classified dataset. While we considered any overlap between classified and reference objects to represent true positives, we recognise that a more conservative overlap criterion would reduce the estimated accuracy of the models. We were less concerned with PVP shape and size compared to PVP location, so this criterion was acceptable for our purposes. To compare the RF and SVM classifiers, we used another F-Score with a β=2 to put more emphasis on false negatives.

To maximize the accuracy of our reference dataset, we included all water bodies encountered through field sampling, and this included extremely small pools (< 50 m^2) that tended to dry out before obligate amphibian larvae could develop. Since these pools were less frequently associated with obligate amphibians and were considerably more difficult to identify, we decided to only include PVPs >50 m^2 to calculate producer's accuracy. We also investigated possible effect of pool size and distance from roads on true positives, false positives, and false negatives in our classified results. Due to differences in the average size of PVP objects between the reference and classified datasets, we separately compared the median areas of true positives to false positives and of true positives to false negatives using the reference and classified datasets, respectively. We used a similar approach when comparing the percentage of PVP objects within 100 m of a road.

Results

Within the 23 forested plots (each 500m x 500m) in southeastern Georgian Bay, we encountered 133 PVPs (> 50 m^2) that had not been mapped by the OMNRF as either water body or wetland. These hitherto undocumented wetlands ranged from small pools with open water and sparse vegetation (typically < 500 m^2) to large wetlands dominated by emergent vegetation (typically > 500 m^2), although the median size was relatively small (222 m^2). The large vegetated wetlands (> 500 m^2) we encountered would not be typically designated as vernal pools in the literature, but we found that the edges of these wetlands often contained temporary pools of water that provided similar habitat as traditional vernal pools. In fact, many of these edge pools were opportunistically observed to contain eggs of vernal pool obligate amphibians.

Although our use of the Sentinel band 8 threshold and the OMNRF wetlands/waterbodies resulted in omission of two reference PVPs, this procedure reduced the area for analysis by 75%, and saved a lot of time and processing power. In trials without the use of Sentinel imagery to screen for potentially inundated areas, we found unacceptably high errors of commission that would have falsely indicated an abundance of vernal pool habitat.

The segmentation procedure created 13106 objects with a mean size of 178 m^2. Of these objects, 95% overlapped a reference layer class by more than 60% and were labelled with this class. We used 7518 objects in 14 training plots to train the machine learning classifiers. Most of these objects were labelled as forest (84%), while a smaller portion were labelled as covered water (7%), impervious surfaces (5%), open water (2.5%), and shadows (1.5%).

The RFE procedure selected a subset of 32 unique object features that were deemed important for model training (Table 2.3). Roughly 60% of the selected features were derived from means and standard deviations of SCOOP imagery bands or band derivatives. Of the remaining features, four were derived from Sentinel imagery bands, four from the SCOOP DTM derivatives, two from the GLCM and GLDV, two from the object shape, and one from the existence of neighbouring OMNRF waterbodies.

We used cross-validation to select for the optimal model parameterization for the RF and SVM machine learning classifiers. The optimal parameterization for the RF model used an mtry of 2, an ntree of 200, and achieved cross-validated F-Score of 0.65 (precision=0.44, recall=0.75). The optimal parameterization for the SVM model used sigma of 0.05 and a cost of 1. This parameterization yielded an F-Score of 0.58 (precision=0.39, recall=0.68). It is worth noting that using a custom F-Score that puts more weight on the class of interest resulted in different parameterizations for each model when compared to using the default accuracy metrics. When we used more common accuracy metrics of Overall Accuracy and Kappa Score, we ended up with lower final accuracies for our vernal pool classification. Overall Accuracy and Kappa Score weigh classes evenly, so, while the classifier may perform better overall, most of the resultant classes are not relevant for the accuracy of vernal pool delineation.

The trained RF and SVM models were applied to the objects within the validation plots to produce 5129 classified objects for each classifier. Both classifiers produced similar proportions of classes: 5-6% open water, 5-9% shadows, 7-9% impervious surfaces, 22% covered water, and 57-58% forest. The increased proportion of open-water and covered-water classes compared to the reference dataset was mainly a result of extensions of the OMNRF waterbodies that were missed during the digitization of the reference dataset. Similarly, the increased proportion of shadows in the training dataset compared to the reference dataset was because the lighter-coloured shadows in the SCOOP imagery had not been identified as such in the reference dataset. The majority of the objects classified as open water were adjacent to other water objects: open water, covered water, or OMNRF waterbodies (90% and 77% for RF and SVM respectively). When using the post-classification procedure to group these spatially associated objects, we obtained 59 objects classified as PVPs for the RF classifier and 44 for the SVM classifier (Figure 2.4). The median size of the PVP objects classified by RF was only slightly larger than that in the reference dataset (285m^2 vs 222m^2, respectively), whereas those classified by SVM were twice as large (442 m^2 vs. 222m^2, respectively).

We assessed the accuracy of the PVP objects from the post-classification procedure using the F-Score, a weighted average of precision (user's accuracy) and recall (producer's accuracy). The F-Score we used in this study placed a higher weight on false positives, meaning that producer's accuracy had a stronger influence than user's accuracy. There appeared to be a direct trade-off between producer's and user's accuracies between the two classifiers. Whereas the RF classifier had higher producer's accuracies, the SVM classifier had comparatively higher user's accuracies (Table 2.4). Since producer's accuracy was weighted higher, the RF classifier produced higher F-Scores when compared to the SVM classifier (Table 2.4). Consequently, our optimal model using the RF classifier had an omission rate of 15% and a commission rate of 23% for reference PVPs.

Approximately 85% of the correctly classified PVP objects overlapped PVP objects from the reference dataset by more than 50%. The remaining 15% of correctly classified PVP objects overlapped the reference dataset by more than 20% but less than 50%. These PVPs tended to be highly vegetated with few areas of open water. The median size of PVPs that were correctly mapped in both the reference and classified datasets were larger than those that had been missed or were the result of commission error (Table 2.5). Another factor that may have affected mapping accuracy was difficulty in distinguishing between PVPs and the shadow class due to spectral confusion. This was supported by the fact that only 24% of the PVPs in the reference dataset occurred within 100 m of roads, while 38% of false positives occurred within this buffer (Table 2.5). Closer inspection of SCOOP imagery confirmed that false positives were always associated with dark shadows, which were especially common near roadways and on exposed bedrock. False negative PVPs included small PVPs (< 100 m^2), those with high canopy cover, and those on slopes or exposed bedrock with small drainage basins. These PVPs were difficult to distinguish from the shadow class.

Discussion

Remote sensing approaches for mapping small ephemeral wetlands have been prevalent during the last decade (Bourgeau-Chavez et al.,2016; Carpenter et al. 2011; Cormier et al. 2013; DiBello et al. 2016; Julian et al. 2009; Leonard et al., 2012; Riley et al. 2017; Wu et al. 2014). This study contributes to this growing knowledge base by combining documented wetland mapping techniques to develop an efficient classifier for vernal pools in a remote area of Ontario. We confirmed that groups of pixels representing vernal pools in high-resolution leaf-off CIR imagery can be separated from surrounding land-use with OBIA. Further, we found that machine learning was able to classify objects from OBIA based on the spectral, texture, shape, and neighbourhood characteristics of each object. Our object-based machine learning approach was able to accurately predict the locations of PVPs with a minimum size threshold of 50 m^2, with a corresponding producer's accuracy of 85% and user's accuracy of 77%. It is noteworthy that past studies that have achieved similar or better results for forested wetlands of comparable size required the use of LiDAR-derived data products, which are expensive and not yet available for remote regions in Ontario (Leonard et al. 2012; Riley et al. 2017; Wu et al. 2014; but see Bourgeau-Chavez et al. 2016).

This study also highlights the shortcomings of previous wetland mapping projects in the province. The wetland and waterbody datasets from the OMNRF accounted for 116 individual wetlands that intersected the 23 study plots. Our field surveys uncovered an additional 133 undocumented wetlands, more than doubling the estimate of wetland density for the region. While the provincial wetland and waterbody datasets remain very important for planning and watershed management, our study can supplement these data to provide an even stronger understanding of the water resources in this region of Ontario.

The RF and SVM classifiers performed similarly well in our study, though RF had a higher user's accuracy and tended to produce PVPs of more comparable size to the reference data. Neither classifier assumes any particular distribution of data and both are robust to noise and errors (Ma et al. 2017a). Studies that have reported RF outperforming SVM argue that SVM is more prone to overfitting the data because it is trained on the entire dataset whereas RF is trained on random subsets for each decision tree (Amani et al. 2017; Tian et al. 2016). Our use of repeated CV to subset the data during model training likely reduced the discrepancies between the RF and SVM classifiers with respect to overfitting the data. Though we did not compare computation time in our study, RF has generally been found to be faster when compared to SVM, thus making RF the better option when the two methods produce similar accuracies.

While our classification produced acceptable results for our purposes, we recognize that there are other options for model optimization that we did not explore. For example, we used a single feature selection algorithm and measure of importance to decide on an optimum feature subset. Given the diversity of feature selection methods available for remote sensing applications (e.g. Ma et al. 2017a), it is possible that different feature selection algorithms and importance measures could provide a more accurate prediction of the optimal feature set (Jović et al. 2015; Ma et al. 2017b). We also relied on a down-sampling approach to account for the issue of class imbalances. Though down-sampling is simple to implement, it can miss important discriminatory characteristics of the majority class and reduce the likelihood of capturing class variance, leading to potentially large inaccuracies in the model (He and Garcia 2009). Other resampling methods, such as up-sampling and the synthetic minority over-sampling technique, manipulate the data in different ways and may allow for a more accurate representation of the PVP classes in the model (Chawla et al. 2002; Douzas et al. 2019;

Maxwell et al. 2018). Cost-sensitive measures, which assign higher costs to misclassifications of the minority classes compared to those of the majority classes, are also common approaches for dealing with class imbalances and avoid resampling the data (He and Garcia 2009). While these alternative approaches also come with their own drawbacks, it is certainly possible that a comparison of approaches could yield a more accurate classification of vernal pools.

Our classifier had trouble differentiating between small pools and shadows on impervious surfaces. Conifer shadows on bedrock or roadsides shared similar spectral properties with areas of inundation and accounted for all errors of commission in our models. Similarly, small pools or pools with high canopy cover were often misclassified as shadows. Past vernal pool detection studies have had similar trouble differentiating small pools from shadows (Cormier et al. 2013; Faccio et al. 2013), though the inclusion of LiDAR-derived products has been found to reduce these errors (Leonard et al. 2012; Reutebach et al. 2003). A smaller proportion of reference pools were missed because of the Sentinel 2 screening process. These pools were typically perched on exposed bedrock and had small drainage basins that accounted for only a fraction of the Sentinel 2 pixel size. We found these pools to be less prevalent across our study region and they only accounted for a small fraction of the false negatives in our accuracy analysis.

We focussed our mapping efforts on the coast of southeastern Georgian Bay because it is a highly coveted area for current and future development. Our approach should be informative for our region of interest as the topography and land cover are relatively homogenous (Crins et al. 2009). If this approach were to be applied over the larger SCOOP extent, which stretches 150 km to the south and east of our study region (Figure 2.1), additional samples would be required. Our study sites are clustered and localized for our specific region, so it is unlikely that we have accounted for the full variation in topography and land cover encompassed by the SCOOP products. Similarly, it may be possible to obtain comparable mapping accuracies for regions covered by different Ontario Orthoimagery Projects (OOP), but models would need to be retrained with new field data. The suite of OOPs have differences in spectral characteristics stemming from the time of year when images were acquired, weather conditions before and during image capture, and the camera system used to take the images (OMNRF 2020). The accuracy of these new classifiers may be improved or reduced due to differences in landscape characteristics, such as topography and forest composition (Lathrop et al. 2005), or differences in the characteristics of the OOP, such as image quality and number of years of imagery.

Though the orthoimagery projects of Ontario were not intended to be used for supervised image classification, we believe they are a useful tool for small-scale mapping projects especially when combined with OBIA. Vernal pools are small, obscure, and critically understudied in Ontario. They provide essential habitat for amphibian species, such as mole salamanders (*Ambystoma jeffersonianum*, *A. laterale*, *A. maculatum*) and wood frogs (*Lithobates sylvaticus*), and act as important secondary habitat and stopover sites for species at risk, including Blanding's turtles (*Emydoidea blandingii*; Markle and Chow-Fraser 2014). Vernal pools also provide many ecosystem services including water retention, energy transfer, and nutrient cycling (Hunter 2008; Leibowitz 2003). The conservation of these ecosystems will be important for Ontario, especially in regions that are under high development pressure, such as southeastern Georgian Bay. The classification models from this study should be used to identify vernal pool hotspots and narrow down locations to conduct ground surveys for verification of vernal pools. To verify the locations of vernal pools based on the classified maps, we suggest that surveyors confirm not only the presence of water, but also the presence of obligate amphibian breeders. This would involve either egg or larvae surveys, depending on the time of year. Though Ontario does not have a strict definition of vernal pools, these constraints are consistent with other N. American jurisdictions that have existing vernal pool mapping programs (Brooks et al. 1998; Faccio et al. 2013; DiBello et al. 2016). While field verification is certainly feasible for projects focussed on small areas of interest, the detailed and time-consuming nature of the field work may make this methodology infeasible for large areas of interest.

To date, there are no comprehensive databases of vernal pools for forests of southeastern Georgian Bay or any forested region in Ontario. Knowledge of vernal pool distributions would fill a notable gap in understanding the importance of habitat connectivity for wildlife that are associated with wetland networks. This knowledge will also be important for deciphering the potential impacts of climate change and land-use changes on water resources, including earlier drying and loss of landscape connectivity (Brooks 2009). Although inclusion of high-resolution LiDAR and RADAR data would no doubt improve our mapping, this technology is too expensive to be widely available, and the need for information on vernal pool distribution is too great to hold out for these data.

Conclusion

The study presented here showed that an OBIA approach using high-resolution multispectral imagery and machine learning classification is a promising approach for the detection of potential vernal pools in heavily forested regions of central Ontario. Vernal pools are often overlooked in regional wetland mapping projects due to their small size and the obstruction from the forest canopy. The use of image segmentation to identify homogenous regions of sub-meter-resolution aerial imagery allowed for the detection of forested vernal pools down to a visible surface area of 50 m^2. Our study found that the RF classifier marginally outperformed the SVM classifier, providing final producer’s and user’s accuracies of 85% and 79% respectively.

Errors encountered in this study were the result of misclassifications between small pools and shadows from coniferous trees. It is unlikely that an approach relying exclusively on multispectral imagery can eliminate these types of errors. Once high-resolution LiDAR and/or RADAR become available for these remote regions, the accuracy and precision of this approach will likely improve. In the meantime, this approach provides an efficient method to identify potential vernal pools, thus greatly reducing the time, financial commitment and human resources needed to improve upon local-scale databases of wetland resources.

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References

Amani, M., Salehi, B., Mahdavi, S., Granger, J.E., Brisco, B., and Hanson, A. 2017. "Wetland Classification Using Multi-Source and Multi-Temporal Optical Remote Sensing Data in Newfoundland and Labrador, Canada." *Canadian Journal of Remote Sensing*, 43(4): pp. 360–73. doi:10.1080/07038992.2017.1346468.

Baldwin, R.F., and deMaynadier, P.G. 2009. "Assessing Threats to Pool-Breeding Amphibian Habitat in an Urbanizing Landscape." *Biological Conservation*, 142(8): pp. 1628–38. doi:10.1016/j.biocon.2009.02.039.

Blaschke, T. 2010. "Object Based Image Analysis for Remote Sensing." *ISPRS Journal of Photogrammetry and Remote Sensing*, 65(1): pp. 2–16. doi:10.1016/J.ISPRSJPRS.2009.06.004.

Bourgeau-Chavez, L., Lee, Y., Battaglia, M., Endres, S., Laubach, Z., and Scarbrough, K. 2016. "Identification of Woodland Vernal Pools with Seasonal Change PALSAR Data for Habitat Conservation." *Remote Sensing*, 8(6): pp. 490. doi:10.3390/rs8060490.

Brooks, R.T. 2009. "Potential Impacts of Global Climate Change on the Hydrology and Ecology of Ephemeral Freshwater Systems of the Forests of the Northeastern United States." *Climatic Change*, 95: pp. 469–83. doi:10.1007/s10584-008-9531-9.

Brooks, R.T., Stone, J., and Lyons, P. 1998. “An Inventory of Seasonal Forest Ponds on the Quabbin Reservoir Watershed, Massachusetts.” *Northeastern Naturalist*, 5(3): pp. 219. doi:10.2307/3858622.

Calhoun, A.J.K., Walls, T.E., Stockwell, S.S., and Mccollough, M. 2003. “Evaluating Vernal Pools as a Basis for Conservation Strategies: A Maine Case Study.” *Wetlands*,. Vol. 23. Springer. doi:10.1672/0277-5212(2003)023[0070:EVPAAB]2.0.CO;2.

Calhoun, A.J.K., Mushet, D.M., Bell, K.P., Boix, D., Fitzsimons, J.A., and Isselin-Nondedeu, F. 2017. “Temporary Wetlands: Challenges and Solutions to Conserving a ‘disappearing’ Ecosystem.” *Biological Conservation*, 211(July): pp. 3–11. doi:10.1016/j.biocon.2016.11.024.

Carpenter, L., Stone, J., and Griffin, C.R. 2011. “Accuracy of Aerial Photography for Locating Seasonal (Vernal) Pools in Massachusetts.” *Wetlands*, 31(3): pp. 573–81. doi:10.1007/s13157-011-0169-4.

Chatziantoniou, A., Psomiadis, E., and Petropoulos, G. 2017. “Co-Orbital Sentinel 1 and 2 for LULC Mapping with Emphasis on Wetlands in a Mediterranean Setting Based on Machine Learning.” *Remote Sensing*, 9(12): pp. 1259. doi:10.3390/rs9121259.

Chawla, N. V., Bowyer, K.W., Hall, L.O., and Kegelmeyer, W.P. 2002. “SMOTE: Synthetic Minority over-Sampling Technique.” *Journal of Artificial Intelligence Research*, 16(June): pp. 321–57. doi:10.1613/jair.953.

Cormier, T.A., Congalton, R.G., and Babbitt, K.J. 2013. “Spatio-Statistical Predictions of Vernal Pool Locations in Massachusetts.” *Photogrammetric Engineering & Remote Sensing*, 79(1): pp. 25–35. doi:10.14358/PERS.79.1.25.

Crins, W.J., Gray, P.A., Uhlig, P.W.C., and Wester, M.C. 2009. “The Ecosystems of Ontario, Part 1: Ecozones and Ecoregions.” Sault Ste. Marie: Ontario Ministry of Natural Resources.

DiBello, F.J., Calhoun, A.J.K., Morgan, D.E., and Shearin, A.F. 2016. “Efficiency and Detection Accuracy Using Print and Digital Stereo Aerial Photography for Remotely Mapping Vernal Pools in New England Landscapes.” *Wetlands*, 36(3): pp. 505–14. doi:10.1007/s13157-016-0759-2.

Douzas, G., Bacao, F., Fonseca, J., and Khudinyan, M. 2019. “Imbalanced Learning in Land Cover Classification: Improving Minority Classes’ Prediction Accuracy Using the Geometric SMOTE Algorithm.” *Remote Sensing*, 11(24): pp. 3040. doi:10.3390/rs11243040.

Dronova, I. 2015. “Object-Based Image Analysis in Wetland Research: A Review.” *Remote Sensing*, 7(5): pp. 6380–6413. doi:10.3390/rs70506380.

Evans, J.P., Cecala, K.K., Scheffers, B.R., Oldfield, C.A., Hollingshead, N.A., Haskell, D.G., and McKenzie, B.A. 2017. “Widespread Degradation of a Vernal Pool Network in the Southeastern United States: Challenges to Current and Future Management.” *Wetlands*, 37(6): pp. 1093–1103. doi:10.1007/s13157-017-0943-z.

Faccio, S.D., Lew-Smith, M., and Worthley, A. 2013. “Vermont Vernal Pool Mapping Project: Final Report to the Natural Heritage Information Project of the Vermont Department of Fish and Wildlife.” Huntington: Arrowwood Environmental. http://vtecostudies.org/wp-content/uploads/2014/08/vce-vernal-pool-mapping-final-report.pdf.

Fu, B., Wang, Y., Campbell, A., Li, Y., Zhang, B., Yin, S., Xing, Z., and Jin, X. 2017. “Comparison of Object-Based and Pixel-Based Random Forest Algorithm for Wetland Vegetation Mapping Using High Spatial Resolution GF-1 and SAR Data.” *Ecological Indicators*, 73: pp. 105–17. doi:10.1016/j.ecolind.2016.09.029.

Grenier, M., Labrecque, S., Garneau, M., and Tremblay, A. 2008. “Object-Based Classification of a SPOT-4 Image for Mapping Wetlands in the Context of Greenhouse Gases Emissions: The Case of the Eastmain Region, Québec, Canada.” *Canadian Journal of Remote Sensing*, 34: pp. 398–413. doi:10.5589/m08-049.

Harken, J., and Sugumaran, R. 2005. “Classification of Iowa Wetlands Using an Airborne Hyperspectral Image: A Comparison of the Spectral Angle Mapper Classifier and an Object-Oriented Approach.” *Canadian Journal of Remote Sensing*, 31(2): pp. 167–74. doi:10.5589/m05-003.

He, H., and Garcia, E.A. 2009. “Learning from Imbalanced Data.” *IEEE Transactions on Knowledge and Data Engineering*, 21(9): pp. 1263–84. doi:10.1109/TKDE.2008.239.

Hunter, M.L. 2007. “Valuing and Conserving Vernal Pools as Small-Scale Ecosystems.” In *Science and Conservation of Vernal Pools in Northeastern North America*, edited by A.J.K. Calhoun and P.G. Demaynadier, 1–10. Boca Raton, FL: CRC Press.

Jansujwicz, J.S., Calhoun, A.J.K., and Lilieholm, R.J. 2013. “The Maine Vernal Pool Mapping and Assessment Program: Engaging Municipal Officials and Private Landowners in Community-Based Citizen Science.” *Environmental Management*, 52(6): pp. 1369–85. doi:10.1007/s00267-013-0168-8.

Julian, J.T., Young, J.A., Jones, J.W., Snyder, C.D., and Wright, C.W. 2009. “The Use of Local Indicators of Spatial Association to Improve LiDAR-Derived Predictions of Potential Amphibian Breeding Ponds.” *Journal of Geographical Systems*, 11(1): pp. 89–106. doi:10.1007/s10109-008-0074-4.

Jović, A., Brkić, K., and Bogunović, N. 2015. “A Review of Feature Selection Methods with Applications.” In *2015 38th International Convention on Information and Communication Technology, Electronics and Microelectronics, MIPRO 2015 - Proceedings*, 1200–1205. Institute of Electrical and Electronics Engineers Inc. doi:10.1109/MIPRO.2015.7160458.

Kavzoglu, T., and Colkesen, I. 2009. “A Kernel Functions Analysis for Support Vector Machines for Land Cover Classification.” *International Journal of Applied Earth Observation and Geoinformation*, 11(5): pp. 352–59. doi:10.1016/j.jag.2009.06.002.

Kuhn, M. 2019. “The Caret Package.” http://topepo.github.io/caret/index.html.

Kuhn, M., and Johnson, K. 2013. *Applied Predictive Modelling*. New York: Springer.

Lang, M.W., and McCarty, G.W. 2009. “Lidar Intensity for Improved Detection of Inundation below the Forest Canopy.” *Wetlands*, 29(4): pp. 1166–78. doi:10.1672/08-197.1.

Lathrop, R.G., Montesano, P., Tesauro, J., and Zarate, B. 2005. “Statewide Mapping and Assessment of Vernal Pools: A New Jersey Case Study.” *Journal of Environmental Management*, 76(3): pp. 230–38. doi:10.1016/J.JENVMAN.2005.02.006.

Leibowitz, S.G. 2003. “Isolated Wetlands and Their Functions: An Ecological Perspective.” *Wetlands*, 23(3): pp. 517–31. doi:10.1672/0277-5212(2003)023[0517:IWATFA]2.0.CO;2.

Leonard, P.B., Baldwin, R.F., Homyack, J.A., and Wigley, T.B. 2012. “Remote Detection of Small Wetlands in the Atlantic Coastal Plain of North America: Local Relief Models, Ground Validation, and High-Throughput Computing.” *Forest Ecology and Management*, 284(November): pp. 107–15. doi:10.1016/J.FORECO.2012.07.034.

Lindsay, J.B. 2005. “The Terrain Analysis System: A Tool for Hydro-Geomorphic Applications.” *Hydrological Processes*, 19(5): pp. 1123–30. doi:10.1002/hyp.5818.

Lindsay, J.B. 2016. “Whitebox Geospatial Analytical Tools.” https://jblindsay.github.io/ghrg/Whitebox/download.shtml.

Ma, L., Cheng, L., Li, M., Liu, Y., and Ma, X. 2015. “Training Set Size, Scale, and Features in Geographic Object-Based Image Analysis of Very High Resolution Unmanned Aerial Vehicle Imagery.” *ISPRS Journal of Photogrammetry and Remote Sensing*, 102(April): pp. 14–27. doi:10.1016/J.ISPRSJPRS.2014.12.026.

Ma, L., Fu, T., Blaschke, T., Li, M., Tiede, D., Zhou, Z., Ma, X., and Chen, D. 2017a. “Evaluation of Feature Selection Methods for Object-Based Land Cover Mapping of Unmanned Aerial Vehicle Imagery Using Random Forest and Support Vector

Machine Classifiers." *ISPRS International Journal of Geo-Information*, 6(2): pp. 51–62. doi:10.3390/ijgi6020051.

Ma, L., Li, M., Ma, X., Cheng, L., Du, P., and Liu, Y. 2017b. "A Review of Supervised Object-Based Land-Cover Image Classification." *ISPRS Journal of Photogrammetry and Remote Sensing*, 130(August): pp. 277–93. doi:10.1016/J.ISPRSJPRS.2017.06.001.

Markle, C.E., and Chow-Fraser, P. 2014. "Habitat Selection by the Blanding's Turtle (Emydoidea Blandingii) on a Protected Island in Georgian Bay, Lake Huron." *Chelonian Conservation and Biology*, 13(2): pp. 216–26. doi:10.2744/ccb-1075.1.

Maxwell, A.E., Warner, T.A., and Fang, F. 2018. "Implementation of Machine-Learning Classification in Remote Sensing: An Applied Review." *International Journal of Remote Sensing*, 39(9): pp. 2784–2817. doi:10.1080/01431161.2018.1433343.

Millard, K., and Richardson, M. 2013. "Wetland Mapping with LiDAR Derivatives, SAR Polarimetric Decompositions, and LiDAR–SAR Fusion Using a Random Forest Classifier." *Canadian Journal of Remote Sensing*, 39(4): pp. 290–307. doi:10.5589/m13-038.

Ontario Ministry of Natural Resources and Forestry. 2014. "Ontario Wetland Evaluation System: Southern Manual, 3rd Edition." https://files.ontario.ca/environment-and-energy/parks-and-protected-areas/ontario-wetland-evaluation-system-southen-manual-2014.pdf.

Ontario Ministry of Natural Resources and Forestry. 2020. “Imagery User Guide.” https://www.sdc.gov.on.ca/sites/MNRF-PublicDocs/EN/CMID/Ontario_Imagery_User_Guide.pdf.

Rampi, L.P., Knight, J.F., and Pelletier, K.C. 2014. “Wetland Mapping in the Upper Midwest United States: An Object-Based Approach Integrating Lidar and Imagery Data.” *Photogrammetric Engineering & Remote Sensing*, 80(5): pp. 439–49. doi:10.14358/PERS.80.5.439.

Reutebuch, S.E., Mc Gaughey, R.J., Andersen, H.E., and Carson, W.W. 2003. “Accuracy of a High-Resolution Lidar Terrain Model under a Conifer Forest Canopy.” *Canadian Journal of Remote Sensing*, 29(5): pp. 527–35. doi:10.5589/m03-022.

Riley, J.W., Calhoun, D.L., Barichivich, W.J., and Walls, S.C. 2017. “Identifying Small Depressional Wetlands and Using a Topographic Position Index to Infer Hydroperiod Regimes for Pond-Breeding Amphibians.” *Wetlands*, 37: pp. 325–38. doi:10.1007/s13157-016-0872-2.

Semlitsch, R.D., and Bodie, J.R. 1998. “Are Small, Isolated Wetlands Expendable?” *Conservation Biology*, 12(5): pp. 1129–33. doi:10.1046/j.1523-1739.1998.98166.x.

Tian, S., Zhang, X., Tian, J., and Sun, Q. 2016. “Random Forest Classification of Wetland Landcovers from Multi-Sensor Data in the Arid Region of Xinjiang, China.” *Remote Sensing*, 8(11): pp. 954–68. doi:10.3390/rs8110954.

Wu, Q., Lane, C., and Liu, H. 2014. “An Effective Method for Detecting Potential Woodland Vernal Pools Using High-Resolution LiDAR Data and Aerial Imagery.” *Remote Sensing*, 6(11): pp. 11444–67. doi:10.3390/rs61111444.

Table 2.1: Description of datasets and how they were used in this study.

Data Set	Coverage	Resolution	Acquisition Dates	Used to
Sentinel 2	Worldwide	10 m	April 27, 2016 May 7, 2018 May 5, 2019	Screen areas of interest for classification; derive features for model development
Ontario Ministry of Natural Resources and Forestry (OMNRF) Wetlands/Waterbodies	Ontario-wide	NA	NA	Refine areas of interest for classification
South Central Ontario Orthophotography Project (SCOOP) true colour/near-infrared imagery	~36000 km^2 north of Toronto and east of Georgian Bay	20 cm	May 5-7, 2013 May 14-17, 2018	Derive image objects in areas of interest for classification; derive features for model development
SCOOP stereo-derived DEM	Same as SCOOP imagery	1 m	May 5-7, 2013	Derive depression and slope data to be used as features for model development
Reference Vernal Pool Dataset (23 sites)	575 ha (25 ha per plot) See Figure 2.1	NA	April/May, 2016/2017/2019	Train and validate models

Table 2.2: Features extracted for each object following image segmentation. RGB = Red Green Blue; NIR = Near Infrared; NDWI = Normalized Difference Water Index; DTM = Digital Terrain Model; GLCM = Gray Level Cooccurrence Matrix; GLDV = Gray Level Difference Vector; OMNRF = Ontario Ministry of Natural Resources and Forestry

Data	Object Features
SCOOP imagery	RGB/NIR band means and standard deviations for 2013 and 2018 imagery Mean Brightness (mean of all bands) for 2013 and 2018 Means of averaged 2013 and 2018 RGB/NIR bands NDWI mean and standard deviation GLCM and GLDV statistics for NIR bands: angular second moment, contrast, correlation, dissimilarity, entropy, homogeneity, mean, standard deviation
SCOOP DTM	DTM standard deviation Mean and standard deviation of slope and depression likelihood
Sentinel 2 imagery	RGB/NIR band means
OMNRF Wetlands/ Waterbodies	Existence of neighbouring waterbodies (binary)
Object Shape	Area, asymmetry, border index, border length, compactness, density, elliptic fit, length, length/thickness, length/width, roundness, shape index

Table 2.3: Features selected for model training in order of estimated importance (Imp). The years in brackets (2013 or 2018) correspond to the imagery dataset year for the South Central Orthophotography Project. NDWI = Normalized Difference Water Index; NIR = Near Infrared; SD = standard deviation; OMNRF = Ontario Ministry of Natural Resources and Forestry; GLCM = Gray Level Cooccurrence Matrix

Object Features	Imp	Object Features	Imp	Object Features	Imp
Mean NDWI (2013)	10.4	SD Green (2013)	6.5	Mean Red (2018)	4.7
Mean Green (Sentinel)	9.9	Mean Blue (average of 2013 and 2018)	6.4	Mean Slope	4.1
Mean Red (Sentinel)	9.3	Mean NIR (2018)	6.3	SD Depression	3.3
Mean NIR (Sentinel)	9.2	Mean Blue (2013)	6.3	SD Blue (2013)	3.2
Mean NIR (average of 2013 and 2018)	8.9	Mean Red (2013)	5.6	SD NIR (2018)	3.2
Mean Blue (Sentinel)	8.5	Mean Green (2013)	5.4	GLCM Angular Second Moment	3.1
Brightness	7.2	SD Slope	5.2	Border Index	3.0
Mean NIR (2013)	7.1	Mean Green (2018)	5.0	SD NDWI (2013)	3.0
Mean Depression Likelihood	7.1	Mean Blue (2018)	4.9	Shape Index	2.9
Mean Green (average of 2013 and 2018)	6.7	Existence of OMNRF waterbodies as neighbour	4.8	GLCM Entropy	2.9
Mean Red (average of 2013 and 2018)	6.5			SD NIR (2013)	2.9

Table 2.4: Comparison of accuracy metrics for each model. Accuracy was assessed based on the proportion of overlapping potential vernal pool polygons between the reference and classified datasets. User's accuracy was calculated based on 59 classified potential vernal pool objects (PVP) objects for the Random Forest model and 44 classified PVP objects for the Support Vector Machine model. Producer's accuracy was calculated based on 48 reference PVP objects for both models. F-Score was calculated using a β value of 2 to place a higher emphasis on minimizing errors of omission.

Model	**Random Forest**	**Support Vector Machines**
User's Accuracy	78.0%	84.1%
Producer's Accuracy	85.4%	79.1%
F-Score (β=2)	0.83	0.81

Table 2.5: Comparison of potential vernal pool (PVP) polygon size and percentage found within 100 m of roads for reference and classified datasets using the Random Forest model. Mapped reference PVPs were those correctly classified by the model, while omitted reference PVPs were those missed by the model.

	Reference PVPs		**Classified PVPs**	
	Mapped (N=41)	Omitted (N=7)	True Positives (N=46)	False Positives (N=13)
Median size (m^2)	217	93	369	82
Within 100 m of road	24%	14%	28%	38%

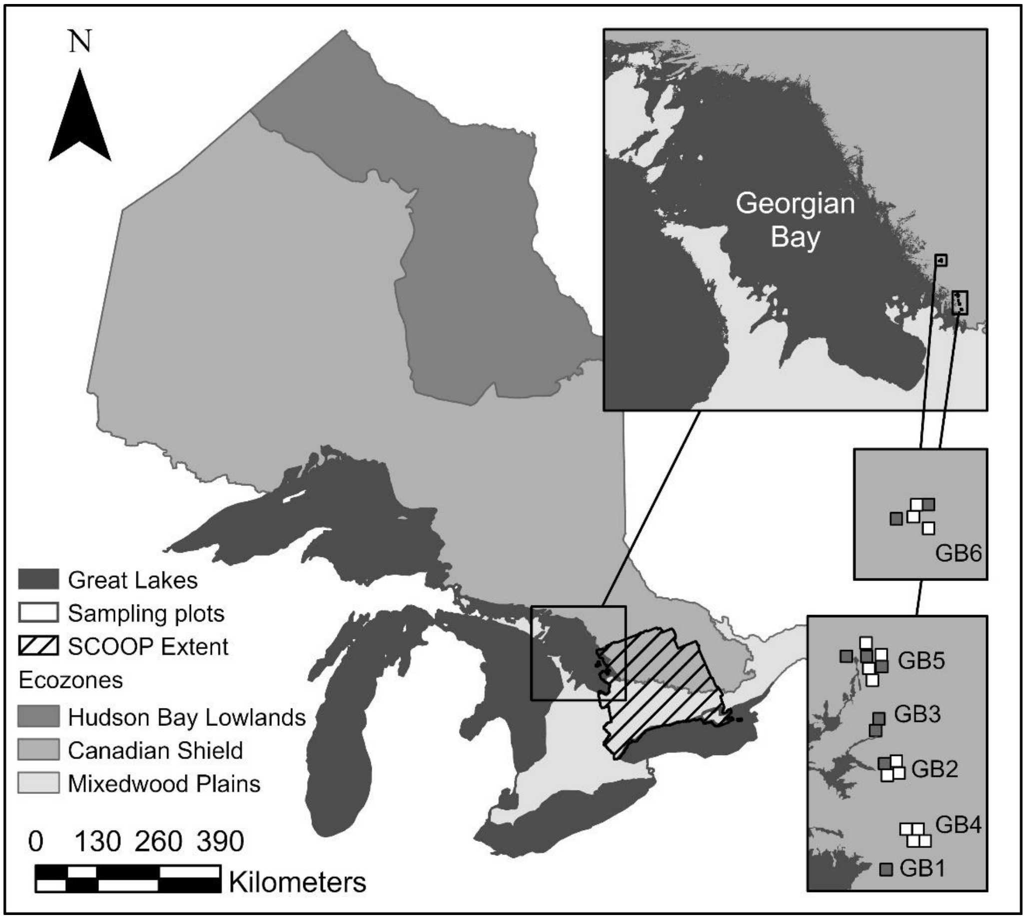

Figure 2.1: Location of the 23 sampling plots in the six sites (GB1, GB2, GB3, GB4, GB5, and GB6) in the Canadian Shield ecozone of Ontario, Canada. Plots used for model training are depicted as white squares, while plots used for validation are depicted as grey squares. The extent of the orthoimagery used in the study is shown with hatched markings. SCOOP = South Central Orthophotography Project

Figure 2.2: Example of a plot with transects (dashed lines) and the route taken by surveyors (solid line) to follow the transects while avoiding major barriers, such as roads and lakes. Basemap source: Ontario Ministry of Natural Resources and Forestry

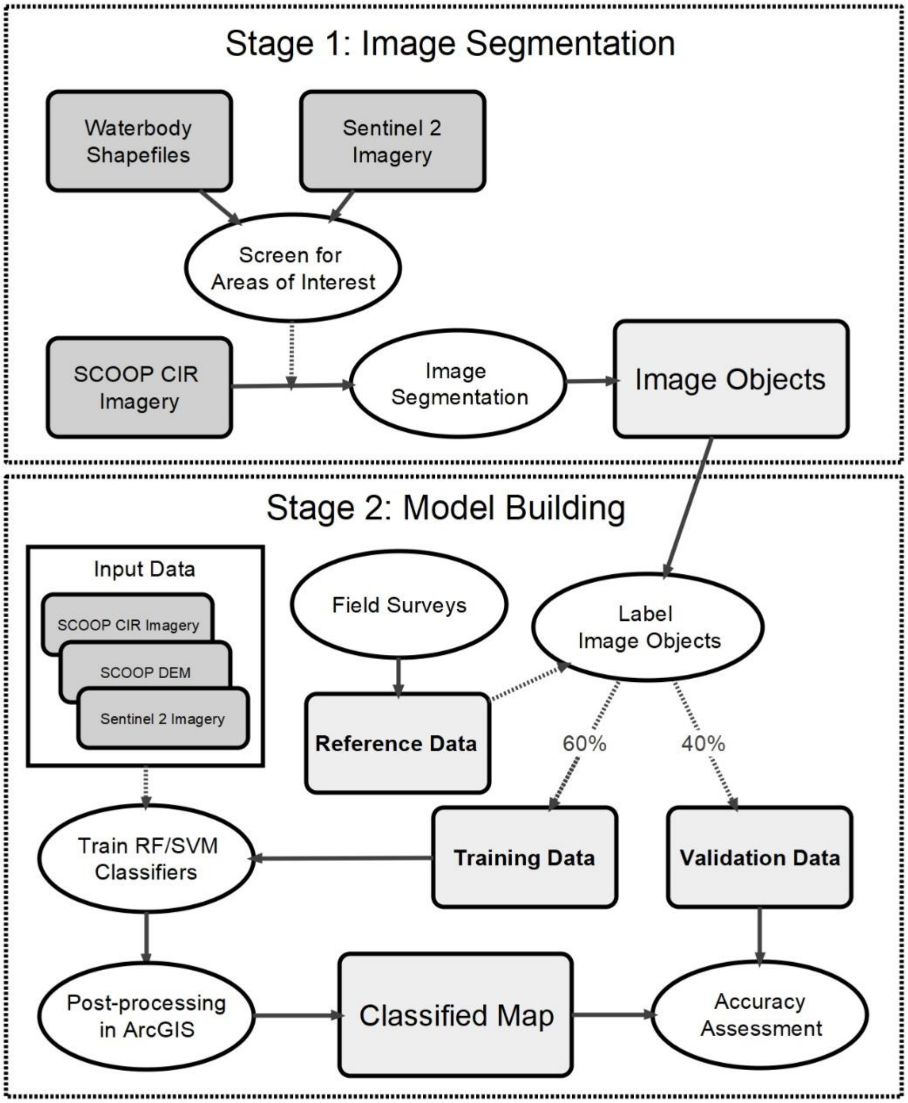

Figure 2.3: A flowchart depicting the two stages of our model development: stage 1 (top panel) image segmentation of high-resolution leaf-off CIR imagery and stage 2 (bottom panel) model building through training and validation of RF and SVM classifiers using reference data from field surveys. SCOOP = South Central Ontario Orthophotography Project; CIR = Colour-Infrared; DEM = Digital Elevation Model; RF = Random Forest; SVM = Support Vector Machines.

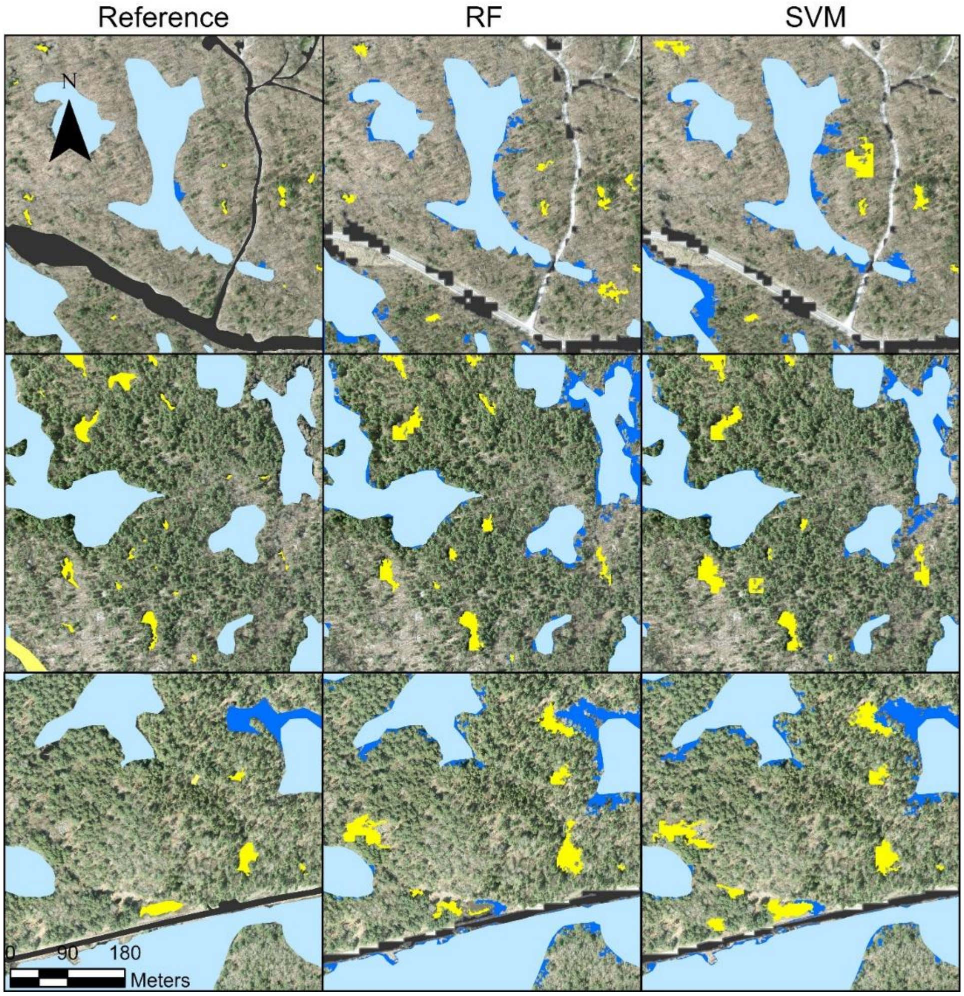

Figure 2.4: Comparison of classification results from each model (RF = Random Forest, SVM = Support Vector Machines) with the reference dataset for three sites: GB2 plot 2 (top), GB3 plot 1 (middle), and GB6 plot 4 (bottom). The light blue polygons represent land previously identified as waterbodies by the Ontario Ministry of Natural Resources and Forestry (OMNRF). The black polygons represent areas classified as roads. The dark blue polygons represent areas classified as being "OMNRFwater" (i.e. missed portions of the OMNRF identified waterbodies). The yellow polygons represent areas classified as "PVP" (potential vernal pools). Basemap source: Ontario Ministry of Natural Resources and Forestry

CHAPTER 3: DETERMINANTS OF AMPHIBIAN COMMUNITY COMPOSITION IN VERNAL POOLS OF SOUTH-CENTRAL ONTARIO: CONSIDERATIONS FOR CONSERVATION UNDER CHANGING CLIMATE AND LAND-USE

In submission: Luymes N and Chow-Fraser P. (2021) Determinants of amphibian community composition in vernal pools of south-central Ontario: Considerations for conservation under changing climate and land-use. Submitted to *Canadian Journal of Fisheries and Aquatic Sciences*

Abstract

Forested vernal pools serve an integral role in the recruitment of amphibians in glaciated northeastern North America. In south-central Ontario, vernal pools exist in relatively unimpacted forest networks, but the amphibian communities face uncertain challenges from climate change and planned provincial land-use development. We surveyed amphibian larvae and collected measurements of habitat characteristics from vernal pools to investigate drivers of changes in amphibian communities and species abundance. Amphibian communities were influenced by hydroperiod length and canopy openness, and the relative abundances of early breeding amphibians were affected by changes in the structure of vegetation communities within pools. Our study suggests that, even across moderate ranges of breeding habitat characteristics, the structure of amphibian communities is dynamic. With climate change leading to more drought-prone summers, the conservation of wetland diversity will become increasingly essential. Management of ephemeral wetlands targeted for future land-use development should be informed by mistakes made in more developed regions of Ontario, where similarly valuable habitats have disappeared due to lack of legislative protection.

Introduction

Global amphibian declines represent one of the hallmarks of the current biodiversity crisis. Amphibians are impacted by numerous stressors, including habitat loss and alteration, invasive species, disease, environmental pollution; however, one of the least well understood threats is global climate change because its effects have yet to be fully realized (Li, Cohen, and Rohr, 2013). Shifts in seasonal timings may cause uneven changes in the phenological cycles of interacting species (Blaustein et al., 2001; Ficetola and Maiorano, 2016; Green, 2017), and environmental stress may increase the susceptibility of amphibians to disease (Blaustein et al., 2010; Rollins-Smith, 2017). In addition, amphibians often have very specific breeding requirements that are tied to aquatic resources, so changes to climate that affect the availability of these resources could have detrimental effects on recruitment (Corn, 2005; Brooks, 2009). Our ability to predict and react to potential effects of climate change on amphibians will rely on efforts to understand the diversity of amphibian habitats and how communities change across environmental gradients.

In glaciated northeastern North America, forested wetlands comprise a large proportion of amphibian breeding habitat. These wetlands vary in a number of qualities that dictate patterns in amphibian diversity across the landscape, including hydroperiod length (i.e. the amount of time a wetland is inundated; Babbitt, Baber, and Tarr, 2003; Baber et al., 2004), supported food chains (detritus vs. grazing; Werner and Glennemeier, 1999; Werner et al., 2007), and surrounding land cover (Hecnar and M’Closkey, 1998; Findlay, Lenton, and Zheng, 2001; Houlahan and Findlay, 2003). Despite these differences leading to unique amphibian assemblages, conservation efforts are often unequal across wetland types. Certain protections are provided to large and conspicuous permanent wetlands, but seasonal wetlands are frequently overlooked in legislature due to their relative obscurity in forested landscapes (Mahaney and Klemens, 2008). In northeastern North America, small seasonal wetlands (vernal pools) are primary breeding habitat for a number of amphibians that are intolerant of predatory fish populations, including salamanders in the genus *Ambystoma* and wood frogs (*Lithobates sylvaticus*). In order to better conserve amphibian diversity on regional scales, underrepresented habitats like vernal pools need greater research and management attention.

Our study focusses on vernal pool habitat in an intact forested region of south-central Ontario. This region is characterized by rugged terrain from exposed Precambrian Shield bedrock, which has led to numerous landscape depressions for wetlands to form, including vernal pools. The highly forested landscape of south-central Ontario supports robust recreational and cottage development that is rapidly expanding as the aging population in southern Ontario swap their urban homes for year-round cottages. Unfortunately, little is known about the aquatic resources in this region compared to more urbanized and agricultural regions in the southern part of the province. This is especially true for small wetlands like vernal pools, which are largely absent in the aerial imagery-derived wetland databases for this region. While breeding habitat associations for amphibians are well studied for northeastern North America, there are considerable differences in climate, landscape characteristics, wetland types, and amphibian ranges that could affect the reliability of these relationships across the province. There is an urgent need to identify key habitat characteristics that structure amphibian communities in vernal pools of south-central Ontario, and to better understand how these ecological interactions may be affected by projected changes associated with global climate change.

Methods

Study area

We conducted our study in forests along the eastern coast of Georgian Bay, Ontario. This area lies within the Georgian Bay Ecoregion (GBE) and marks the western extent of south-central Ontario. GBE is characterized by a mixture of deciduous, coniferous, and mixed forests and common species include white pine, red pine, eastern hemlock, yellow birch, sugar maple, beech, black cherry, and white ash (Crins et al., 2009). The rugged terrain from the underlying Precambrian Shield creates numerous landscape depressions where wetlands form, including lakes, marshes, beaver ponds, thicket swamps, peatland, and vernal pools. The climate is cool-temperate with average annual temperature and precipitation of 4.5 °C and 950 mm (Crins et al., 2009). The land cover is dominated by forests, with only a small proportion (<10%) classified as agriculture or urban (Crins et al., 2009). The shorelines of lakes and rivers are popular areas for cottage development and the eastern shore of Georgian Bay supports large cottage communities. Approximately half of the forests in GBE are on public land are a managed according to sustainable forestry practices (Davidson, 2015).

Amphibian surveys

We conducted larvae surveys in 2019 and 2020 at 35 pools across five public (crown land) forest plots along the eastern coast of Georgian Bay: three plots along southeastern Georgian Bay (SEG) and two plots along mid-eastern Georgian Bay (MEG; Figure 3.1). Forest plots differed in the percent cover of different land cover types (Table 3.1) but were generally characterized by low anthropogenic disturbances and a mixture of deciduous and coniferous species. Our criteria for wetland selection included easy access from roads or trails, a temporary hydroperiod, and signs of breeding amphibians. To account for early- and late-breeding amphibians, we surveyed amphibian larvae twice per year. In 2019, early surveys took place during the last week of May and first week of June, and late surveys took place during the first and second weeks of July. In 2020, early surveys took place during the second-to-last week of June, and late surveys took place during the second week of July. Some pools with short hydroperiods were dry during the later survey periods. Survey timing changed between years due to differences in the onset of warm weather and the perceived rate of pool drying.

To mitigate biases associated with any one survey method, we used two techniques to estimate species abundance at each pool: bucket traps and dip-netting

(Figure 3.2). Bucket traps were 19 L buckets (31 cm diameter) with bottoms removed and dip nets were 350-µm mesh nets on a D frame (30 x 30 x 60 cm). For each bucket trap sample, the bucket trap was driven into the pool substrate and any trapped larvae were extracted with a mesh strainer. For each dip net sample, the net was swept just above the substrate for a 1-m transect. The net was then inverted into a bucket of water and larvae were extracted with a mesh strainer. Extracted larvae were placed in gridded trays, where they were photographed and identified to species.

Each survey consisted of one bucket trap sample and one dip net sample. The total number of surveys per pool was proportional to pool surface area: the smallest pools (<50m^2) were surveyed 5 times and the number of surveys increased in increments of 5 surveys per 150m^2 up to a maximum of 25 surveys for the largest pools (>500m^2). Amphibian species are known to prefer different microhabitat types within wetlands (Heyer et al., 1994), so we stratified survey locations by the proportion of different microhabitat types (e.g., open leaf litter, emergent herbaceous vegetation, shrub thicket, pool edge, pool center) to get a representative sample for each pool. In the case of bucket trap samples, we were unable to survey locations deeper than 1 m, so survey locations were stratified by microhabitat types in accessible regions of each pool. Larvae were identified to species in the field following Altig, McDiarmid, and Bauer (2017) and Mills (2016) and released after photographs were taken.

Habitat surveys

We sampled multiple habitat variables for each pool that were thought to be important predictors of species presence and abundance, including hydroperiod length, pool surface area, maximum depth, percent cover of microhabitat types, percent canopy openness, and road density. To approximate hydroperiod length, we placed HOBO Pendant MX Water Temperature Data Loggers (Onset, Bourne MA, U.S.A.) in the deepest location of each vernal pool and set the recording interval to every hour. We retrieved the loggers in late August when most pools had dried; we then visually assessed the temperature traces to estimate the dates when each pool dried (Baldwin, Calhoun, and deMaynadier, 2006). Using depth data from Leveloggers (Solinst, Georgetown ON, Canada) placed alongside temperature loggers in three pools, we found estimates from the temperature data were accurate to within one day of the Levelogger estimates. Due to logistical constraints, we were only able to estimate pool drying dates in 2020. We were also unable to measure timing of ice-off for each pool but have found most pools to be ice-free by mid-April in previous years. While the timing of ice-off likely differs among pools, we assumed that differences in the timing of pool drying were greater, and thus had a greater effect on hydroperiod length. Consequently, we estimated the average day of ice-off to be April 15 and characterized hydroperiod length to be the number of days between average ice-off and the date that each pool had dried. Future studies may benefit from actual observation of pool ice-off to better approximate differences in hydroperiod length. For analytical purposes, we classified hydroperiod length into three categories: short (<3 months; 19 pools), moderate (3 – 4 months; 7 pools), and long (>4 months – they had not dried before we took out the temperature loggers; 9 pools).

We measured pool length along the longest wetted transect and pool width along the longest wetted transect perpendicular to the length transect. We then used these measurements in an equation for an ellipse to calculate pool surface area. For irregularly shaped pools, we calculated the surface area of individual basins and added these together to calculate the total surface area. Maximum pool area and depth were measured in the early spring when pools were maximally inundated. We also estimated pool surface area during each survey period to correct for differences in larvae density as the pools dried and became smaller.

Canopy openness is known to affect resource availability for anuran larvae. Open canopy wetlands primarily support grazing food chains starting with nutrient-rich algae, while closed canopy wetlands primarily support detritus food chains starting with nutrient-poor leaf litter (Werner and Glennemeier, 1999; Skelly, Friedenburg, and Kiesecker, 2002). We measured canopy openness during leaf-on conditions using a spherical densiometer (Model C; Forestry Suppliers, Jackson MS, U.S.A.) at four cardinal directions for five points within each pool: one point at the intersection of the length and width transects and four points near the pool edge at each end of the length and width transects. We then averaged these measures to come up with an estimate of overall canopy openness.

We visually assessed the percent cover of microhabitat types in early summer when most plant species had become established. We classified microhabitat types into four categories: open leaf litter or mud, herbaceous vegetation (emergent, floating, and submerged), shrub thicket, and moss. Several of the species in this region use persistent woody and non-woody vegetation as egg attachment sites (Egan and Paton, 2004) and the four-toed salamander is known to deposit eggs within mossy hummocks along the pool margins (Chalmers and Loftin, 2006). Herbaceous vegetation such as grass may also represent a high-quality food source for anuran tadpoles (Williams, Rittenhouse, and Semlitsch, 2008) and the presence of complex structure from vegetation communities may provide cover for developing larvae (Hecnar and M’Closkey, 1997).

Road density was estimated with ArcGIS software. A 300-m diameter buffer size was chosen to encompass the approximate home ranges of common amphibian species in this study (Vasconcelos and Calhoun, 2004; Semlitsch and Skelly, 2008) and to limit spatial correlation of road density measurements between pools in the same forest plot.

Statistical analysis

Drivers of community structure.

We quantified catch per unit effort (CPUE) of larval amphibians by dividing the total number of larvae captured during a survey period by the number of surveys during that period. We combined abundances from bucket trap and dip net surveys in our calculations of CPUE. We also wanted to account for changes in larvae density due to changes in pool surface area across survey periods, so we multiplied CPUE by the proportional pool area during the survey period (i.e. relative to the maximum area of the pool). We used these area-standardized CPUE values to calculate an average CPUE across all survey periods.

To visualise differences in the structure of amphibian assemblages, we used non-metric multidimensional scaling (NMDS) plots. We used the Bray-Curtis dissimilarity matrix of the average CPUE values for each amphibian species encountered to create NMDS plots. We used “stress” of the NMDS solution to decide on the number of ordination axes and interpreted the stress values according to common rules of thumb (Clarke, 1993). We used permutational multi-variate analysis of variance (PERMANOVA) to assess amphibian community composition across three habitat variables: hydroperiod length and canopy openness. These habitat variables have been found to have strong effects on species richness and community composition in previous studies (Skelly, Werner, and Cortwright, 1999; Werner et al., 2007). PERMANOVAs were run with the Bray-Curtis dissimilarity matrix and 999 permutations. We created separate univariate models for each habitat variable and a marginal-effects model for all variables combined. Significance was assessed at a 0.05 confidence level based on p-values derived from the pseudo-F scores. For categorical variables that were found to be significant, we performed pairwise PERMANOVA comparisons and used the similarity percentage routine (SIMPER) to assess the contribution of each species to the average dissimilarity between different categories. PERMANOVA and SIMPER analyses can be disproportionately influenced by species or habitat categories with high variances in abundance (Warton, Wright, and Wang, 2012). To overcome these biases, we used results from the species-specific models to validate results from the community analysis. All community analyses were performed in R with the vegan package (Oksanen et al., 2020).

Species-specific relationships with habitat variables.

We used generalized linear mixed models (GLMM) to evaluate the relationship between habitat variables and the larval abundance of any species that were encountered in >30% of the study pools. Species encountered less frequently (<30% of pools) were also characterized by low abundances. Low detection rates may have biased abundance estimates for these species, so we chose not to fit models for these species. We fit our models with a Poisson distribution and a log link to accommodate the count data. We standardized the count data in our models using a log-transformed offset term with the number of surveys as the numerator and proportional pool area as the denominator. Our habitat variable set included hydroperiod length, maximum pool surface area, maximum depth, percent canopy openness, percent herbaceous vegetation cover, presence of thicket cover, and road density within a 300-m diameter buffer around each pool. We also included year and day of year in our models to account for annual and seasonal fluctuations in larval abundances. We checked for collinearity in our variable set using Spearman's rank correlation coefficient (rho) and variance inflation factors (VIF). We used conservative cut-offs of *rho* > 0.7 or VIF > 8 to consider removing variables from our analysis. All variables were scaled and centered prior to model development to allow for comparisons of effect sizes. We created 23 models for each species, including a null model, a global model, models for each habitat variable, and models with different *a priori* combinations of habitat variables.

Since pools in the same forest plot were often in close proximity to one another (< 1km apart), the abundance of larvae at one pool may be influenced by abundances in nearby pools. To account for potential spatial autocorrelation, we specified forest plot as a random effect in all of our models. We also specified pool ID and year nested within pool ID as random effects to account for repeated samples of each pool and unequal sampling of pools across years, respectively. Lastly, we found evidence of overdispersion in initial model formulations, so we specified an observation-level random effect for all models (Harrison, 2014). We fit GLMM using the blme package in R (Dorie et al., 2020) and assigned weak priors to the random effects.

We used an information theoretic approach with Akaike's Information Criterion corrected for small samples sizes (AICc) to compare and select models with the best support from our data. We considered models with the lowest AICc to have the best support. The difference between the AICc value of a model and the best-supported model is ΔAICc. Models with ΔAICc <2 were considered to have strong support and were retained to derive final model sets for each species. For each habitat variable in the final model sets, we calculated a variable importance measure by summing the relative weights of all the models where that variable occurred. Habitat variables with importance measures equal to 1.0 are found in all models in the final model set, while variables with importance measures close to 0 are found in only one or a few models. AICc values and model weights were calculated using R package MuMIn (Bartoń, 2020). Variable estimates and confidence intervals were compared across models for consistency and were reported for the model with the lowest AICc for that variable, unless otherwise specified. Confidence intervals were estimated with the Wald method. To investigate the effect of influential habitat variables, we used the top models to estimate the change in CPUE across the range of the habitat variable while setting other modelled variables to their average values.

To evaluate whether the relative rank of amphibian abundances across pools was preserved between years, we tested the correlation of average CPUE between 2019 and 2020 for species encountered in >50% of pools. We would expect the CPUE of species with relatively consistent annual breeding effort, embryo survival, and/or larvae survival to be highly correlated between years, while those with large population fluctuations and/or strong dependence on annual weather conditions to be less correlated between years. All analyses were performed in R (R Core Team, 2020).

Ethics approval

Animal handling for this study followed guidelines recommended by the Canadian Council on Animal Care and was approved by McMaster University's Animal Research and Ethics Board (Animal Use Protocol: 17-01-06).

Results

Wetland characteristics

Pools varied in size and appearance (Table 3.2), with substrate ranging from hard packed leaf litter to loose muddy bottoms, and vegetation from dense thicket cover to emergent grasses and sedges. All variables had VIF < 8 and Spearman's rank correlation coefficients for most of the within-pool variables considered in this study fell between -0.2 and 0.2 with p-values greater than 0.05. Pool vegetation cover and canopy openness were the only variables strongly correlated with one another (rho=0.72. P<0.00001), while hydroperiod length was moderately correlated with canopy openness (rho=0.59, *P*<0.001) and maximum depth (*rho*=0.49, *P*<0.01). Since the mechanism by which key factors affect the amphibian community may vary, all correlated variables were retained for further statistical analyses.

Larvae assemblages

During 2019 and 2020, we captured 2830 amphibian larvae from eight amphibian species (five anurans and three caudates). Of the 35 pools included in our study, 21 (60%) were surveyed four times, twice each year; eight (23%) were surveyed three times because the sites had dried before the second survey period in 2020; one (3%) was surveyed only twice in 2019 because it was dry during both 2020 survey periods; and five (14%) were surveyed only once because they only had standing water during the early part of the 2019 survey period. From these 35 pools, we excluded the 5 pools with extremely short hydroperiods from further analysis because they dried before any larvae could be captured (i.e. we found unhatched eggs of spotted salamander (*Ambystoma maculatum*)). The early surveys were generally characterized by early breeding species, including the spotted salamander, blue-spotted salamander (*Ambystoma laterale*), and wood frog (*Lithobates sylvaticus*), while later surveys were characterized by fewer encounters of wood frogs and more encounters of species with prolonged breeding periods, including the spring peeper (*Pseudocris crucifer*).

In pools with larvae, richness varied from one to six, with a mean of 3.5. The most commonly encountered species, the spotted salamander, was captured in all but two pools (93% of the pools). Other common species included the blue-spotted salamander, wood frog, and spring peeper, which were found in more than 60% of the study pools. Together, spotted salamanders, blue-spotted salamanders, wood frogs, and spring peepers accounted for 95% of all larvae captured. Gray treefrogs (*Hyla versicolor*) and four-toed salamanders (*Hemidactylium scutatum*) were less common but were still found in more than 20% of the study pools. American toads (*Anaxyrus americanus*) and green frogs (*Lithobates clamitans*) were the least common species in our study (one and two pools, respectively). Both of these rarely encountered species were excluded from the community analysis so as not to disproportionately influence overall trends.

Drivers of community structure

The stress of the two-dimensional NMDS model was 0.11, indicating a good representation of the dissimilarities between pools in reduced dimensions. Species with close phylogenetic relationships tended to be associated in ordination space. For instance, centroids for the two mole salamanders (spotted and blue-spotted salamanders) and for the two treefrogs (gray treefrogs and spring peepers) were found closer to each other than with centroids of other taxa (Figure 3.3). The centroid for the four-toed salamander (upper right corner of the Figure 3.3) was isolated from the other species in ordination space and this species tended to be captured in pools where other species were either absent or captured at low abundances. They were also the only species found in two sphagnum-dominated pools, which had environmental characteristics that were very different from those of the other pools. These two pools had a disproportionate influence on the appearance of initial NMDS ordinations and were excluded from further statistical analyses.

Results of the between-sites PERMANOVAs indicated that larvae community structure changed across hydroperiod classes (*pseudo-F*=2.27, *P*=0.01; Figure 3.3) and across the gradient of canopy openness (*pseudo-F*=2.25, *P*=0.04); however, neither variable had significant independent effects on community structure in the marginal-effects model (hydroperiod: *pseudo-F*=1.59, *P*=0.09; canopy openness: *pseudo-F*=1.00, *P*=0.4). Pairwise comparisons of hydroperiod classes revealed community structures in pools with short hydroperiods were dissimilar from those in pools with long hydroperiods (*pseudo-F*=3.79, *P*=0.003) and pools with moderate hydroperiods (*pseudo-F*=2.06, *P*=0.05). Community structures in pools with moderate hydroperiods were not significantly different from those in pools with long hydroperiods (*pseudo-F*=0.51, *P*=0.8). Spring peepers contributed the most to average dissimilarity (SIMPER) between pools with short hydroperiods and pools with moderate and long hydroperiods (32% and 50% respectively), followed by wood frogs (25% and 22%) and spotted salamanders (20% and 14%). The remaining species contributed <20% to average dissimilarity between hydroperiod classes. The proportion of spring peepers in total larvae captured increased with hydroperiod (Figure 3.4A); spring peepers had a low average CPUE in pools with short hydroperiods, while spring peepers in pools with moderate and long hydroperiods had the highest average CPUE (Figure 3.4B). Average percent canopy openness also increased across hydroperiod classes and was twice as large in pools with long hydroperiods compared to those with short hydroperiods. The other three common species (spotted salamanders, blue-spotted salamanders, and wood frogs) did not appear to differ as strongly between hydroperiod classes, though wood frogs and spotted salamanders appeared to have a somewhat higher average CPUE in pools with moderate hydroperiods.

Species-specific relationships with habitat variables

Generalized linear mixed models were used to test the effect of habitat variables on the relative abundances of spotted salamanders, blue-spotted salamanders, wood frogs, and spring peepers. Gray treefrogs and four-toed salamanders were excluded because they were found in less than 30% of the study pools. Models for each species were found to fit the data well according to deviance tests and diagnostic plots. Variables in models with strong support (ΔAICc<2) were considered potential drivers of species abundances (Table 3.3). Models without any habitat variables were strongly supported for two species (blue-spotted salamanders and wood frogs), but the best supported model for all species included some combination of habitat variables. The combinations of habitat variables in models with strong support did not overlap appreciably between species (Figure 3.5). Since parameter estimates for the most influential variables were mostly conserved across models, we did not perform model averaging. The exceptions to this were canopy openness and hydroperiod, where parameter estimates were slightly weaker in models including both variables. Parameter estimates for canopy openness and herbaceous vegetation cover were also substantially different in models including both variables, but these models were relatively weak for all four species and not included in the final model set.

Herbaceous vegetation cover, thicket cover, and canopy openness were the only habitat variables that were important for more than one species. Higher vegetation cover and canopy openness were strongly and positively correlated with the CPUE of spring peepers and weakly and negatively correlated with CPUE of blue-spotted salamanders. Herbaceous vegetation was also weakly correlated with CPUE of wood frogs and spotted salamanders; however, these relationships appear to be tied to the strong effects of other variables included in the same models. Accounting for the effect of hydroperiod length, CPUE of spring peepers in pools with the most open canopies was estimated to be 50 times greater than in pools that were almost completely shaded by canopy. Similarly, spring peeper CPUE was estimated to be 20 times greater in pools with near-complete cover of herbaceous vegetation compared to pools with no herbaceous vegetation. The presence of thicket cover in pools was strongly associated with CPUE of spotted salamanders and wood frogs, and weakly associated with CPUE of blue-spotted salamanders. The CPUE of wood frogs and spotted salamanders was 6 and 4 times greater, respectively, in pools with thicket cover compared to pools with no thicket cover.

Models including hydroperiod were only strongly supported for spring peepers and had a positive effect on spring peeper CPUE. Accounting for the effect of canopy openness, the CPUE of spring peepers was estimated to be five times higher in pools with moderate hydroperiods compared to pools with short hydroperiods and twice as large in pools with long hydroperiods compared to pools with moderate hydroperiods.

Wood frogs were the only species with strongly supported models that included pool size and road density. Increases in pool size were associated with increases in the CPUE of wood frogs, though the effect did not appear to be very strong. Road density within a 300-m diameter buffer appeared to have a negative effect on CPUE of wood frogs in our study; CPUE of wood frogs was 16 times lower in pools that had high road density (2.7 km/km^2) compared with pools without any roads in the 300-m buffer.

All species had lower CPUE in 2020 compared to 2019, though this effect was weak for spotted salamanders (Figure 3.5). We used spearman’s rank correlation coefficients to investigate whether the rank order of CPUE in pools was conserved across years. CPUE exhibited a strong and positive correlation across years for spring peepers (*rho*=0.82 *P*<0.0001) but was only weakly correlated for wood frogs (*rho*=0.35 *P*=0.2), spotted salamanders (*rho*=0.30 *P*=0.2), and blue-spotted salamanders (*rho*=0.26 *P*=0.3).

Discussion

The influence of hydroperiod on larval amphibian assemblages in our study is consistent with the large body of literature documenting the strong influence of hydroperiod on numerous aspects of pond-breeding amphibian communities. In previous studies, hydroperiod significantly affected species richness and diversity (Snodgrass et al., 2000; Werner et al., 2007), community composition (Babbitt, Baber, and Tarr, 2003), recruitment (Pechmann et al., 1989; Karraker and Gibbs, 2009), breeding effort (Egan and Paton, 2004; Baldwin, Calhoun, and deMaynadier, 2006; Veysey, Mattfeldt, and Babbitt, 2011), species occupancy (Guderyahn, Smithers, and Mims, 2016), and amphibian density (Babbitt, Baber, and Tarr, 2003; Semlitsch et al., 2015).

While pond-breeding amphibians benefit from increases in hydroperiod length (Babbitt, Baber, and Tarr, 2003; Veysey, Mattfeldt, and Babbitt, 2011), they are also more vulnerable to predation by fish and predaceous invertebrates that inhabit pools with longer hydroperiods (Egan and Paton, 2004; Semlitsch et al., 2015). The trade-off between tolerance to rapid pool drying and vulnerability to predation risk appears to be the major driver explaining species' preference for wetlands with different hydroperiod lengths (Semlitsch et al., 2015). Certain species (e.g. wood frogs and *Ambystoma* salamanders) have evolved high tolerance to rapid pool drying by breeding in early spring and maintaining fast rates of larval development and growth (Rowe and Dunson, 1995; Wellborn, Skelly, and Werner, 1996). Other species (e.g. spring peepers and gray treefrogs) are moderately tolerant of pool drying and predator presence and make use of a variety of wetlands over a prolonged breeding period (Skelly, 1996). Lastly, some species (e.g. green frogs) are intolerant of wetland drying, but have evolved traits and behaviours, such as unpalatability or reduced movement, that help them avoid predation in permanent wetlands (Petranka, and Sih, 1988; Rubbo and Kiesecker, 2005).

Only the abundance of spring peepers showed a strong positive relationship with hydroperiod length in our wetlands; however, we only found the rarely encountered gray treefrog (*Hyla versicolor*) in pools with moderate or long hydroperiods, and this suggests that we may have found a similar positive relationship between hydroperiod length and abundance for this species if we had sampled more sites and for a longer duration. The apparent lack of an effect of hydroperiod on the other amphibian species may be explained by our limited range in hydroperiod length. We focussed our study on forested ephemeral pools and did not sample any wetlands that were permanently inundated such as beaver ponds, thicket swamps and marshes. At the other end of the spectrum, we lost seven of our pools with the shortest hydroperiods because of unexpectedly hot weather conditions that dried up the pools before larvae hatched from their eggs. By expanding our sampling to include other wetland types and increasing sampling effort on pools that dry up quickly, future investigations may be able to obtain a more pronounced effect of hydroperiod on abundances of the vernal pool obligate amphibian species.

Canopy-openness was positively correlated with herbaceous vegetation cover and hydroperiod length in our study and likely contributed to differences in amphibian community composition across hydroperiod classes. Spring peepers, in particular, had the highest larval densities in open canopy pools with high herbaceous vegetation cover. By comparison, we found no evidence to suggest a strong effect of canopy openness or herbaceous cover on the density of wood frogs, spotted salamanders, or blue-spotted salamanders. The discrepancy between species responses to canopy openness is thought to be primarily linked to differences in resource requirements (Earl et al., 2011). Caudate larvae are carnivores and are considered canopy generalists because prey species are available across a gradient of canopy cover (Earl et al., 2011). Anurans in this region, however, are primarily herbivores/detritivores that feed on a range of food quality (Schiesari, 2006). For instance, filamentous algae and decaying non-woody plants in open canopy pools are of higher nutritional quality than the decaying leaf litter that covers closed canopy pools (Skelly, Freidenburg, and Kiesecker, 2002; Williams, Rittenhouse, and Semlitsch, 2008). Lower food quality can depress growth and development rates of larvae, which can reduce fitness and increase the risk of mortality from pond drying (Werner and Glennemeier, 1999; Skelly, Freidenburg, and Kiesecker, 2002). Compared to other anurans, wood frogs are more efficient at metabolizing lower quality detritus and regularly breed in heavily shaded wetlands (Schiesari, 2006).

The presence of vegetation may also influence breeding effort by increasing the number of available attachment sites for amphibian eggs. The anuran species and *Ambystoma* salamanders are known to attach their eggs to a variety of sturdy structures within the pool basin, including floating logs and branches, submerged thicket, and the remnants of herbaceous vegetation from the previous year (Egan and Paton, 2004). Anecdotally, we have found the large egg masses of wood frogs and *Ambystoma* salamanders to be more frequently attached to submerged branches of shrubs compared to herbaceous vegetation, which may explain the positive relationship we observed between the presence of thicket cover and the densities of wood frogs and *Ambystoma* salamanders. Unlike the other species encountered in our surveys, four-toed salamanders are known to lay their eggs in mossy clumps at the edges of pools (Chalmers and Loftin, 2006). Though they were not included in our species-specific analysis, four-toed salamanders appeared to be associated with sphagnum moss cover, and were the only species encountered in the two pools that were completely covered by sphagnum moss.

Changes in canopy openness and vegetation communities can occur as a result of human intervention, as is the case with forest management practices and land-use changes, or from disturbance events such as forest fires. These changes cause shifts in amphibian communities and, in the case of natural fire disturbances, can be important for maintaining breeding habitat for some species (Skelly, Werner, and Cortwright, 1999; Gorman, Haas, and Himes, 2013). Studies that have examined the effect of fires on pond-breeding amphibians have found some species to be resilient (Hossack and Corn, 2007); however, increases in the frequency and severity of fires from climate change may lead to more severe consequences, especially for species that depend on upland forest habitat for most of the year (Blaustein et al., 2010).

Decreases in forest health from prolonged periods of drought and more frequent disease outbreaks are also expected to affect forest health (Sturrock et al., 2011), which may further reduce the prevalence of closed canopy habitats. More research is needed to determine the effect of widespread canopy changes on the performance of amphibians in this region. Although changes in canopy openness in our study were modest, we saw obvious shifts in vegetation communities and dominant amphibian species. More pronounced canopy gradients have been associated with larger shifts in amphibian communities and can lead to drastically different ecosystems (Werner et al., 2007). Regardless of the resiliency of pond-breeding amphibians to widespread canopy changes, closed canopy wetlands represent unique ecosystems and efforts should be made to ensure sufficient land is protected to allow patches of these wetlands to persist in the event of forest loss.

Forests in our study region have had relatively minor changes in surrounding land-use. Roads accounted for the highest percentage of urban land-use and the only buildings within a kilometer radius of each forest plot were cottages or marinas. Despite low levels of anthropogenic stress, we found evidence to suggest that road density had negative effects on the density of wood frogs. These results are consistent with those in more impacted regions (Findlay, Lenton, and Zheng, 2001; Veysey, Mattfeldt, and Babbitt, 2011) and suggest wood frogs may be particularly sensitive to land-use changes that disrupt seasonal migration and dispersal. Impacts of land-use changes have also been found to extend to other species of pond-breeding amphibians in more anthropogenically impacted regions (Houlahan and Findlay, 2003; Veysey, Mattfeldt, and Babbitt, 2011). Many pond-breeding amphibians are hypothesized to be governed by metapopulation dynamics on large spatial scales and may rely on landscape connectivity between wetlands for gene flow and population rescue (Marsh and Trenham, 2001; Semlitsch, 2008). South-central Ontario has low-intensity land-use and high wetland density, and despite the small impact on wood frog abundance, impacts to amphibian dispersal are likely minimal (Bauer, Paton, and Swallow, 2010). Future development plans for this region may necessitate the inclusion of the protection of landscape connectivity to surrounding wetlands in management plans.

Our results agree with the large body of evidence for the important role that wetland hydroperiods have in structuring amphibian communities. This relationship is especially important to consider in the face of global climate change. In northeastern North America, climate change is projected to lead to increased temperatures along with more frequent and longer periods of drought (Bush and Lemmen, 2019). While there is evidence that shifts in the timing of spring melt may promote earlier migration to breeding pools (Blaustein et al., 2001; Todd et al., 2011; Green, 2016), the average length of hydroperiods is predicted to decrease and low recruitment years for amphibians are expected to occur more frequently (Brooks, 2009; Blaustein et al., 2010).

How amphibian populations will respond to these changes remains unclear. The pools with the shortest hydroperiods will likely become unreliable for most amphibians, while wetlands that previously held fish populations may dry more frequently and become suitable for a greater range of amphibians. On the landscape level, reductions in wetland density could limit dispersal across the landscape, leading to reduced genetic diversity and slower rates of recolonization following local extinctions (Willson and Hopkins, 2013; Coster et al., 2015). Within breeding pools, differences in species-specific phenological responses to earlier warming could alter predator-prey dynamics and lead to competitive advantages for species that have high adaptive capacity (Todd et al., 2009; Blaustein et al., 2010; Walls, Barichivich, and Brown, 2013). While the specific adaptive response of vernal pool communities to climate change is unknown, the adaptive capacity of individual species may depend on their ability to colonize wetlands that become suitable due to climate-induced changes in hydroperiod or canopy openness.

Conservation should prioritize the preservation of diverse assemblages of wetlands at the landscape scale.

This study represents one of the first to explore the diversity of pond-breeding amphibian communities in south-central Ontario. While we did find associations between amphibians and habitat characteristics, it is clear that vernal pools and their communities are dynamic and short-term studies will undoubtedly miss information. Hydroperiod length in this study is only useful as a relative measure, as annual weather conditions will ultimately affect the duration that pools are available in any given year (Brooks, 2009). Longer-term studies could examine the effect of year-to-year fluctuations in hydroperiods on changes in the composition of species assemblages. Since our surveys took place during drier than average conditions, we acknowledge the possibility that we did not obtain a complete list of species that use these vernal pools on a long-term basis. In addition, the relative performance of species in one year can have trickle-down effects in subsequent years; population booms and busts for short-lived species like wood frogs or spring peepers have the potential to affect breeding effort in subsequent years (Berven, 1990; Werner et al., 2009).

Amphibian communities can be monitored with different surveying techniques, in addition to or instead of the larval survey techniques used here. For example, egg surveys or call surveys can be used to estimate breeding population size (Grant et al., 2005;

Shirose et al., 1997), but these are usually best for targeting specific amphibian groups. If the project includes a few wetlands or many field personnel, pitfall-trap surveys could be used to obtain accurate estimates of the population size of breeding individuals or recent metamorphs from many different species (Gibbons et al., 2006; Karraker and Gibbs, 2009). The advantage of larval surveys is that they are relatively easy to implement in remote locations and have the potential to target a broad range of amphibian species for a larger number of sites; however, they reveal only a snapshot of amphibian development that reflects a combination of breeding effort, embryo survival, and larval survival. Therefore, larval surveys may be particularly relevant for long-term monitoring (i.e. over at least 10 years), where multi-year averages of species occupancy and abundance can provide better estimates of community structure, and changes across years can give insight into population and community-level dynamics. Less invasive monitoring techniques, such as those requiring the collection of eDNA, are becoming more accessible, but are generally species-specific and are not suited for abundance data.

Vernal pools in south-central Ontario vary with respect to their suitability as breeding habitat for amphibian species. They are critically under-protected by legislation despite accounting for over half of the wetlands in the study region (Luymes and Chow-Fraser, 2021). They are also likely to be disproportionally impacted by climate change since their ecology revolves around weather-dependent hydrological cycles (Brooks, 2009). Vernal pools and forested wetlands in general are responsible for a considerable amount of energy and biomass that is then transferred to terrestrial habitats (Leibowitz, 2003). While amphibians in this area are not imperiled, their contributions to this transfer of resources should warrant consideration for increased protection. The future health of forest ecosystems in this region will likely depend on the maintenance of the ecological functions provided by forested wetlands (Nyman, 2011). Forest health, in turn, impacts the amphibians that spend a majority of their lives in terrestrial environments, as is the case for most of the amphibians encountered in this study (Skidds et al., 2007; Todd et al., 2009).

The importance of hydroperiod length on the assemblages and richness of amphibian species highlights the urgent need to understand how climate change will alter the hydrology and physical characteristics of breeding habitats in these forested seasonal wetlands of south-central Ontario. A shift in timing of rain events or onset of hot weather could severely reduce the reproductive success of species with constrained breeding periods, such as wood frogs or *Ambystoma* salamanders (Brooks, 2009; Blaustein et al., 2010). In addition, climate change may also affect forest health in this region, as well as increased frequency of forest fires and disease (Gillett et al., 2004; Bush and Lemmen, 2019). Since the density of vernal pool obligate amphibians (wood frogs, spotted salamanders, blue-spotted salamanders) appeared to be influenced by changes in the composition of vegetation communities within the pool basin, any change in forest health/canopy may also affect the assemblages of these species. Unfortunately, most climate investigations have focused on regions where the effect of climate change is difficult to separate from other amphibian stressors (Li, Cohen, and Rohr, 2013). Large remote forests like those in south-central Ontario are generally not at immediate risk of degradation but are thought to provide indispensable environmental values (Watson et al., 2018). Small-scale ecosystems in these forests, such as forested wetlands, have not been explored as extensively as those in more urban areas, but may provide opportunities to study the effects of climate change in the absence of other major stressors. There is clearly a need for more research focused on forested ecosystems in these regions that support high diversity of wetland types and wetland-dependent biota.

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References

Altig, R., McDiarmid, R.W., and Bauer, A.M. 2017. Handbook of Larval Amphibians of the United States and Canada, Handbook of Larval Amphibians of the United States and Canada. Cornell University Press. https://doi.org/10.7591/9780801456084

Babbitt, K.J., Baber, M.J., and Tarr, T.L. 2003. Patterns of larval amphibian distribution along a wetland hydroperiod gradient. Can. J. Zool. 81(9): 1539–1552. https://doi.org/10.1139/z03-131

Baber, M.J., Fleishman, E., Babbitt, K.J., and Tarr, T.L. 2004. The relationship between wetland hydroperiod and nestedness patterns in assemblages of larval amphibians and predatory macroinvertebrates. Oikos 107(1): 16–27. https://doi.org/10.1111/j.0030-1299.2004.12968.x

Baldwin, R.F., Calhoun, A.J.K., and DeMaynadier, P.G. 2006. The significance of hydroperiod and stand maturity for pool-breeding amphibians in forested landscapes. Can. J. Zool. 84(11): 1604–1615. https://doi.org/10.1139/Z06-146

Bartoń, K. 2020. MuMIn: Multi-Model Inference. R package version 1.43.17 [online]. Available from https://cran.r-project.org/web/packages/MuMIn/index.html.

Bauer, D.M., Paton, P.W.C., and Swallow, S.K. 2010. Are wetland regulations cost effective for species protection? a case study of amphibian metapopulations. Ecol. Appl. 20(3): 798–815. https://doi.org/10.1890/08-2209.1

Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71(4), 1599–1608. https://doi.org/10.2307/1938295

Blaustein, A.R., Belden, L.K., Olson, D.H., Green, D.M., Root, T.L., and Kiesecker, J.M. 2001. Amphibian breeding and climate change. Conserv. Biol. 15(6): 1804–1809. https://doi.org/10.1046/j.1523-1739.2001.00307.x

Blaustein, A.R., Walls, S.C., Bancroft, B.A., Lawler, J.J., Searle, C.L., and Gervasi, S.S. 2010. Direct and indirect effects of climate change on amphibian populations. Diversity 2(2): 281–313. https://doi.org/10.3390/d2020281

Brooks, R.T. 2009. Potential impacts of global climate change on the hydrology and ecology of ephemeral freshwater systems of the forests of the northeastern United States. Clim. Change 95: 469–483. https://doi.org/10.1007/s10584-008-9531-9

Bush, E. and Lemmen, D.S. 2019. Canada’s changing climate report. Government of Canada.

Chalmers, R.J. and Loftin, C.S. 2006. Wetland and microhabitat use by nesting four-toed salamanders in Maine. J. Herpetol. 40(4): 478–485. https://doi.org/10.1670/0022-1511(2006)40[478:WAMUBN]2.0.CO;2

Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18(1): 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Corn, P.S. 2005. Climate change and amphibians. Anim. Biodivers. Conserv. 28(1): 59–67.

Coster, S.S., Babbitt, K.J., Cooper, A., and Kovach, A.I. 2015. Limited influence of local and landscape factors on finescale gene flow in two pond-breeding amphibians. Mol. Ecol. 24(4): 742–758. https://doi.org/10.1111/mec.13062

Crins, W.J., Gray, P.A., Uhlig, P.W.C., and Wester, M.C. 2009. The ecosystems of Ontario, Part 1: Ecozones and ecoregions. Ontario Ministry of Natural Resources, Sault Ste. Marie.

Davidson, B. 2015. French-Severn Forest, Ontario, Canada, in: Forest Plans of North America. Elsevier Inc. pp. 367–376. https://doi.org/10.1016/B978-0-12-799936-4.00041-2

Dorie, V., Bates, D., Maechler, M., Bolker, B., and Walker, S. 2020. blme: Bayesian Linear Mixed-Effects Models. R package version 1.0-5 [online]. Available from https://cran.r-project.org/web/packages/blme/index.html.

Earl, J.E., Luhring, T.M., Williams, B.K., and Semlitsch, R.D. 2011. Biomass export of salamanders and anurans from ponds is affected differentially by changes in canopy cover. Freshw. Biol. 56(12): 2473–2482. https://doi.org/10.1111/j.1365-2427.2011.02672.x

Egan, R.S. and Paton, P.W.C. 2004. Within-pond parameters affecting oviposition by wood frogs and spotted salamanders. Wetlands 24(1): 1–13. https://doi.org/10.1672/0277-5212(2004)024[0001:WPAOBW]2.0.CO;2

Ficetola, G.F. and Maiorano, L. 2016. Contrasting effects of temperature and precipitation change on amphibian phenology, abundance and performance. Oecologia 181(3): 683–693. https://doi.org/10.1007/s00442-016-3610-9

Findlay, C.S., Lenton, J., and Zheng, L. 2001. Land-use correlates of anuran community richness and composition in Southeastern Ontario wetlands. Ecoscience 8(3): 336–343. https://doi.org/10.1080/11956860.2001.11682661

Gibbons, J.W., Winne, C.T., Scott, D.E., Willson, J.D., Glaudas, X., Andrews, K.M., Todd, B.D., Fedewa, L.A., Wilkinson, L., Tsaliagos, R.N., Harper, S.J., Greene, J.L., Tuberville, T.D., Metts, B.S., Dorcas, M.E., Nestor, J.P., Young, C.A., Akre, T., Reed, R.N., Buhlmann, K.A., Norman, J., Croshaw, D.A., Hagen, C., and Rothermel, B.B. 2006. Remarkable amphibian biomass and abundance in an isolated wetland: Implications for wetland conservation. Conserv. Biol. 20(5): 1457–1465. https://doi.org/10.1111/j.1523-1739.2006.00443.x

Gillett, N.P., Weaver, A.J., Zwiers, F.W., and Flannigan, M.D. 2004. Detecting the effect of climate change on Canadian forest fires. Geophys. Res. Lett. 31(18): 4. https://doi.org/10.1029/2004GL020876

Gorman, T.A., Haas, C.A., and Himes, J.G. 2013. Evaluating methods to restore amphibian habitat in fire-suppressed pine flatwoods wetlands. Fire Ecol. 9(1): 96–109. https://doi.org/10.4996/fireecology.0901096

Grant, E.H.C., Jung, R.E., Nichols, J.D., and Hines, J.E. 2005. Double-observer approach to estimating egg mass abundance of pool-breeding amphibians. Wetl. Ecol. Manag. 13(3): 305–320. https://doi.org/10.1007/s11273-004-7524-7

Green, D.M. 2017. Amphibian breeding phenology trends under climate change: predicting the past to forecast the future. Glob. Chang. Biol. 23(2): 646–656. https://doi.org/10.1111/gcb.13390

Guderyahn, L.B., Smithers, A.P., and Mims, M.C. 2016. Assessing habitat requirements of pond-breeding amphibians in a highly urbanized landscape: implications for management. Urban Ecosyst. 19(4): 1801–1821. https://doi.org/10.1007/s11252-016-0569-6

Harrison, X.A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. PeerJ 19. https://doi.org/10.7717/peerj.616

Hecnar, S.J. and M'Closkey, R.T. 1997. The effects of predatory fish on amphibian species richness and distribution. Biol. Conserv. 79(2-3): 123–131. https://doi.org/10.1016/S0006-3207(96)00113-9

Hecnar, S.J. and M'Closkey, R.T. 1998. Species richness patterns of amphibians in southwestern Ontario ponds. J. Biogeogr. 25(4): 763–772. https://doi.org/10.1046/j.1365-2699.1998.2540763.x

Hossack, B.R. and Corn, P.S. 2007. Responses of pond-breeding amphibians to wildfire: Short-term patterns in occupancy and colonization. Ecol. Appl. 17(5): 1403–1410. https://doi.org/10.1890/06-2037.1

Houlahan, J.E. and Findlay, C.S. 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. Can. J. Fish. Aquat. Sci. 60(9): 1078–1094. https://doi.org/10.1139/f03-095

Karraker, N.E. and Gibbs, J.P. 2009. Amphibian production in forested landscapes in relation to wetland hydroperiod: A case study of vernal pools and beaver ponds. Biol. Conserv. 142(10): 2293–2302. https://doi.org/10.1016/j.biocon.2009.05.002

Leibowitz, S.G. 2003. Isolated wetlands and their functions: an ecological perspective. Wetlands 23(3): 517–531. https://doi.org/10.1672/0277-5212(2003)023[0517:IWATFA]2.0.CO;2

Li, Y., Cohen, J.M., and Rohr, J.R. 2013. Review and synthesis of the effects of climate change on amphibians. Integr. Zool. 8(2): 145–161. https://doi.org/10.1111/1749-4877.12001

Luymes, N. and Chow-Fraser, P. 2021. Detection of Potential Vernal Pools on the Canadian Shield (Ontario) Using Object-Based Image Analysis in Combination with Machine Learning. Can. J. Remote Sens. 1–16. https://doi.org/10.1080/07038992.2021.1900717

Mahaney, W. and Klemens, M. 2008. Vernal Pool Conservation Policy: The Federal, State, and Local Context, in: Science and Conservation of Vernal Pools in Northeastern North America. CRC Press, Boca Raton. pp. 193–212.

Marsh, D.M. and Trenham, P.C. 2001. Metapopulation dynamics and amphibian conservation. Conserv. Biol. 15(1): 40–49. https://doi.org/10.1046/j.1523-1739.2001.00129.x

Mills, P. 2016. Metamorphosis. Ontario, Canada.

Oksanen, J., Blanchet, F.G., Legendre, P., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H., Szoecs, E., and Wagner, H. 2020. vegan: Community Ecology Package. R package version 2.5-7 [online]. Available from https://cran.r-project.org/web/packages/vegan/index.html.

Pechmann, J.H.K., Scott, D.E., Whitfield Gibbons, J., and Semlitsch, R.D. 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. Wetl. Ecol. Manag. 1(1): 3–11. https://doi.org/10.1007/BF00177885

R Core Team. 2020. R: A Language and Environment for Statistical Computing. [online.] R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.r-project.org/.

Rollins-Smith, L.A. 2017. Amphibian immunity–stress, disease, and climate change. Dev. Comp. Immunol. 66: 111–119. https://doi.org/10.1016/j.dci.2016.07.002

Rowe, C.L. and Dunson, W.A. 1995. Impacts of hydroperiod on growth and survival of larval amphibians in temporary ponds of Central Pennsylvania, USA. Oecologia 102(4): 397–403. https://doi.org/10.1007/BF00341351

Rubbo, M.J. and Kiesecker, J.M. 2005. Amphibian breeding distribution in an urbanized landscape. Conserv. Biol. 19(2): 504–511. https://doi.org/10.1111/j.1523-1739.2005.000101.x

Schiesari, L. 2006. Pond canopy cover: A resource gradient for anuran larvae. Freshw. Biol. 51(3): 412–423. https://doi.org/10.1111/j.1365-2427.2005.01497.x

Semlitsch, R.D. 2008. Differentiating Migration and Dispersal Processes for Pond-Breeding Amphibians. J. Wildl. Manage. 72(1): 260–267. https://doi.org/10.2193/2007-082

Semlitsch, R. and Skelly, D. 2008. Ecology and Conservation of Pool-Breeding Amphibians, in: Science and Conservation of Vernal Pools in Northeastern North America. CRC Press, Boca Raton. pp. 127–148.

Semlitsch, R.D., Peterman, W.E., Anderson, T.L., Drake, D.L., and Ousterhout, B.H. 2015. Intermediate pond sizes contain the highest density, richness, and diversity of pond-breeding amphibians. PLoS One 10(4): 20. https://doi.org/10.1371/journal.pone.0123055

Shirose, L.J., Bishop, C.A., Green, D.M., MacDonald, C.J., Brooks, R.J., and Helferty, N.J. 1997. Validation Tests of an Amphibian Call Count Survey Technique in Ontario, Canada. Herpetologica 53(3): 312–320.

Skelly, D.K. 1996. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. Copeia 1999(3): 599–605. https://doi.org/10.2307/1447523

Skelly, D.K., Werner, E.E., and Cortwright, S.A. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. Ecology 80(7): 2326–2337. https://doi.org/10.1890/0012-9658(1999)080[2326:LTDDOA]2.0.CO;2

Skelly, D.K., Freidenburg, L.K., and Kiesecker, J.M. 2002. Forest canopy and the performance of larval amphibians. Ecology 83(4): 983–992. https://doi.org/10.1890/0012-9658(2002)083[0983:FCATPO]2.0.CO;2

Skidds, D.E., Golet, F.C., Paton, P.W.C., and Mitchell, J.C. 2007. Habitat correlates of reproductive effort in wood frogs and spotted salamanders in an urbanizing watershed. J. Herpetol. 41: 439–450. https://doi.org/10.1670/0022-1511(2007)41[439:HCOREI]2.0.CO;2

Snodgrass, J.W., Komoroski, M.J., Bryan, A.L., and Burger, J. 2000. Relationships among isolated wetland size, hydroperiod, and amphibian species richness:

Implications for wetland regulations. Conserv. Biol. 14(3): 414–419. https://doi.org/10.1046/j.1523-1739.2000.99161.x

Sturrock, R.N., Frankel, S.J., Brown, A. V., Hennon, P.E., Kliejunas, J.T., Lewis, K.J., Worrall, J.J., and Woods, A.J. 2011. Climate change and forest diseases. Plant Pathol. 60(1): 133–149. https://doi.org/10.1111/j.1365-3059.2010.02406.x

Todd, B.D., Luhring, T.M., Rothermel, B.B., and Gibbons, J.W. 2009. Effects of forest removal on amphibian migrations: Implications for habitat and landscape connectivity. J. Appl. Ecol. 46(3): 554–561. https://doi.org/10.1111/j.1365-2664.2009.01645.x

Todd, B.D., Scott, D.E., Pechmann, J.H.K., and Gibbons, J.W. 2011. Climate change correlates with rapid delays and advancements in reproductive timing in an amphibian community. Proc. R. Soc. B Biol. Sci. 278: 2191–2197. https://doi.org/10.1098/rspb.2010.1768

Vasconcelos, D. and Calhoun, A.J.K. 2004. Movement patterns of adult and juvenile *Rana sylvatica* (LeConte) and *Ambystoma maculatum* (Shaw) in three restored seasonal pools in Maine. J. Herpetol. 38(4): 551–561. https://doi.org/10.1670/157-03A

Veysey, J.S., Mattfeldt, S.D., and Babbitt, K.J. 2011. Comparative influence of isolation, landscape, and wetland characteristics on egg-mass abundance of two pool-breeding amphibian species. Landsc. Ecol. 26(5): 661–672. https://doi.org/10.1007/s10980-011-9590-6

Walls, S.C., Barichivich, W.J., and Brown, M.E. 2013. Drought, deluge and declines: The impact of precipitation extremes on amphibians in a changing climate. Biology 2(1): 399–418. https://doi.org/10.3390/biology2010399

Warton, D.I., Wright, S.T., and Wang, Y. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods Ecol. Evol. 3(1): 89–101. https://doi.org/10.1111/j.2041-210X.2011.00127.x

Watson, J.E.M., Evans, T., Venter, O., Williams, B., Tulloch, A., Stewart, C., Thompson, I., Ray, J.C., Murray, K., Salazar, A., McAlpine, C., Potapov, P., Walston, J., Robinson, J.G., Painter, M., Wilkie, D., Filardi, C., Laurance, W.F., Houghton, R.A., Maxwell, S., Grantham, H., Samper, C., Wang, S., Laestadius, L., Runting, R.K., Silva-Chávez, G.A., Ervin, J., and Lindenmayer, D. 2018. The exceptional value of intact forest ecosystems. Nat. Ecol. Evol. 2(4): 599–610. https://doi.org/10.1038/s41559-018-0490-x

Wellborn, G.A., Skelly, D.K., and Werner, E.E. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annu. Rev. Ecol. Syst. 27(1): 337–363. https://doi.org/10.1146/annurev.ecolsys.27.1.337

Werner, E.E. and Glennemeier, K.S. 1999. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. Copeia 1999(1): 1–12. https://doi.org/10.2307/1447379

Werner, E.E., Skelly, D.K., Relyea, R.A., and Yurewicz, K.L. 2007. Amphibian species richness across environmental gradients. Oikos 116(10): 1697–1712. https://doi.org/10.1111/j.0030-1299.2007.15935.x

Werner, E.E., Relyea, R.A., Yurewicz, K.L., Skelly, D.K., and Davis, C.J. 2009. Comparative landscape dynamics of two anuran species: Climate-driven interaction of local and regional processes. Ecol. Monogr. 79(3): 503–521. https://doi.org/10.1890/08-1047.1

Williams, B.K., Rittenhouse, T.A.G., and Semlitsch, R.D., 2008. Leaf litter input mediates tadpole performance across forest canopy treatments. Oecologia 155(2): 377–384. https://doi.org/10.1007/s00442-007-0920-y

Willson, J.D. and Hopkins, W.A. 2013. Evaluating the Effects of Anthropogenic Stressors on Source-Sink Dynamics in Pond-Breeding Amphibians. Conserv. Biol. 27(3): 595–604. https://doi.org/10.1111/cobi.12044

Table 3.1: Percent cover of major land classes in a 2 km buffer around the center of each forest plot. Land classes are derived from the Ontario Land Cover Compilation dataset.

Study Plot	**Open Water**	**Forest**				**Bedrock**	**Urban**
		Sparse	**Deciduous**	**Mixed**	**Coniferous**		
Southeastern Georgian Bay 1 (**SEG1**)	18	32	11	24	1	7	7
Southeastern Georgian Bay 2 (**SEG2**)	15	50	12	7	1	9	7
Southeastern Georgian Bay 3 (**SEG3**)	8	13	19	46	12	1	0
Mid-eastern Georgian Bay 1 (**MEG1**)	8	33	24	27	1	8	0
Mid-eastern Georgian Bay 2 (**MEG2**)	11	41	2	36	4	5	2

Table 3.2: Within-pool and landscape variables measured at survey pools (N=35) during 2019 and 2020

Variable	**Description**	**Mean**	**Range**
Canopy openness	Percent canopy openness; measured using a densiometer.	24	4 – 57
Herbaceous vegetation cover	Percent of pool covered by herbaceous vegetation; measured via ocular estimation.	34	0 – 95
Area	Pool area in square meters; estimated using length and width.	180	13 – 590
Maximum depth	Maximum depth of pool in centimeters.	58	27 – 110
Hydroperiod*	Estimated number of ice-free, wetted days from April 15th – August 26th, 2020; maximum of 101 days.	72	44 – 101+
Road density	Length of roads (km) per squared kilometer in a 300 m buffer around pool.	2.0	0 – 2.8

*Pools were estimated to be ice-free around mid-April based on observations from previous years

Table 3.3: Results of generalized linear mixed modelling relating relative abundance of amphibian larvae to local- and landscape-level environmental variables of vernal pools in eastern Georgian Bay, Ontario. Second order Akaike's Information Criteria values (AICc) are shown for the top models for each species and for models with small differences in AICc (ΔAICc < 2). K = number of estimated parameters.

Model	**K**	**AICc**	**ΔAICc**
Blue-spotted salamander (*A. laterale*)			
Herb. Veg Cover + Year + Julian Day	8	412.6	0
Year + Julian Day	7	413.6	1.0
Canopy Openness + Year + Julian Day	8	413.7	1.1
Herb. Veg. Cover + Thicket Cover + Year + Julian Day	9	414.4	1.8
Spotted salamander (*A. maculatum*)			
Thicket Cover + Year + Julian Day	8	548.1	0
Herb. Veg. Cover + Thicket Cover + Year + Julian Day	9	549.7	1.7
Wood frog (*L. sylvaticus*)			
Thicket Cover + Year + Julian Day	8	334.9	0
Herb. Veg. Cover + Thicket Cover + Road Density + Year + Julian Day	10	336.1	1.2
Year + Julian Day	7	336.2	1.4
Area + Year + Julian Day	8	336.5	1.6
Herb. Veg. Cover + Thicket Cover + Year + Julian Day	9	336.9	1.9
Spring peeper (*P. crucifer*)			
Canopy Openness + Year + Julian Day	8	491.4	0
Hydroperiod + Vegetation Cover + Year + Julian Day	10	491.6	0.2

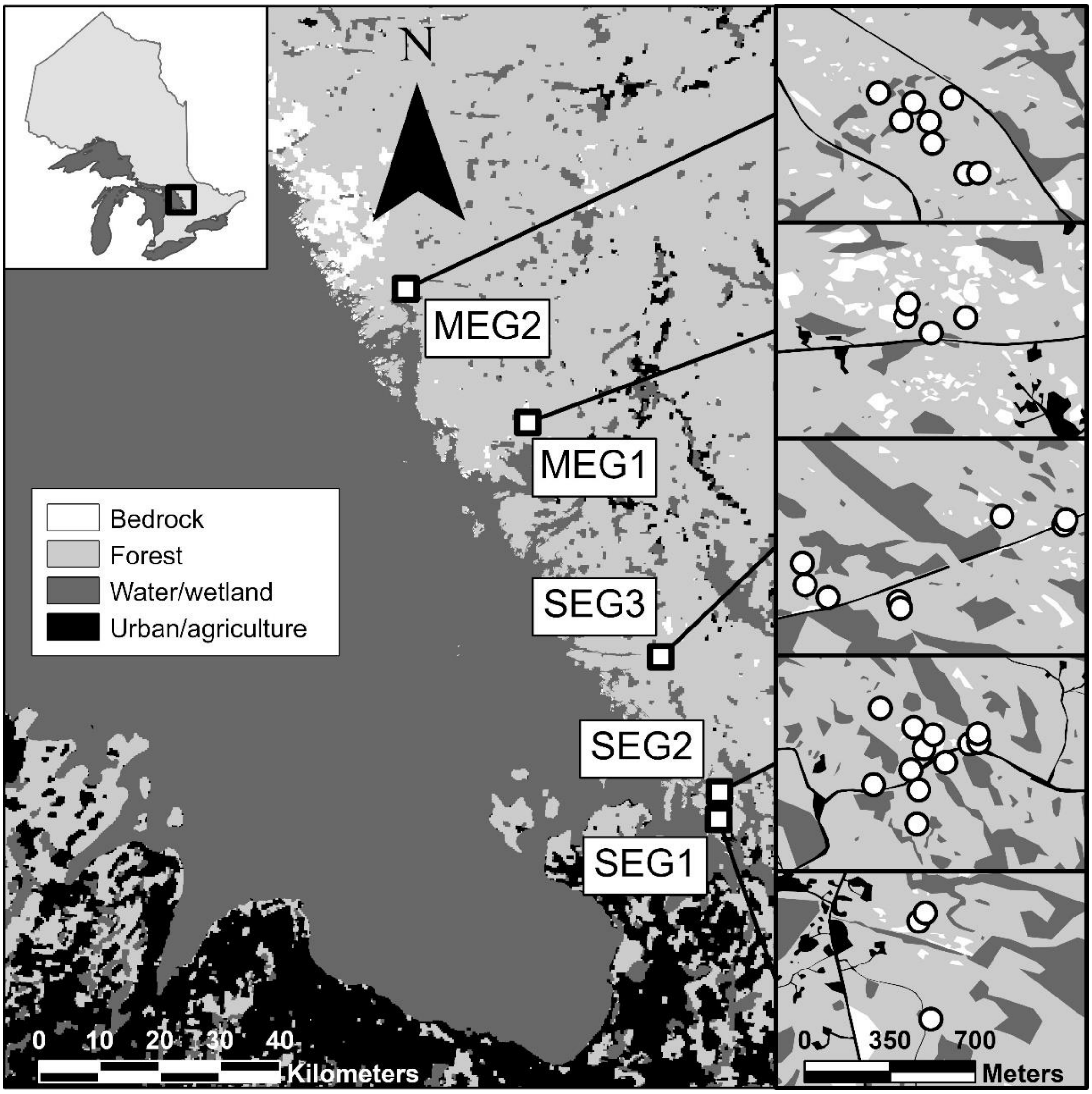

Figure 3.1: Map of the eastern Georgian Bay, Ontario showing the locations of the five forest plots (white squares) and the 35 vernal pools (white circles) included in the study. The map shows the predominant land cover classes surrounding each forest plot. Created using ArcGIS Pro software. SEG = Southeastern Georgian Bay; MEG = mid-eastern Georgian Bay.

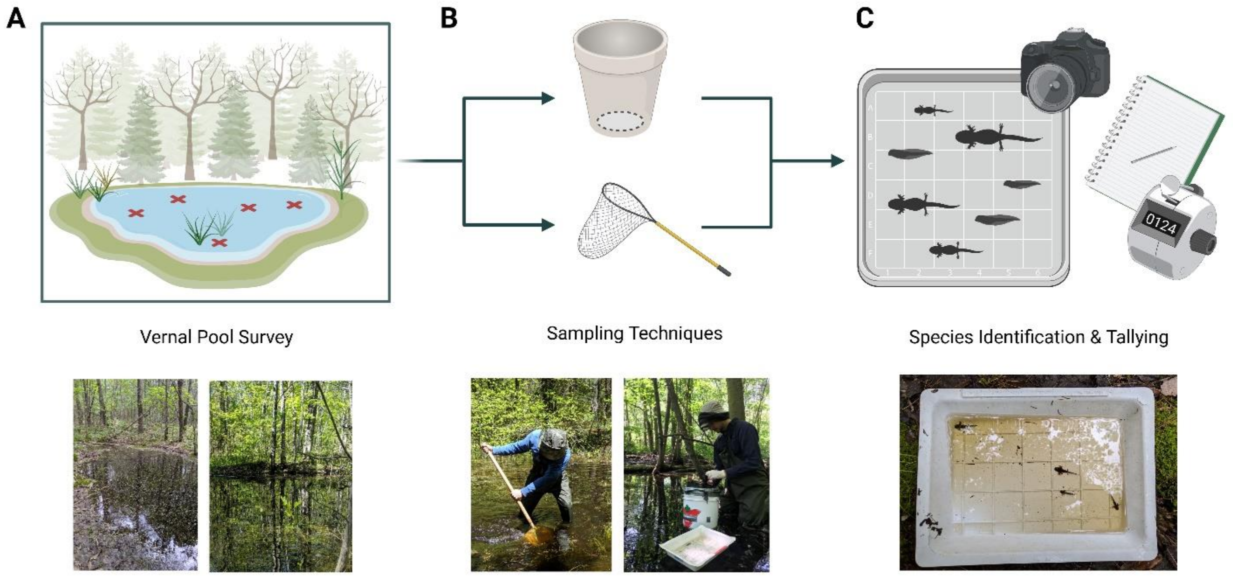

Figure 3.2: Strategy for amphibian larvae surveys in vernal pools: A) Identification of sampling locations within each pool based on pool area, depth, and the distribution of microhabitats; B) Larvae collection at each location using bucket-trap and dip-net techniques; and C) Photographs of larvae on gridded sampling trays to identify species and tally individuals. Created by Samantha Lau using BioRender

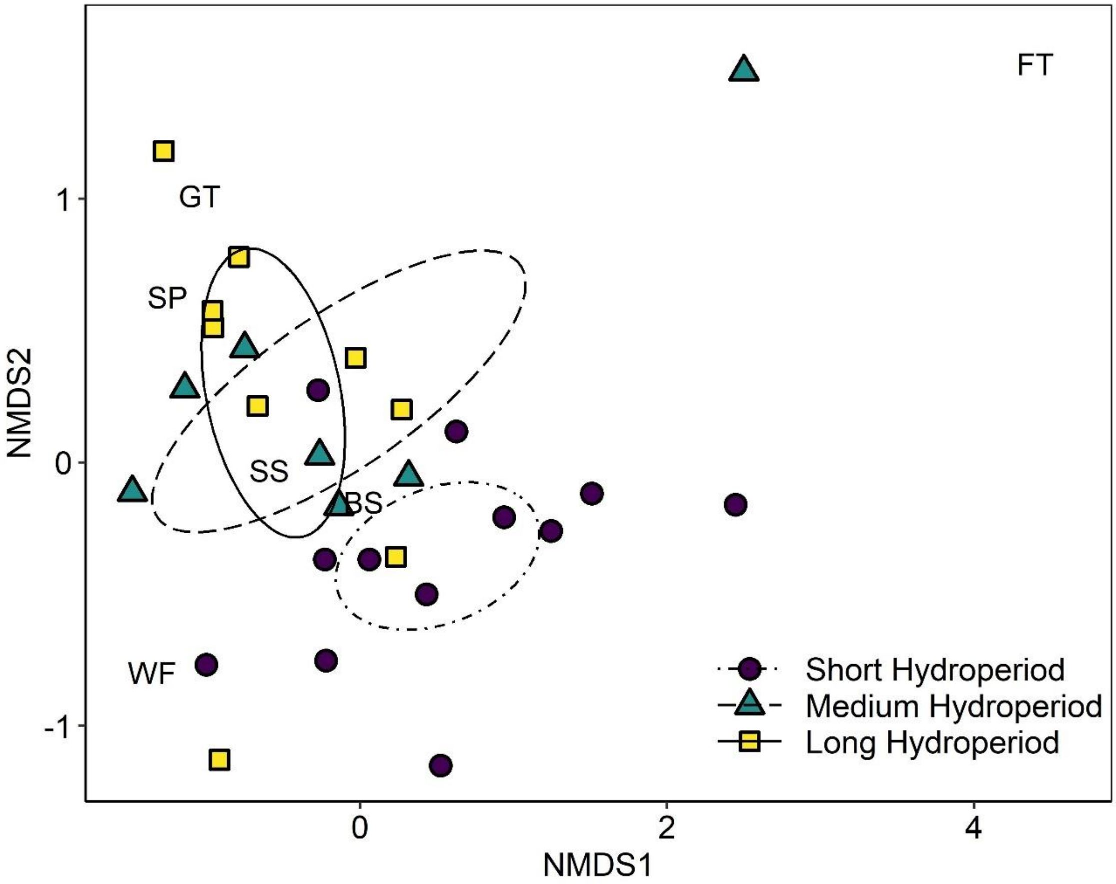

Figure 3.3: Ordination plot of the first and second axes from non-metric multidimensional scaling (NMDS) on amphibian communities within 28 forested vernal pools in eastern Georgian Bay, Ontario sampled during 2019 and 2020. Vernal pools were divided based on relative hydroperiod. Shapes represent different wetlands, and letters represent species codes. Confidence ellipses were plotted for each of the vernal pool groups to visualise differences in species composition. FT = four-toed salamander; BS = blue-spotted salamander; SS = spotted salamander; WF = wood frog; SP = spring peeper; GT = gray treefrog

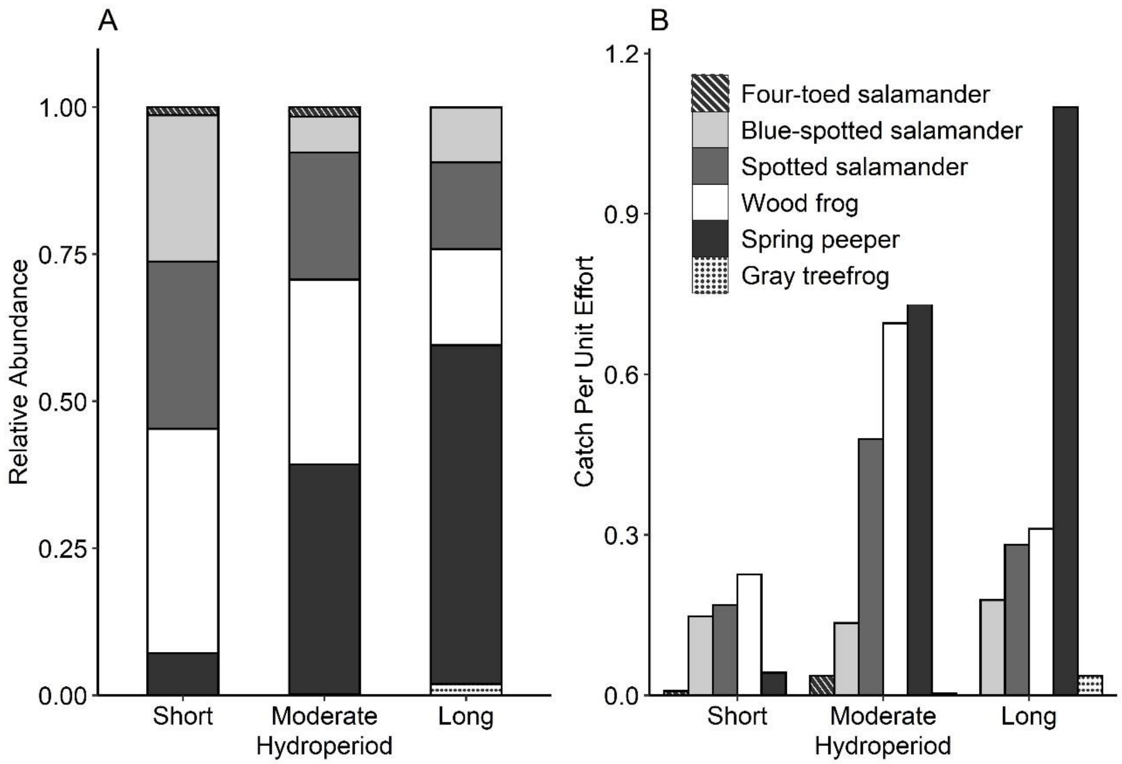

Figure 3.4: Relative abundance (A) and average catch per unit effort (B) of amphibian larvae in short- (< 2 months; N=12), moderate- (2 – 3 months; N=7), and long-hydroperiod (> 3 months; N=9) vernal pools surveyed in eastern Georgian Bay, Ontario during 2019 and 2020.

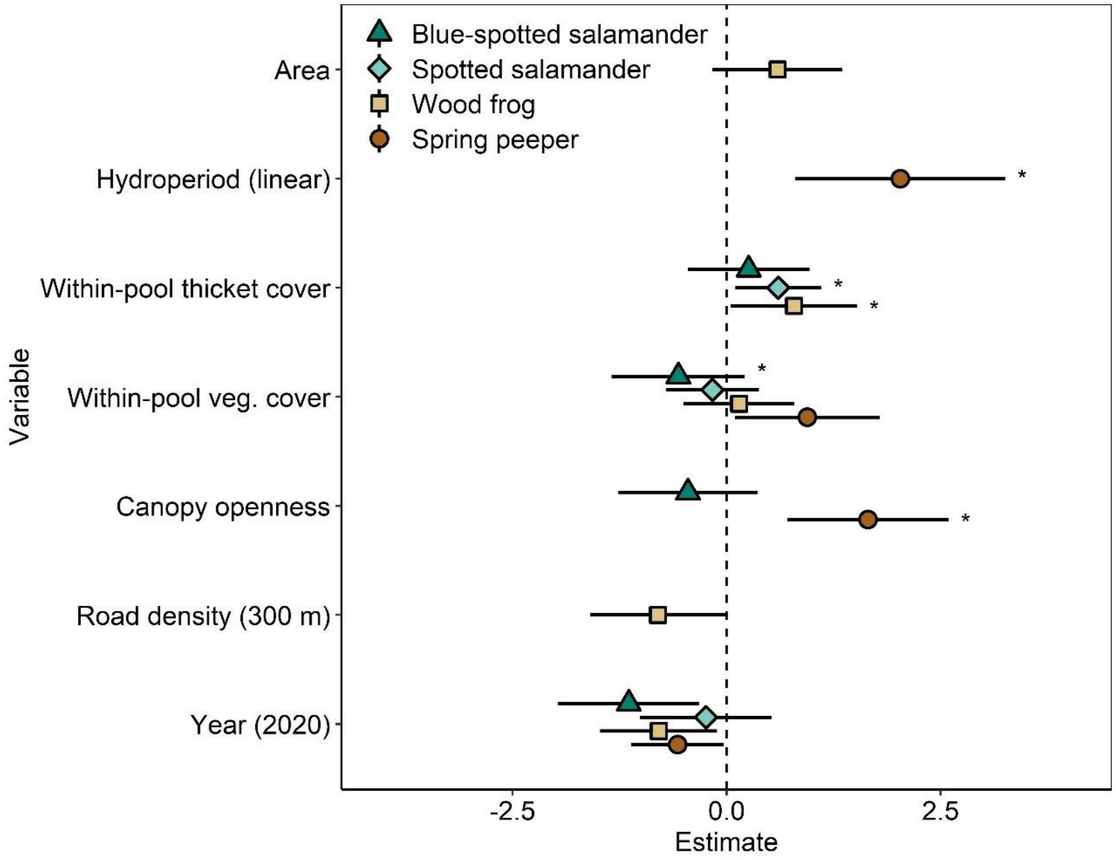

Figure 3.5: Coefficient plot of generalized linear mixed modelling results relating relative abundance of amphibian larvae to habitat and landscape-level variables of 28 vernal pools in eastern Georgian Bay, Ontario sampled during 2019 and 2020. Variables from the top models (ΔAICc < 2) were plotted for each species. Only estimates from the best supported model for each variable were plotted because estimates were consistent between the top models. Estimates with a star (*) indicate that the associated variable has strong support from the top models (combined model weights > 0.5). Error bars represent 95% confidence intervals estimated using the Wald method.

CHAPTER 4: FINE-SCALE HABITAT SUITABILITY MODELLING FOR JEFFERSON SALAMANDERS (*AMBYSTOMA JEFFERSONIANUM*) USING SPATIALLY BIASED SPECIES OCCURRENCE DATA IN SOUTHERN ONTARIO, CANADA

Abstract

Species distribution models (SDMs) have become widely used ecological tools thanks to the increasing availability of geographic datasets containing species occurrences and environmental conditions. SDMs are especially important for rare and imperiled species where habitat conservation is a top priority. In multi-authored datasets, occurrence data are seldom collected in a standardized way, and therefore suffer from sampling biases and lack true absence data. Fortunately, many techniques such as subsampling and pseudo-absence selection, have been developed to account for biased data. We use known locations of the endangered Jefferson salamander (*Ambystoma jeffersonianum*) in southern Ontario (Canada) to test the effectiveness of different SDM techniques. We used two techniques to account for the lack of absence data in datasets: 1) pseudo-absences based on randomly selected background data and 2) pseudo-absences based on locations of a target species with comparable sampling biases. Both techniques yielded models with good discriminatory performance but differed slightly in the locations of forests that were deemed most suitable. Deciduous/mixed forest cover, terrain ruggedness, and agriculture cover had the strongest influence on habitat suitability, and these effects appeared to be strongest on the spatial scale representing salamander home ranges. Projected habitat suitability maps based on these models may help uncover undocumented populations of Jefferson salamanders, especially if combined with fine-scale maps of potential breeding wetlands. Based on the similar performance of the two

techniques used to account for biased data in this study, we recommend that choice of pseudo-absences in future studies consider both the conservation/management goals and the nature of spatial biases in the data.

Introduction

Understanding species distributions and the habitat requirements that drive these distributions is a major goal of conservation biology. As the current biodiversity crisis intensifies and ecosystems continue to be lost at an unprecedented rate, fast and efficient methods to assess the suitability of habitats for species at risk have become a priority. Species distribution models (SDMs) have quickly become the predominant technique for achieving this goal due to accessibility of spatial data and species distribution databases (Guisan and Thuiller, 2005). SDMs relate spatially derived environmental predictors to species occurrences to predict occupancy or habitat suitability across a region of interest. Despite the widespread use of SDMs, species distribution datasets are rarely collected with distribution modelling in mind. To generate reliable results for conservation and management, methods are needed to account for the sampling biases of the data and the specific ecology of the species of interest (e.g. Millar and Blouin-Demars, 2012; Van Strien et al., 2013; Beck et al., 2014; Johnston et al., 2020).

Sampling biases are common in species distribution datasets and are one of the most important considerations in the modelling design process (Bystriakova et al., 2012;

Gaspard, Kim, and Chun, 2019). The lack of standardized approaches for data collection leads to a patchy distribution of observations centered around accessible areas, areas with high population densities of the species, and areas with high biodiversity (Tye et al., 2016). SDMs that do not properly account for spatial biases can lead to autocorrelated residuals, inflated performance metrics, and misinterpretations of species distributions and habitat correlates (Veloz, 2009; Bystriakova et al., 2012; Gaspard, Kim, and Chun, 2019).

Reliable information on species absence is rarely collected for distribution datasets, especially for inconspicuous species; and while there are techniques to estimate detection probability from presence-only data (Lele, Moreno, and Bayne, 2012; Sólymos, Lele, and Bayne, 2012), many studies rely on selection of pseudo-absences to improve model performance (Elith et al., 2006; Phillips et al., 2009). The selection of pseudo-absences can have an impact on SDM predictions and techniques tend to vary in effectiveness across studies (Barbet-Masin et al., 2012; Ranc et al., 2016; Marx and Quillfeldt, 2018). For example, one of the most common techniques involves the selection of pseudo-absences from unsampled background data. While straightforward, this technique does not consider the sampling bias of the occurrence records (Phillips et al., 2009). Methods used to account for sampling bias usually involve occurrence records of a target species or group of species that have been collected using similar methods. While target-species pseudo-absence approaches can improve model performances, there is a high risk of overestimating habitat suitability since the combined distribution of species observations rarely covers the entire region under consideration (Ranc et al., 2016).

The objectives of this study were to identify undocumented habitat of the Jefferson salamander (*Ambystoma jeffersonianum*) in Ontario and determine important environmental drivers of habitat suitability. The Jefferson salamander has been negatively impacted by habitat loss and degradation in Ontario and is listed provincially and federally as a species at risk (Linton, McCarter, and Fotherby, 2018; Environment Canada, 2016). Its current distribution is limited to fragmented forest plots within agriculture and urban matrices. One of the major goals of the current recovery strategy for the Jefferson salamander is to identify undocumented populations, particularly within the Greenbelt and Oak Ridges Moraine regions in Ontario. While forests in these regions are protected to a certain extent, new development projects, such as the construction of a six-lane highway, threaten to decrease available forested habitat. Improved knowledge of the distribution of Jefferson salamanders and other species at risk in these regions is especially important for protecting suitable habitat from the potential threats of land-use changes.

Jefferson salamanders, along with the related blue-spotted salamander (*A. laterale*), are part of a unique unisexual hybrid complex. The unisexual salamanders in this complex evolved from a separate ancestral lineage but rely on sperm from true breeding (non-hybrid) salamanders to stimulate the development of embryos (Bogart et al., 2007). The nuclear genome of unisexual salamanders in Ontario consists of chromosome complements from both true-breeding Jefferson and blue-spotted salamanders and is dominated by chromosomes from the true breeding species they rely on for reproduction (Bi, Bogart, and Fu, 2008). Due to similarities in appearance between Jefferson salamanders and their unisexual dependents, genetic testing of specimens remains the most common method for compiling observations (Linton, McCarter, and Fotherby, 2018). We used records from a long-term dataset of genotyped specimens to train SDMs for Jefferson salamanders. While this dataset contains prominent sampling biases, dedicated sampling regimes for distribution modelling can be time-intensive and costly and distribution information represents an urgent need for Jefferson salamanders.

To develop SDMs we chose predictor variables based on a literature review of environmental correlates of occupancy and abundance and examined how importance of these variables differed across three spatial scales corresponding to estimated home ranges, dispersal distances, and large-scale land use impacts. Lastly, we examined the effect of pseudo-absence selection on model fit, performance, and spatial autocorrelation by employing two techniques: a random background approach and a target-species approach using occurrences of the related blue-spotted salamander (*A. laterale*).

Methods

Distribution data and study area

Occurrence records of Jefferson salamanders, blue-spotted salamanders, and their unisexual dependents were collected between 1982 and 2020, and genetic techniques were used to identify the constituent genotype of each specimen (for a list of techniques see Mills, Hossie, and Murray, 2020). We omitted all observations collected before 1990 because there was no land cover information available. We also limited the dataset to all observations collected south of the Canadian Shield Ecoregion in Ontario as Jefferson salamanders have not been observed north of this boundary (Figure 4.1a). This left us with 326 observations of true-breeding Jefferson salamanders, 663 observations of true-breeding blue-spotted salamanders, and 3815 observations of unisexual salamanders. Some observations of blue-spotted salamanders in south-eastern Ontario were still far away from the nearest observations of Jefferson salamanders. We retained these observations in the study to increase sample size but recognize that they may represent an additional source of bias because changes in selected environmental variables over these large spatial scales may not represent drivers of suitability at the local scale (Lobo, Jiménez-Valverde, and Real, 2007).

Unisexual salamanders are dependent on the presence of sperm-donors from true-breeding species (i.e. Jefferson or blue-spotted), often outnumbering them in areas of geographic overlap such as Ontario (Bogart and Klemens, 1997). Those containing a nuclear genome dominated by chromosomes incorporated from the Jefferson salamander were assumed to be Jefferson dependent unisexuals ($\text{Unisexual}_{\text{Jefferson}}$), whereas those with a majority of chromosomes incorporated from the blue-spotted salamander were assumed to be blue-spotted dependent unisexuals ($\text{Unisexual}_{\text{Blue-spotted}}$). In this study, we grouped Jefferson together with $\text{Unisexual}_{\text{Jefferson}}$ and blue-spotted salamanders with $\text{Unisexual}_{\text{Blue-spotted}}$. Unisexuals with an equal number of chromosomes from each species were omitted from this study.

Data collectors revisited locations across years, but observations often had slightly different geographic coordinates. As such, we used a raster grid (cells of 100 m^2) to group adjacent observations into 607 unique sites. Since observations for this study were highly clustered, we also used spatial thinning to subsample the dataset so that that all sites where Jefferson salamanders or $\text{Unisexual}_{\text{Jefferson}}$ were observed were at least 5 km apart. Based on a preliminary analysis, a critical distance of 5 km limited residual spatial autocorrelation while providing a sufficiently large dataset to train and evaluate models. This distance also exceeds typical movement distances for individual salamanders (Semlitsch, 1998; Committee on the Status of Endangered Wildlife in Canada (COSEWIC), 2010; Van Drunen et al., 2020[1]; Van Drunen et al., 2020[2]). The thinning approach resulted in 47 sites for presence data, of which 32 (68%) were used for model training and 15 (32%) were retained for model evaluation.

Pseudo-absences

Since absence data were not collected as part of the salamander dataset, we used two techniques to define pseudo-absences. The first technique derived pseudo-absences from random background data (RAs). RAs were restricted to forested land because agriculture and urban land were already assumed to be unsuitable habitat. We also ensured RAs were separated by at least 2 km and no more than 10 km from sites with Jefferson salamanders. The lower limit of 2 km ensured that presences and pseudo-absences were separated by distances that exceeded maximum movement distances of Jefferson salamanders and Unisexual$_{\text{Jefferson}}$ (COSEWIC, 2010), and the upper limit of 10 km was selected to reduce biases associated with environmentally distant pseudo-absences. The use of presences and absences separated by large geographic distances in SDMs has been criticized for inflating performance metrics and misrepresenting drivers of local habitat suitability (Lobo, Jiménez-Valverde, and Real, 2007). Since the number of available RA points was considerably larger than the number of presence points, we created subsamples of the RA data. We developed preliminary models to test a range of subsample repetitions (1, 2, 5, 10, and 20) and RAs per subsample (32, 64, and 128), and found that performance according to area under the receiver operating characteristic curve (AUC) for most models reached an asymptote with 10 subsamples of 64 RAs each.

Our second technique derived target-species pseudo-absences (TAs) from observations of the related blue-spotted salamander and Unisexual$_{\text{Blue-spotted}}$. Given that blue-spotted salamanders were part of the same dataset as that for Jefferson salamanders, they were assumed to better represent the sampling bias of the data. Although Jefferson and blue-spotted salamanders seldom use the same breeding wetlands (Bogart et al., 2007; Bogart and Klemens, 2008), we found that 6% of the sites defined in this study had evidence of both species. To limit the possibility of undocumented occupancy of Jefferson salamanders at sites with blue-spotted salamanders, TAs were limited to sites that had at least 7 observations of blue-spotted salamanders or Unisexual$_{\text{Blue-spotted}}$ and no observations of Jefferson salamanders or Unisexual$_{\text{Jefferson}}$. Only three sites (< 1%) had a ratio of blue-spotted to Jefferson salamanders that exceeded this cut-off, so we had high confidence that these sites were not occupied by Jefferson salamanders. Similar to the RAs, we also limited TAs to sites that were at least 2 km away from the nearest sites with Jefferson salamanders. Lastly, in order to reduce clustering, we thinned this dataset so that remaining sites were at least 5 km apart. This left us with 33 sites for TAs, of which 22 (67%) were used for model training and 11 (33%) were retained for model evaluation.

Predictor variables

Since climate was similar across our study area, we focussed on fine resolution land cover and terrain variables as predictors of habitat suitability for Jefferson salamanders. We obtained land cover information from the Southern Ontario Land Resource Information System (SOLRIS) 3.0, which defines 31 different land cover types at a 15-m resolution (Ontario Ministry of Natural Resources and Forestry, 2019). We grouped land cover types from the SOLRIS dataset to derive four unique land cover classes: agriculture, deciduous/mixed forest, coniferous forest, and urban (Figure 4.1b). The literature suggests that deciduous and mixed forests are the preferred habitat for Jefferson salamander (Petranka, 1998; Faccio, 2003; COSEWIC, 2010; Linton, McCarter, and Fotherby, 2018), and that increases in agriculture and urban cover can have negative effects on occupancy and connectivity (Thompson, Gates, and Taylor, 1980; Peterman, Crawford, and Kuhns, 2013; Crawford et al., 2016). By comparison, coniferous forests are less frequently associated with Jefferson salamanders (Faccio, 2003).

We used two provincial waterbody datasets to define potential breeding wetlands since *Ambystoma* salamanders in glaciated northeastern North America, including Jefferson salamanders, require suitable wetlands for breeding and recruitment. Final retained wetlands from these datasets consisted of marshes, ponds, and pools, all of which have been associated with Jefferson salamanders (Nyman, Ryan, and Anderson, 1988; Petranka, 1998). Small, fish-free wetlands (vernal pools), which represent some of the most important breeding habitat for Jefferson salamanders (Petranka, 1998; Porej, Micacchion, and Hetherington, 2004), were largely missing from waterbody datasets due to difficulties detecting these wetlands from aerial imagery. As such, we recognize that this dataset does not represent the full range of breeding habitat present in Ontario forests. We also recognize that there is overlap in the breeding wetland preferences of Jefferson and blue-spotted salamanders (Petranka, 1998); therefore, models using TAs derived from blue-spotted salamanders may underrepresent the importance of wetland habitat.

Since Jefferson salamanders require forest uplands for overwintering sites and landscape depressions for breeding wetlands (Thompson, Gates, and Taylor, 1980; Petranka, 1998), we hypothesized that suitable forests would have hilly topographies. Blue-spotted salamanders have similar requirements for overwintering sites and breeding wetlands but the affinity of Jefferson salamanders for upland habitat surrounding breeding pools tends to be stronger (Petranka, 1998). To estimate topographic variability, we used a provincial digital elevation model (DEM) with a 10 m resolution to derive a terrain ruggedness index (TRI) layer in QGIS. TRI measures the change in elevation between a central cell of a DEM and all its neighbouring cells, with higher values indicating a more rugged terrain.

We resampled land cover and TRI data to a 100-m resolution to match the resolution of the aggregated salamander data. We then used the focal statistics tool in ArcGIS to calculate averages for each cell based on the values of all cells within specified buffer distances. We chose buffer diameters of 300 m, 1000 m, and 5000 m, which corresponded to rough estimates of home ranges, dispersal distances, and large-scale land use impacts, respectively (COSEWIC, 2016; Van Drunen et al., 2020[1]; Table 4.1).

Given the irregular shape of forest patches of southern Ontario, circular buffers may not be able to adequately characterize the amount of available habitat. To better approximate characteristics of individual forest patches, we calculated three patch metrics using FRAGSTATS: area, perimeter-area ratio, and proximity index. The area and perimeter-area ratio of forest patches have been identified as important drivers of Jefferson salamander occupancy, with occupancy increasing in larger forest patches and in forest patches with less complex boundaries (lower perimeter-area ratio; (Weyrauch and Grubb, 2004). The proximity index is used to estimate forest patch isolation and fragmentation based on the proximity and size of neighbouring forest patches. Since *Ambystoma* salamanders are known to exhibit source-sink metapopulation dynamics (Marsh and Trenham, 2008), we hypothesized that Jefferson salamanders would more likely occur in forest patches with higher proximity index scores. To account for sites that were not located within forest patches, we created a raster layer where each cell was assigned patch metric values of the closest forest patch (Table 4.1).

Model development

Modelling algorithms

We developed SDMs using six modelling algorithms that have had success in previous studies: Classification Tree Analysis (CTA), Flexible Discriminant Analysis (FDA), Generalized Boosted Model (GBM), Generalized Linear Model (GLM), Maximum Entropy (ME), and Random Forest (RF; summarized in Table 4.2). Model tuning, training, and evaluation were accomplished using the biomod2 modelling package in R (Thuiller et al., 2021). Parameter tuning was carried out for the default parameters in the biomod2 package for all models except GLM and ME (Table 4.2), which have been found to be less sensitive to changes in parameter settings (Hallgren et al., 2019).

To further reduce the possibility of residual spatial autocorrelation, a five-fold cross-validation approach was used to fit each model. Presence training data were spatially aggregated based on distance and geography to create the five subsets for the cross-validation approach. RAs were assigned to subsets based on proximity to presence sites, whereas TAs were randomly assigned to each subset, as they were less evenly distributed across the five subset regions. For RA models, we repeated the cross-validation approach ten times, once for each of the ten RA subsamples. Since we did not have enough TA data to create multiple subsamples, the cross-validation approach was performed once.

Variable selection

We applied a variable selection framework similar to Bellamy and Altringham (2015) to reduce dimensionality and improve model fit. Using our model fitting approach, we first created univariate models for all 24 predictor variables and evaluated their performance using the average AUC of the training dataset (AUC_{train}) across all modelling algorithms. Variables with no discriminatory ability ($AUC_{train} < 0.5$) were removed and the remaining variables were tested for collinearity using Pearson's Correlation Coefficient ($r > 0.7$). For collinear variables, the variable with the higher AUC_{train} was retained for further analysis. To account for multicollinearity of land cover variables, we iteratively calculated variance inflation scores (VIFs) and removed variables with the highest scores until the scores of all remaining variables were below a conservative threshold ($VIF < 8$). Remining variables were further pruned with a leave-one-out stepwise modelling approach. We first fit a global model using the variables pruned in the previous steps. Next, we pruned variables that had the smallest increase in AUC_{train} when they were removed from the model. This process was repeated until AUC_{train} did not improve after further removal of any remaining variables. We estimated variable importance in the final models using the internal variable importance function of the biomod2 package. This function calculates the correlation between model predictions from the reference data and predictions from data where the values of the variable of interest have been randomly shuffled. The lower the correlation between predictions, the higher the variable importance score.

Model performance

Since TAs were assumed to be a more accurate representation of true absences, we used reserved TA and presence data to evaluate and compare model performance. We assessed performance using four metrics of model discriminatory ability and precision: AUC, sensitivity, specificity, and the minimum acceptable error predicted area (MAE). AUC is a threshold-independent metric of model discriminatory ability that estimates the probability that a randomly chosen presence point will have a higher predicted probability than a randomly chosen absence (or pseudo-absence) point. Models were considered no better than random if AUC of the evaluation dataset (AUC_{eval}) ≤ 0.5, poor if $0.5 < AUC_{eval} < 0.7$, acceptable if $0.7 \leq AUC_{eval} < 0.8$, good if $0.8 \leq AUC_{eval} < 0.9$, and excellent if $AUC_{eval} \geq 0.9$ (ref). We also compared AUC_{eval} with the AUC_{train} to determine if there were signs that models had been overfit to the training data. Though AUC is one of the most common metrics for assessing the discriminatory ability of SDMs, it has been criticized for weighing errors of omission and commission equally and ignoring the spatial distribution of model predictions (Lobo, Jiménez-Valverde, and Real, 2007). We structured our other metrics of model performance to address these limitations but acknowledge we did not cover some aspects of performance, such as the calibration of model predictions.

Since SDMs produce continuous suitability predictions, thresholds are required to convert predictions into binary outputs for differentiating between suitable and unsuitable habitat. We selected two thresholds to evaluate and compare discriminatory ability and precision between models. First, we selected a threshold that maximized the sum of sensitivity and specificity of the evaluation dataset, as has been suggested for models without true absences. Sensitivity and specificity, or the rates of true positives and true negatives, represent more straightforward measures of model discriminatory ability compared to AUC and give a more holistic interpretation of model accuracy.

Our second threshold was designed to maximize specificity while allowing at most one presence from the evaluation dataset to be misclassified (sensitivity > 93%; Gogol-Prokurat, 2011). This conservative threshold is useful for conservation plans for at-risk species, where reducing the risk of missing suitable habitat is especially important (Fielding and Bell, 1997). To evaluate the relative performance of models at this threshold, we used MAE, which measures the proportion of the study area that is predicted to be suitable (Fielding and Bell, 1997). Models with the lowest MAE are considered to be the most precise, as they are able to narrow down the locations of suitable habitat while also minimizing the risk of missing suitable habitat.

We constructed Moran's correlograms to test for significant spatial autocorrelation in model residuals for the combined set of training and evaluation presence sites. For each model, significance of Moran's I was calculated with 1000 Monte Carlo permutations for increments of 5000 m. We also mapped model residuals and visually assessed their distribution to verify the lack of obvious spatial patterns.

Habitat suitability maps

We created three habitat suitability maps for each pseudo-absence technique: (1) a continuous suitability map of the best discriminating model, (2) an ensemble map of binary predictions based on the threshold that maximized the sum of sensitivity and specificity, and (3) an ensemble map of binary predictions based on the MAE threshold. For the continuous suitability map, predictions from the model with the best discriminatory ability according to AUC_{eval} were plotted to create a continuous layer of habitat suitability. While habitat suitability values from these maps are not calibrated, they preserve all information from the model and result in a smooth suitability surface. For the first ensemble map, binary predictions from models with sensitivity and specificity >70% were overlaid to view consensus areas of habitat suitability. The threshold of 70% was selected to ensure that accuracies of both suitable and unsuitable sites were sufficiently high. For the second ensemble map, we overlaid binary predictions from all models with MAE < 75%. We chose a threshold of 75% because predicted suitable areas comprising a larger proportion of the study area would not be useful for prioritizing protected areas (Gogol-Prokurat, 2011).

Final habitat suitability maps were restricted to a 20 km buffer around the Greenbelt region of Ontario because this region has been prioritized under the Jefferson salamander recovery strategy and contains the highest density of recorded salamander observations (Figure 4.1). We also excluded parts of the Greenbelt region that were substantially isolated from Jefferson salamander observations because we had low confidence in the accuracy of predictions for these areas.

Results

Variable selection

Variables tested were often strongly correlated across spatial scales (Pearson's $r > 0.7$), although most were not strongly correlated with each other. The exception was the strong positive correlation between Prox_{CFP} and $\text{DecidMixedForest}_{5000}$ ($r > 0.7$). TRI_{1000} was also moderately correlated with $\text{DecidMixedForest}_{300,1000}$ ($r > 0.6$).

The discriminatory ability ($\text{AUC}_{\text{train}}$) of relevant land cover and terrain variables in univariate models ($\text{AUC}_{\text{train}} > 0.5$) tended to peak at buffer distances of 300–1000 m (Figure 4.2). Models including $\text{DecidMixedForest}_{300,1000,5000}$ or $\text{TRI}_{300,1000,5000}$ had consistently high discriminatory ability across pseudo-absence techniques. Univariate models associated with RAs often had higher discrimination compared to those associated with TAs. Additional variables with high discrimination in the RA models included $\text{Agriculture}_{300,1000,5000}$, $\text{WetlandDensity}_{300,1000}$, A_{CFP}, and PA_{CFP}. After performing our variable selection framework, we were left with a final set of eight variables for the RA models and five variables for the TA models.

Model Performance

All models performed reasonably well according to the chosen performance metrics (Table 4.3). AUC_{eval} results suggested good (>0.8) or excellent (>0.9) discriminatory performance for all models used in this study. Specificity was often higher than sensitivity but both metrics were generally greater than 70%, suggesting good binary discrimination of habitat suitability. The exceptions were the CTA model using TAs and the GLM and ME models using RAs, which estimated either specificity or sensitivity to be between 60 – 70%. MAE was less than 75% for the majority of models; however, two RA models (GLM and ME) and three TA models (CTA, GLM, and ME) had MAE between 75 – 90%, suggesting limited usefulness in prioritizing suitable habitat. The relative rank of models according to AUC_{eval} and MAE was consistent for TA models, suggesting good correspondence between model discriminatory ability and precision. The same was not true for RA models. The CTA model had the highest AUC_{eval} (best discriminatory ability) but a relatively high MAE (low precision) and the opposite was true for the ME model, which had the best precision according to MAE but a relatively low discriminatory ability according to AUC_{eval}.

Most models using TAs had similar or slightly better discriminatory ability according to AUC_{eval} compared to those using RAs, whereas RA models tended to have better precision according to MAE (see Table 4.3 for exceptions). Comparisons between the AUC_{eval} and AUC_{train} suggested that most models fit the data well; however, the ME and RF models had somewhat larger AUC_{train} relative to AUC_{eval}, suggesting they may have been overfitted to the training data.

We found no evidence of residual spatial autocorrelation in any models based on analyses of Moran's correlograms, and visual assessments of mapped residuals did not reveal noticeable spatial patterns.

Variable contributions

The top explanatory variables were generally consistent across algorithms but differed between pseudo-absence selection techniques (Table 4.4). For the TA models, TRI_{1000} was the most important variable, whereas for RA models, $DecidMixedForest_{300}$ and $Agriculture_{300}$ had the highest variable importance scores. $DecidMixedForest_{300}$ also had high variable importance scores for four of the TA models. Most of the other explanatory variables included in the final models were less consistent across algorithms and often had much lower variable-importance scores when compared to the top predictor variables. Most of the TA models only had one or two important variables, whereas moderately important variables for the RA models generally consisted of some combination of TRI_{300}, TRI_{5000}, and PA_{CFP}.

Response plots produced by the univariate models of the top variables for each dataset had a similar appearance across pseudo-absence selection techniques and for most algorithms (Figure 4.3). The probability of occupancy increased with increasing $DecidMixedForest_{300}$ and TRI_{1000} and this trend was consistent across spatial scales; however, since the RA models were trained on a larger range of TRI_{1000} values, some of the response plots suggested a parabolic relationship with TRI_{1000}. The probability of occupancy tended to decrease with increasing $Agriculture_{300}$, and this was especially prominent for RA models. For less important variables, relationships with occupancy were generally less pronounced and sometimes inconsistent across algorithms. The probability of occupancy for the RA models experienced a slight peak at moderate $WetlandCover_{5000}$ and decreased slightly with $Urban_{1000}$ and PA_{CFP}. Likewise, decreases in $ConifForest_{300}$ and increases in A_{CFP} had slight positive effects on probability of occupancy according to TA models.

Habitat suitability maps

For both pseudo-absence techniques, the continuous suitability map and the first ensemble map were similar in appearance, though the predicted habitat suitability for the ensemble map had more of a binary appearance (Figure 4.4). This is likely a result of the high degree of overlap in the binary predictions of the models used to create the ensemble map. For both pseudo-absence techniques, the second ensemble map predicted a higher proportion of the study area to be suitable compared to the first two maps.

Predicted habitat suitability varied between the TA and RA techniques for all three habitat suitability maps, though differences were less pronounced for the second ensemble map (Figure 4.4). A larger proportion of the region was predicted to have high habitat suitability for the TA maps compared to the RA maps. Part of this discrepancy resulted from forest patches in agriculturally dominated landscapes being predicted to have high suitability in TA models but low suitability in RA models. Despite these discrepancies, all maps generally agreed on areas within the Greenbelt that were predicted to have the highest habitat suitability. All maps suggested the presence of additional suitable habitat in the west, along the Niagara Escarpment, and in the east, along the Oak Ridges Moraine, where Jefferson salamanders had not been previously documented for this dataset. One of these forest patches in the Niagara region had historic populations of Jefferson salamanders and has been undergoing restoration initiatives.

Discussion

Model performance

For classifying suitable habitat of Jefferson salamanders (and Unisexual$_{\text{Jefferson}}$), SDMs based on random background pseudo-absence data (RA) performed as well as those based on pseudo-absences derived from a target species (TA); however, the two approaches differed with respect to the appearance of associated habitat suitability maps and the composition of influential variables. Data thinning and stratified subsampling were able to mitigate some of the biases associated with spatially clustered occurrence data, as evidenced by the apparent lack of substantial residual spatial autocorrelation.

In general, all modeling algorithms used in this study yielded good discriminatory ability and the majority of the AUC measures for training and evaluation data were similar, suggesting that most of the models fit the data well. The good fit for the RA models was a pleasant surprise, given that they had been trained on pseudo-absences obtained from a technique different from those used in the evaluation data. We also used two binary prediction thresholds that prioritized either overall discriminatory ability or the identification of suitable habitat. Both thresholds produced acceptable results for most models according to sensitivity/specificity or MAE, but the models that consistently performed well across all measures of success were the RA models using the FDA and CTA algorithms and the TA models using the FDA and GBM algorithms.

Variable contributions and the effect of spatial scale

Both percent cover of deciduous/mixed forest and TRI were important discriminatory variables for both the RA and TA models. The positive effect of deciduous/mixed forest cover on habitat suitability for Jefferson salamanders has been suggested previously (Faccio, 2003) and is in line with the habitat description for the Jefferson salamander and Unisexual$_{\text{Jefferson}}$ under the current provincial recovery strategy (Linton, McCarter, and Fotherby, 2018). Deciduous leaf litter may be preferred because it provides better cover and reduced desiccation risk for migrating and foraging salamanders (Lee-Yaw, Sechley, and Irwin, 2015; Feuka et al., 2017); at the same time, coniferous forests are thought to be less suitable because the lower pH in the soil and water has negative effects on recruitment and occupancy (Horne and Dunson, 1994[1]; Horne and Dunson, 1994[2]; Feuka et al., 2017). The positive effect of terrain ruggedness on habitat suitability models may be slightly confounded by a moderate correlation with deciduous/mixed forest cover, but there is also a sound explanatory basis. *Ambystoma* salamanders in the glaciated northeastern part of N. America are typically found in forests with variable terrain, where breeding wetlands occur in landscape depressions and overwintering habitat occur in surrounding uplands (Thompson, Gates, and Taylor, 1980). Compared to blue-spotted salamanders that frequently use bottomlands, Jefferson salamanders have shown a stronger affinity for isolated wetlands that exist in highly variable terrain (Petranka, 1998). What might have also contributed to this result is that much of the remaining large patches of deciduous and mixed forest in southern Ontario are currently relegated to highly variable terrain that are difficult for agricultural and/or urban development. Research on potential local-scale habitat associations, such as the topographic position of breeding pools and overwintering sites, should be conducted to provide evidence to support or refute the relationship with terrain variability.

The number of univariate models that had good discriminatory ability was considerably larger for the RA than for the TA dataset. While some of this discrepancy likely results from the biased nature of the occurrence data, the importance of some variables on Jefferson salamander habitat suitability may be masked by overlapping habitat requirements of blue-spotted salamanders. For example, the percent cover of agriculture was an important discriminatory variable in the RA models but not in the TA models. Although previous studies have found negative effects of agriculture on the occupancy of Jefferson and blue-spotted salamanders (Guerry and Hunter, 2002), our results may be an artifact of forests in agriculturally dominant landscapes being inadequately sampled (i.e. rarely revisited and being sampled fewer times) compared to forests located close to major cities that are more accessible and therefore more convenient to sample. Though we limited the locations of random background points to a 10-km-diameter buffer around observations, background points were more frequently located in agriculturally dominant landscapes. We found similar trends across models for effects of other variables, including wetland density, urban cover, wetland area and perimeter-area ratio of the closest forest patch, although most of these had only weak effects in the final models. To allow for a more rigorous test of these variables, future studies should include a greater range of forest sizes and adjacent land uses, especially small forests in agriculturally dominated landscapes.

Despite the importance of wetlands as breeding habitat for Jefferson salamanders, we did not find strong relationships with any of the wetland density or cover variables in TA or RA models. Wetland cover was included in the final models for the RA dataset but was not one of the most important variables. It is important to note that although Jefferson salamanders are able to breed in a variety of forested wetlands, small, fish-free wetlands (vernal pools) are their preferred breeding habitat (Thompson, Gates, and Taylor, 1980; Porej, Micacchion, and Hetherington, 2004) and vernal pools have not been completely mapped in southern Ontario. Therefore, the wetland density and cover variables are not representative of all breeding habitat available for Jefferson salamanders. With next generation remote sensing technology, a more detailed/complete wetland dataset should be used to develop more accurate SDMs for Jefferson salamanders and other *Ambystoma* salamanders. In the meantime, users of habitat suitability maps from this study will benefit from use of aerial photointerpretation techniques to delineate the presence of potential breeding wetlands in forests that are deemed to have high suitability.

It is possible that some of the variables we hypothesized to affect habitat suitability could have acted on a different biological response variable, such as abundance or reproductive effort (Miguet et al., 2016). For example, while wetland density did not appear to influence salamander occupancy in our models, forests with a greater density of wetlands may support larger populations of salamanders. Similarly, forests surrounded by agriculture or urban land may support smaller populations of salamanders.

Most variables with good discriminatory power in univariate models had the largest effects at the 300-m-diameter buffer and many of the variables with large importance measures in the final models were also within this buffer, likely because 300 m is a close representation of the estimated home range size for Jefferson salamanders (Linton, McCarter, and Fotherby, 2018; Van Drunen et al., 2020[1]). Forest patches in southern Ontario are rarely larger than even our smallest buffer distance, so decreases in the importance of land cover variables at higher spatial scales may also reflect decreased heterogeneity in the composition of land cover types. The effect of terrain ruggedness was less impacted by spatial scale; TRI_{1000} was the most important variable for the TA models, while TRI_{300} and TRI_{5000} were important variables for the RA models. Increases in buffer distances may not have the same homogenizing effect on terrain ruggedness compared to land cover since the former varied more gradually over the landscape.

Habitat suitability maps

Although mapped locations of Jefferson salamanders were mostly consistent with forests having high suitability, we found some points in forests that were consistently classified as having low suitability. The majority of these sites were associated with data collected during the earliest decade in this study (i.e.1990 – 1999), and we have no information to confirm whether these populations are still viable. This highlights one of the problems associated with long-term datasets where animals are long-lived, and sites have been inconsistently sampled across time and space. The suitability of these sites may also be defined by environmental variables (micro-habitat characteristics) not included in this study, since comparison with land-cover information do not reveal substantial changes in forest cover over the past several decades. Occupied sites with low predicted suitability may also be characterized by small populations. In fact, for some of the areas of low suitability, blue-spotted salamanders appear to be dominant, and in sympatry, we have noticed that when one congener is abundant, the other tends to be scarce (Bogart et al., 2007; Bogart and Klemens, 2008).

Areas of high suitability were generally consistent between all habitat suitability maps, though the TA maps consistently predicted high habitat suitability for a greater proportion of the total forested area. Most of these additional forests were in agriculturally dominated landscapes, which contained few observations of either species. The choice of habitat suitability map should consider the limitations and biases of the predictions along with the specific conservation goals. Since RA maps appear to be more conservative, they may be beneficial for projects looking to identify forests that share many similarities with forests that are already known to have Jefferson salamanders. On the other hand, TA maps may prove to be more informative for projects aimed at expanding the search for Jefferson salamanders in regions that have not been properly surveyed. Likewise, the choice of threshold (or lack thereof) to map suitability predictions should also be informed by conservation goals. Those interested in narrowing down the list of candidate forests to those most likely to be suitable may benefit from maps with high discriminatory ability, such as the continuous suitability maps or ensemble maps based on the sensitivity/specificity threshold. Lastly, if the protection of all potentially suitable forests is the objective, the most beneficial maps may be those with low rates of false absences, such as the ensemble maps based on the MAE threshold.

Methodological considerations

Although AUC has been used extensively to assess the discriminatory performance of HSMs, it has been criticized for ignoring certain aspects of models such as goodness-of-fit, the magnitude of omission and commission errors, and the spatial distribution of model residuals (Lobo, Jiménez-Valverde, and Real, 2007). For these reasons, we used multiple metrics to assess model performance, including sensitivity, specificity, and MAE. While these latter measures provide different assessments of model performance, none can assess the numerical accuracy of model predictions. Consequently, models with good discriminatory ability may still incorrectly predict the likelihood of occupancy (Vaughan and Ormerod, 2005). Since our goal was to produce habitat suitability maps, we were less concerned with accuracy of model calibration;

therefore, we warn against using habitat suitability values from this study as probability of occupancy by Jefferson salamanders. Similarly, habitat suitability predictions from these models should not be used to make inferences about population sizes, demographics, or population dynamics. Lastly, models are region-specific, and we caution against using our models to predict habitat suitability outside of the Greenbelt, given that only a few points were available from other regions in Ontario to train the models.

We limited our choice of modelling algorithms to those compatible with the biomod2 modelling package that have shown good performance in previous SDM studies. Presence-only modelling algorithms, such as ME, have been used successfully in previous studies, but are typically recommended for datasets that use random background pseudo-absences (Guillera-Arroita, Lahoz-Monfort, and Elith, 2014). As such, the comparison of ME models across datasets may not be indicative of the performance of the pseudo-absence selection techniques. While all of the modelling algorithms we tested generally had good performance and produced similar suitability maps, they differed in the relative contributions of variables and suggested different relative performance of the two pseudo-absence selection techniques. We recommend using multiple modelling algorithms, both to assess the precision of suitability estimates and to mitigate potential biases associated with any one algorithm (Li and Wang, 2013).

Presence and absence data are difficult to collect for the Jefferson salamander due to the fossorial nature of adults for much of the year and its similar appearance to other *Ambystoma* salamanders in the larval and juvenile stages. Genetic techniques used to differentiate between Jefferson salamanders and closely related species are the primary methods for evaluating species occurrences, but the data have not been collected with the goal of defining distribution. This explains why occurrence data in this study are highly clustered and spatially biased towards accessible forests. Although we implemented techniques to limit the effect of spatial biases, including data thinning, spatial subsetting, and target pseudo-absence selection, the low number and locations of occurrence data may have underestimated the habitat niche for this species and may have led to biased predictions of suitability. In addition, while pseudo-absences based on target species can decrease the effects of spatially biased observations, they can also misrepresent the importance of discriminatory variables that have similar effects on both species (Fithian et al., 2015). Future studies may benefit from additional screening of potential sites, including limiting forests to those containing suitable breeding wetlands.

Though we grouped Jefferson salamanders and Unisexual$_{\text{Jefferson}}$ into a single suitability model, we recognize that there are well-documented differences in the types of habitat used by each species (Hoffmann et al., 2018; Van Drunen et al., 2020^{1}; Van Drunen et al., 2020^{2}). We were not concerned with accuracy of the proportion of true-breeding and unisexual salamanders since our goal was to improve the knowledge of the distribution of Jefferson salamanders across Ontario. As more data becomes available, future studies should use SDMs to differentiate between suitable habitat for true-breeding and unisexual salamanders.

Implications for conservation

The results of this study provide an important tool for improving the knowledge of Jefferson salamander habitat and distribution in Ontario. Jefferson salamanders and $\text{Unisexual}_{\text{Jefferson}}$ are designated as species at risk at the provincial and federal level and one of the main conservation priorities discussed in their recovery strategy is to improve on their known distribution (Linton, McCarter, and Fotherby, 2018; Environment Canada, 2016). Our focus on drivers of suitability at spatial scales associated with home ranges and dispersal distances complements the results of previous studies that have focussed on more local or regional spatial scales (Peterman, Crawford, and Kuhns, 2013; Mills, Hossie, and Murray, 2020). The habitat suitability maps produced in this study identify several potentially suitable forests within the known range of Jefferson salamanders that have not been surveyed. In addition, one forest with high predicted suitability was identified as a historical site for Jefferson salamanders post-modelling. Our maps should be used to maximize effort towards confirming the occupancy of Jefferson salamander in unsurveyed breeding ponds and protecting them from development.

Our study provides a framework for the development of SDMs for species that are difficult to key out in the field based on morphological attributes. The recent surge in SDMs has been brought about by the growing abundance of readily available species distribution data from museums, research institutions, environmental groups, and community-sourced applications like iNaturalist or HerpMapper (e.g. Millar and Blouin-Demars, 2012; Bradter et al., 2017; Marx and Quillfeldt, 2018). While increasingly sophisticated techniques continue to be developed to account for biases in these data, a one-size-fits-all approach to modelling species distributions is not appropriate (Elith and Leathwick, 2009). SDMs need to be designed to consider the ecology of the species of interest, the nature of the data collection process, and the intended end-use in order to produce useful results for conservation decisions (Guillera-Arroita et al., 2015). We have demonstrated that species occurrence datasets based on genotyped samples can be used in SDMs at small spatial scales by correcting for spatial bias using subsampling and pseudo-absence selection techniques. As high-resolution spatial data become more readily available and citizen science programs increase in popularity, tools such as those developed in this study will be needed to turn the valuable collection of publicly sourced but spatially biased occurrence data into useful information to assist conservation efforts.

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References

Barbet-Massin M, Jiguet F, Albert CH, and Thuiller W. (2012). Selecting pseudo-absences for species distribution models: How, where and how many? *Methods in Ecology and Evolution*, *3*(2), 327–338.

Beck J, Böller M, Erhardt A, and Schwanghart W. (2014). Spatial bias in the GBIF database and its effect on modeling species' geographic distributions. *Ecological Informatics*, *19*, 10–15.

Bellamy C and Altringham J. (2015). Predicting species distributions using record centre data: Multi-scale modelling of habitat suitability for bat roosts. *PLoS ONE*, *10*(6), 17.

Bi K, Bogart JP, and Fu J. (2008). The prevalence of genome replacement in unisexual salamanders of the genus *Ambystoma* (Amphibia, Caudata) revealed by nuclear gene genealogy. *BMC Evolutionary Biology*, *8*(1), 158.

Bogart JP, Bi K, Fu J, Noble DWA, and Niedzwiecki J. (2007). Unisexual salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. *Genome*, *50*(2), 119–136.

Bogart JP and Klemens MW. (2008). Additional distributional records of *Ambystoma laterale*, *A. jeffersonianum* (amphibia: caudata) and their unisexual kleptogens in northeastern North America. *American Museum Novitates*, *2008*(3627), 1–58.

Bogart JP and Klemens MW. (1997). Hybrids and genetic interactions of mole salamanders (*Ambystoma jeffersonianum* and *A. laterale*) (Amphibia, Caudata) in New York and New England. *American Museum Novitates*, *3218*(3218), 1–78.

Bradter U, Mair L, Jönsson M, Knape J, Singer A, and Snäll T. (2018). Can opportunistically collected Citizen Science data fill a data gap for habitat suitability models of less common species? *Methods in Ecology and Evolution*, *9*(7), 1667–1678.

Breiman L. (2001). Random forests. *Machine Learning*, *45*(1), 5–32.

Breiman L, Friedman J, Stone CJ, and Olshen RA. (1984). *Classification and regression trees*. Wadsworth International Group.

Bystriakova N, Peregrym M, Erkens RHJ, Bezsmertna O, and Harald S. (2012). Sampling bias in geographic and environmental space and its effect on the predictive power of species distribution models. *Systematics and Biodiversity*, *10*(3), 305–315.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). (2010). *COSEWIC Assessment and Status Report on the Jefferson Salamander Ambystoma jeffersonianum in Canada*. https://species-registry.canada.ca/index-en.html#/consultations/2269

Crawford JA, Peterman WE, Kuhns AR, and Eggert LS. (2016). Altered functional connectivity and genetic diversity of a threatened salamander in an agroecosystem. *Landscape Ecology*, *31*(10), 2231–2244.

Elith J and Leathwick JR. (2009). Species distribution models: Ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 677–697.

Environment Canada. (2016). *Recovery Strategy for the Jefferson Salamander (Ambystoma jeffersonianum) in Canada*. Species at Risk Act Recovery Strategy Series. Environment Canada. https://species-registry.canada.ca/index-en.html#/consultations/2733

Faccio SD. (2003). Postbreeding emigration and habitat use by Jefferson and spotted salamanders in Vermont. *Journal of Herpetology*, *37*(3), 479–489.

Feuka AB, Hoffmann KE, Hunter ML, and Calhoun AJK. (2017). Effects of light pollution on habitat selection in post-metamorphic wood frogs (*Rana sylvaticus*) and unisexual blue-spotted salamanders (*Ambystoma laterale* × *jeffersonianum*). *Herpetological Conservation and Biology*, *12*(2), 470–476.

Fielding AH and Bell JF. (1997). A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*, *24*(1), 38–49.

Fithian W, Elith J, Hastie T, and Keith DA. (2015). Bias correction in species distribution models: Pooling survey and collection data for multiple species. *Methods in Ecology and Evolution*, *6*(4), 424–438.

Friedman JH. (2001). Greedy function approximation: A gradient boosting machine. *Annals of Statistics, 29*(5), 1189–1232.

Gaspard G, Kim D, and Chun Y. (2019, May 14). Residual spatial autocorrelation in macroecological and biogeographical modeling: A review. *Journal of Ecology and Environment*, Vol. 43, pp. 1–11.

Gogol-Prokurat M. (2011). Predicting habitat suitability for rare plants at local spatial scales using a species distribution model. *Ecological Applications*, *21*(1), 33–47.

Guerry AD and Hunter ML. (2002). Amphibian distributions in a landscape of forests and agriculture: An examination of landscape composition and configuration. *Conservation Biology*, *16*(3), 745–754.

Guillera-Arroita G, Lahoz-Monfort JJ, Elith J, Gordon A, Kujala H, Lentini PE, Mccarthy MA, Tingley R, and Wintle BA. (2015). Is my species distribution model fit for purpose? Matching data and models to applications. *Global Ecology and Biogeography*, *24*(3), 276–292.

Guisan A and Thuiller W. (2005). Predicting species distribution: Offering more than simple habitat models. *Ecology Letters*, *8*(9), 993–1009.

Hallgren W, Santana F, Low-Choy S, Zhao Y, and Mackey B. (2019). Species distribution models can be highly sensitive to algorithm configuration. *Ecological Modelling*, *408*, 23.

Hastie T, Tibshirani R, and Buja A. (1994). Flexible discriminant analysis by optimal scoring. *Journal of the American Statistical Association*, *89*(428), 1255–1270.

Hoffmann K, Hunter M, Calhoun AJK, and Bogart J. (2018). Post-breeding migration and habitat of unisexual salamanders in Maine, USA. *Journal of Herpetology*, *52*(3), 273–281.

Horne MT and Dunson WA. (1994)[1]. Behavioral and physiological responses of the terrestrial life stages of the Jefferson salamander, Ambystoma jeffersonianum, to low soil pH. *Archives of Environmental Contamination and Toxicology*, *27*(2), 232–238.

Horne MT and Dunson WA. (1994)[2]. Environmental contamination and toxicology exclusion of the Jefferson salamander, *Ambystoma jeffersonianum*, from some potential breeding ponds in Pennsylvania: Effects of pH, temperature, and metals on embryonic development. *Arch. Environ. Contam. Toxicol*, *27*, 323–330.

Johnston A, Moran N, Musgrove A, Fink D, and Baillie SR. (2020). Estimating species distributions from spatially biased citizen science data. *Ecological Modelling*, *422*, 108927.

Lee-Yaw JA, Sechley TH, and Irwin DE. (2015). Conflicting effects of microhabitats on Long-toed Salamander (*Ambystoma macrodactylum*) movement: implications for landscape connectivity. *Canadian Journal of Zoology*, *93*(1), 1–7.

Lele SR, Moreno M, and Bayne E. (2012). Dealing with detection error in site occupancy surveys: what can we do with a single survey? *Journal of Plant Ecology*, *5*, 22–31.

Li X and Wang Y. (2013, June 1). Applying various algorithms for species distribution modelling. *Integrative Zoology*, Vol. 8, pp. 124–135.

Linton J, McCarter J, and Fotherby H. (2018). *Jefferson Salamander (Ambystoma jeffersonianum) and Unisexual Ambystoma Jefferson Salamander dependent population (Ambystoma laterale – (2) jeffersonianum) in Ontario*. Ontario Recovery Strategy Series. Ontario Ministry of Natural Resources and Forestry. https://www.ontario.ca/page/jefferson-salamander-recovery-strategy

Lobo JM, Jiménez-Valverde A, and Real R. (2008). AUC: a misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography*, *17*(2), 145–151.

Marsh DM and Trenham PC. (2001). Metapopulation Dynamics and Amphibian Conservation. *Conservation Biology*, *15*(1), 40–49.

Marx M and Quillfeldt P. (2018). Species distribution models of European Turtle Doves in Germany are more reliable with presence only rather than presence absence data. *Scientific Reports*, *8*(1), 1–13.

McCullagh P and Nelder JA. (1989). *Generalized linear models 2nd Edition*. Chapman and Hall.

Miguet P, Jackson HB, Jackson ND, Martin AE, Fahrig L. (2016). What determines the spatial extent of landscape effects on species? *Landscape Ecology*, *31*, 1177–1194.

Millar CS and Blouin-Demers G. (2012). Habitat suitability modelling for species at risk is sensitive to algorithm and scale: A case study of Blanding's turtle, *Emydoidea blandingii,* in Ontario, Canada. *Journal for Nature Conservation*, *20*(1), 18–29.

Mills PB, Hossie TJ, and Murray DL. (2020). Niche determinants in a salamander complex: Does hybridism or reproductive parasitism explain patterns of distribution? *Ecosphere, 11*(10), 15.

Nyman S, Ryan MJ, and Anderson JD. (1988). The distribution of the *Ambystoma jeffersonianum* complex in New Jersey. In *Source: Journal of Herpetology* (Vol. 22).

Peterman WE, Crawford JA, and Kuhns AR. (2013). Using species distribution and occupancy modeling to guide survey efforts and assess species status. *Journal for Nature Conservation*, *21*(2), 114–121.

Petranka J. (1998). *Salamanders of the United States and Canada*. Smithsonian Institution Press.

Phillips SJ, Dudík M, Elith J, Graham CH, Lehmann A, Leathwick J, and Ferrier S. (2009). Sample selection bias and presence-only distribution models: Implications for background and pseudo-absence data. *Ecological Applications*, *19*(1), 181–197.

Phillips SJ, Anderson RP, and Schapire RE. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3–4), 231–259.

Porej D, Micacchion M, and Hetherington TE. (2004). Core terrestrial habitat for conservation of local populations of salamanders and wood frogs in agricultural landscapes. *Biological Conservation*, *120*(3), 399–409.

Ranc N, Santini L, Rondinini C, Boitani L, Poitevin F, Angerbjörn A, and Maiorano L. (2017). Performance tradeoffs in target-group bias correction for species distribution models. *Ecography*, *40*(9), 1076–1087.

Semlitsch RD. (1998). Biological delineation of terrestrial buffer zones for pond-breeding salamanders. *Conservation Biology*, *12*(5), 1113–1119.

Sólymos P, Lele S, Bayne E. (2012). Conditional likelihood approach for analyzing single visit abundance survey data in the presence of zero inflation and detection error. *Environmetrics*, *23*, 197–205.

Thompson EL, Gates JE, and Taylor GJ. (1980). Distribution and breeding habitat selection of the Jefferson Salamander, *Ambystoma jeffersonianum*, in Maryland. *Journal of Herpetology*, *14*(2), 113.

Thuiller W, Georges D, Gueguen M, Engler R, and Breiner F. (2021). *biomod2: ensemble platform for species distribution modeling R package version 3.5.1*. https://cran.r-project.org/web/packages/biomod2/index.html

Tye CA, McCleery RA, Fletcher RJ, Greene DU, and Butryn RS. (2017). Evaluating citizen vs. professional data for modelling distributions of a rare squirrel. *Journal of Applied Ecology*, *54*(2), 628–637.

Van Drunen SG, Linton JE, Bogart JP, McCarter J, Fotherby H, Sandilands A, and Norris DR. (2020)[1]. Estimating critical habitat based on year-round movements of the endangered Jefferson salamander (*Ambystoma jeffersonianum*) and their unisexual dependents. *Canadian Journal of Zoology*, *98*(2), 117–126.

Van Drunen SG, Linton JE, Bogart JP, and Norris DR. (2020)[2]. Post-emergence survival and dispersal of juvenile Jefferson salamander (*Ambystoma jeffersonianum*) and their unisexual dependents. *Amphibia-Reptilia*, *42*(1), 29–41.

Van Strien AJ, Van Swaay CAM, and Termaat T. (2013). Opportunistic citizen science data of animal species produce reliable estimates of distribution trends if analysed with occupancy models. *Journal of Applied Ecology*, *50*(6), 1450–1458.

Vaughan IP and Ormerod SJ. (2005). The continuing challenges of testing species distribution models. *Journal of Applied Ecology*, *42*(4), 720–730.

Veloz SD. (2009). Spatially autocorrelated sampling falsely inflates measures of accuracy for presence-only niche models. *Journal of Biogeography*, *36*(12), 2290–2299.

Weyrauch SL and Grubb TC. (2004). Patch and landscape characteristics associated with the distribution of woodland amphibians in an agricultural fragmented landscape: An information-theoretic approach. *Biological Conservation*, *115*(3), 443–450.

Table 4.1: Land cover and terrain predictor variables used in the study. Numerical subscripts indicate buffer diameters (m) for land cover and terrain variables. SOLRIS = Southern Ontario Land Resource Information System; OMNRF = Ontario Ministry of Natural Resources and Forestry; DEM = Digital Elevation Model.

Predictor Variable	**Description**	**Data source**
$\text{Agriculture}_{300,\ 1000,\ 5000}$	Percent cover of agriculture within indicated buffer diameters	SORLIS 3.0
$\text{DecidMixedForest}_{300,\ 1000,\ 5000}$	Percent cover of deciduous and mixed forest within indicated buffer diameters	SORLIS 3.0
$\text{ConifForest}_{300,1000,\ 5000}$	Percent cover of coniferous forests within indicated buffer diameters	SORLIS 3.0
$\text{Urban}_{300,\ 1000,\ 5000}$	Percent cover of urban land within indicated buffer diameters	SORLIS 3.0
$\text{WetlandCover}_{300,\ 1000,\ 5000}$	Percent cover of wetlands within indicated buffer diameters	OMNRF
$\text{WetlandDens}_{300,\ 1000,\ 5000}$	Density of wetlands within indicated buffer diameters	OMNRF
$\text{TRI}_{300,1000,\ 5000}$	Average terrain ruggedness index (TRI) within indicated buffer diameters	Provincial DEM
A_{CFP}	Area of the closest forest patch (CFP)	SORLIS 3.0
PA_{CFP}	Perimeter-area ratio of CFP	SORLIS 3.0
Prox_{CFP}	Proximity index of CFP	SORLIS 3.0

Table 4.2: Description of modelling algorithms used in this study. Tuning parameters are listed for relevant models. Abbreviations for algorithms are in brackets.

Modelling algorithm	**Description**	**Advantages**	**Tuning Parameters**
Generalized Linear Model (GLM)	Generalization of general linear models for non-normal data, including species presence/absence data. Building block for more sophisticated modelling algorithms (McCullagh and Nelder, 1989).	Strong statistical foundation, simple interpretation of variable relationships	Did not tune
Flexible Discriminant Analysis (FDA)	An extension of linear discriminant analysis that uses multiple linear and non-linear combinations of predictor variables to discriminate between presence/absence observations (Hastie, Tibshirani, and Buja, 1994).	Works with many variables, automatically detects interactions	Maximum polynomial degree for interactions; maximum number of model terms
Classification Tree Analysis (CTA)	Creates a decision tree with nodes that iteratively split data into smaller subsets using predictor variable thresholds (Breiman et al., 1984).	Makes use of complex interactions between predictor variables	Complexity parameter; maximum depth of tree
Random Forest (RF)	Ensemble classifier consisting of many decision trees, where each tree votes on the final classification of the observations (Breiman, 2001).	Efficient, handles collinear variables, robust with unbalanced data	Number of variables for each node
Generalized Boosted Model (GBM)	Ensemble technique consisting of many predictive trees, where each successive tree is fit to the residuals of the previous tree and final predictions are based on the average of all trees (Friedman, 2001).	Overcomes inaccuracies of single models, able to fit complex relationships	Number of model trees; maximum depth of trees; shrinkage parameter
Maximum Entropy (ME)	Presence-only approach that estimate species distribution by identifying the most uniform distribution of occupancy probability subject to constraints set by the predictor variables (Phillips. Anderson, and Schapire, 2006).	Does not require absence data, robust to parameter settings	Did not tune

Table 4.3: Model performance of models using six modelling algorithms for datasets with random background pseudo-absences and target-species pseudo-absences. The threshold independent area under the receiver operating characteristic curve is reported for the average model predictions of the evaluation (AUC_{eval}) and training data (AUC_{train}). Sensitivity and specificity are calculated from the model predictions based on a threshold that maximized the sum of sensitivity and specificity. The minimum acceptable error predicted area (MAE) is the proportion of the study area predicted to be suitable based on a threshold that allows for at most one false absence in the evaluation dataset. Bold entries under the columns for AUC and MAE identify the best performing models according to each metric. See Table 4.2 for full names of algorithms.

Algorithm	**Random background pseudo-absences**				**Target-species pseudo-absences**			
	AUC_{eval} (AUC_{train})	**Sensitivity**	**Specificity**	**MAE**	**AUC_{eval} (AUC_{train})**	**Sensitivity**	**Specificity**	**MAE**
CTA	**0.90** (0.90)	0.87	0.91	0.75	0.83 (0.81)	0.67	0.91	0.89
FDA	0.87 (0.87)	0.73	0.91	0.68	0.90 (0.83)	1.00	0.82	0.71
GBM	0.87 (0.89)	0.87	0.82	0.76	**0.91** (0.87)	0.87	0.91	**0.67**
GLM	0.83 (0.86)	0.93	0.64	0.73	0.86 (0.82)	0.80	0.91	0.86
ME	0.84 (0.91)	0.67	0.91	**0.51**	0.86 (0.93)	0.73	0.91	0.81
RF	0.85 (0.95)	0.73	0.91	0.79	0.90 (0.98)	0.87	0.91	0.73

Table 4.4: Variable importance measures calculated for the six modelling algorithms for each Jefferson salamander pseudo-absence selection technique. Variables with the highest importance for each model are highlighted in bold and underlined. Importance measures are based on the correlation between reference model predictions and model predictions made after shuffling the values of the variable of interest. The lower the correlation, the higher the variable importance score. See Table 4.2 for full names of algorithms. TRI = Terrain Ruggedness Index; A = Area; PA = Perimeter-area ratio; CFP = Closest forest patch.

Variable	**Random background pseudo-absences**						**Target-species pseudo-absences**					
	CTA	**FDA**	**GBM**	**GLM**	**ME**	**RF**	**CTA**	**FDA**	**GBM**	**GLM**	**ME**	**RF**
Agriculture$_{300}$	0.33	0.25	0.21	0.23	**0.28**	**0.16**	0	0	0	0	0.11	0.02
Agriculture$_{5000}$	0.05	0.04	0.01	0.03	0.12	0.02						
DecidMixedForest-$_{300}$	**0.56**	**0.50**	**0.33**	**0.36**	0.21	0.15	0	0.40	0.24	0	0.29	0.18
ConifForest$_{300}$							0	0	0	0	0.13	0.01
Urban$_{1000}$	0.02	0.02	0	0.02	0.08	0.02						
WetlandCover$_{5000}$	0.02	0.01	0	0.03	0.10	0.02						
TRI$_{300}$	0.07	0.11	0.05	0.04	0.12	0.07						
TRI$_{1000}$							**0.93**	**0.51**	**0.46**	**0.96**	**0.45**	**0.39**
TRI$_{5000}$	0.08	0.11	0.06	0.11	0.16	0.07						
A$_{CFP}$							0	0	0.01	0	0.10	0.03
PA$_{CFP}$	0.04	0.06	0.02	0.09	0.17	0.07						

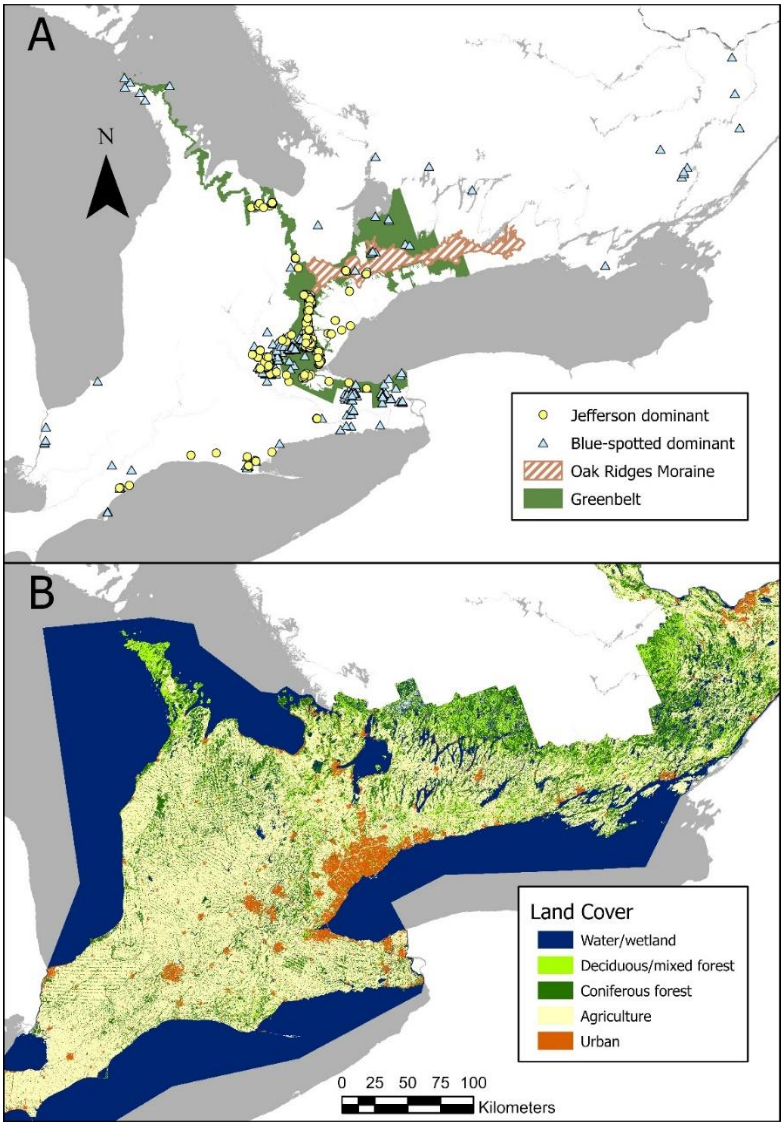

Figure 4.1: Map of southern Ontario showing a) the boundaries of the Greenbelt and Oak Ridges Moraine overlaid by the recorded locations of Jefferson salamanders (*Ambystoma jeffersonianum*) and blue-spotted salamanders (*A. laterale*), and a) major land cover classes used as explanatory variables in the habitat suitability models. Overlap between salamander records masks many sample locations at the scale used for this map.

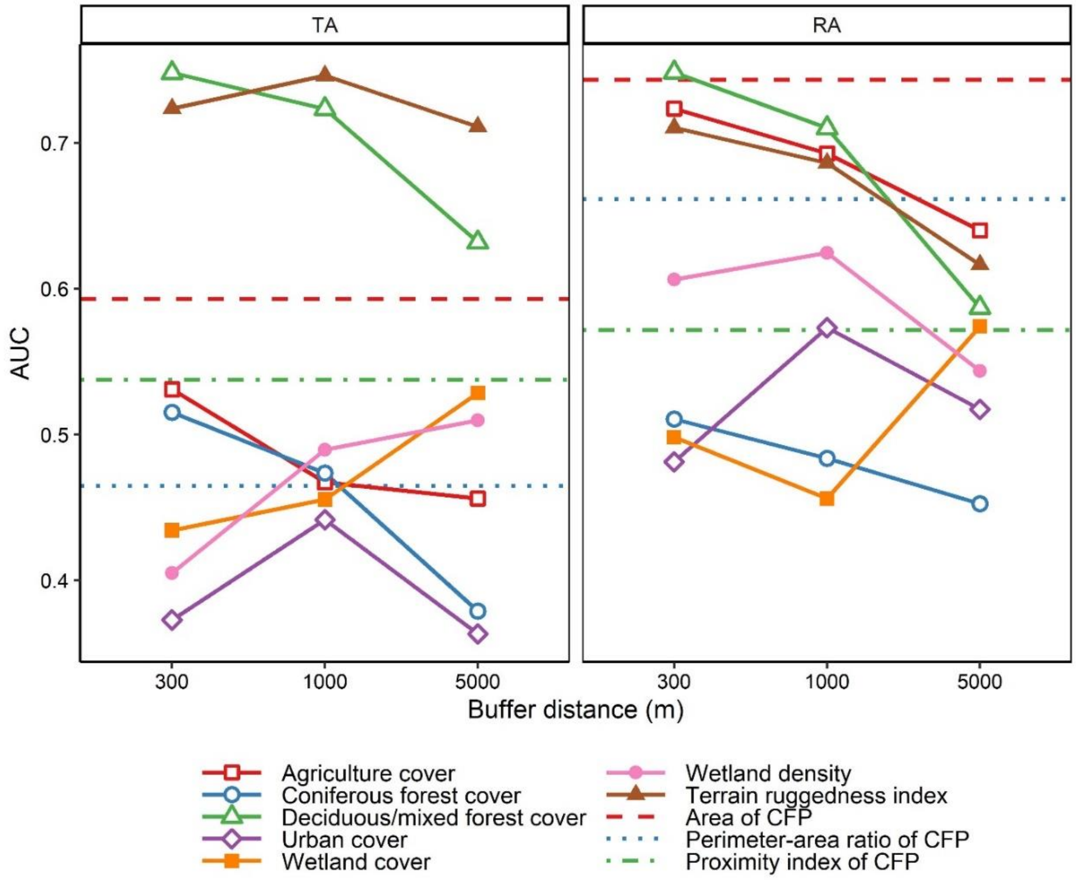

Figure 4.2: The relative ability of explanatory variables to discriminate between presences and pseudo-absences of Jefferson salamanders (as measured by AUC_{train}) at different spatial scales. Graphs are shown for models using pseudo-absences derived from random background data (RA) and for those derived from the locations of a target species (TA). AUC values were averaged across six different modelling algorithms. Variables represented by horizontal lines were scale-independent. Models with discriminatory ability < 0.5 are considered to be no better than random.

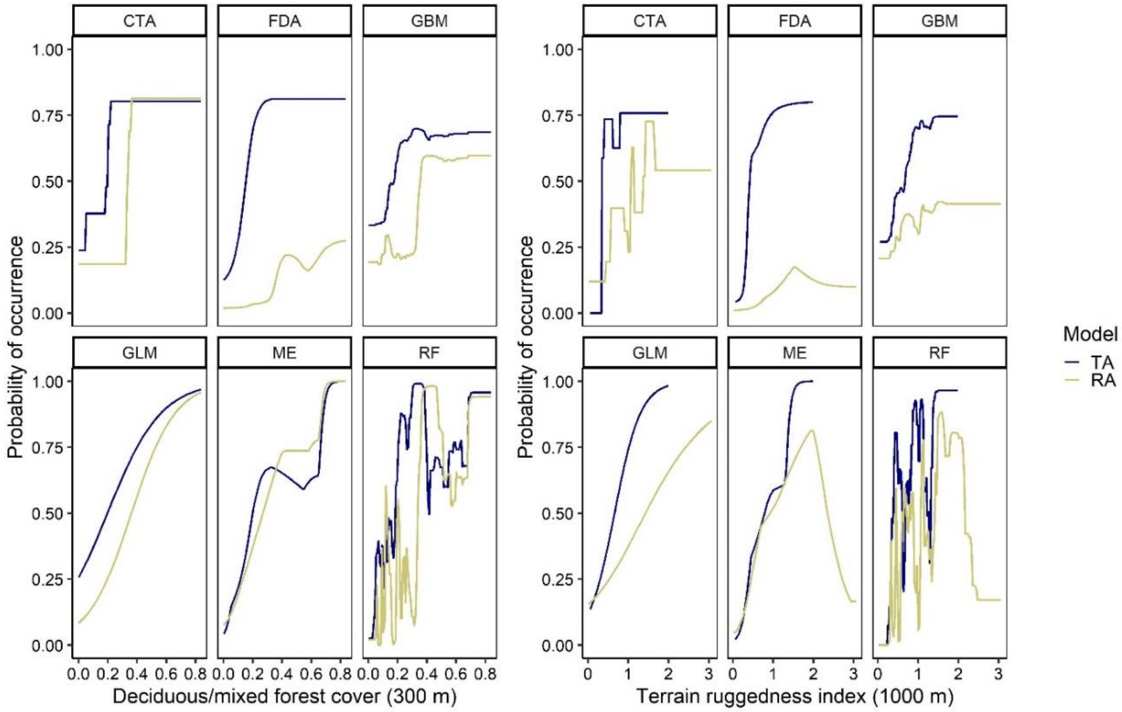

Figure 4.3: Response plots produced by univariate models of the strongest explanatory variables for Jefferson salamander habitat suitability models. Response plots are shown for six modelling algorithms involving either pseudo-absences derived from random background data (RA) or from a target species (TA). Similar response curves were obtained at other buffer sizes for each variable. Y axes represent the predicted probability of Jefferson salamanders being present at a particular location based on the effect of each explanatory variable. Full names associated with abbreviations of modelling algorithms appear in Table 4.2.

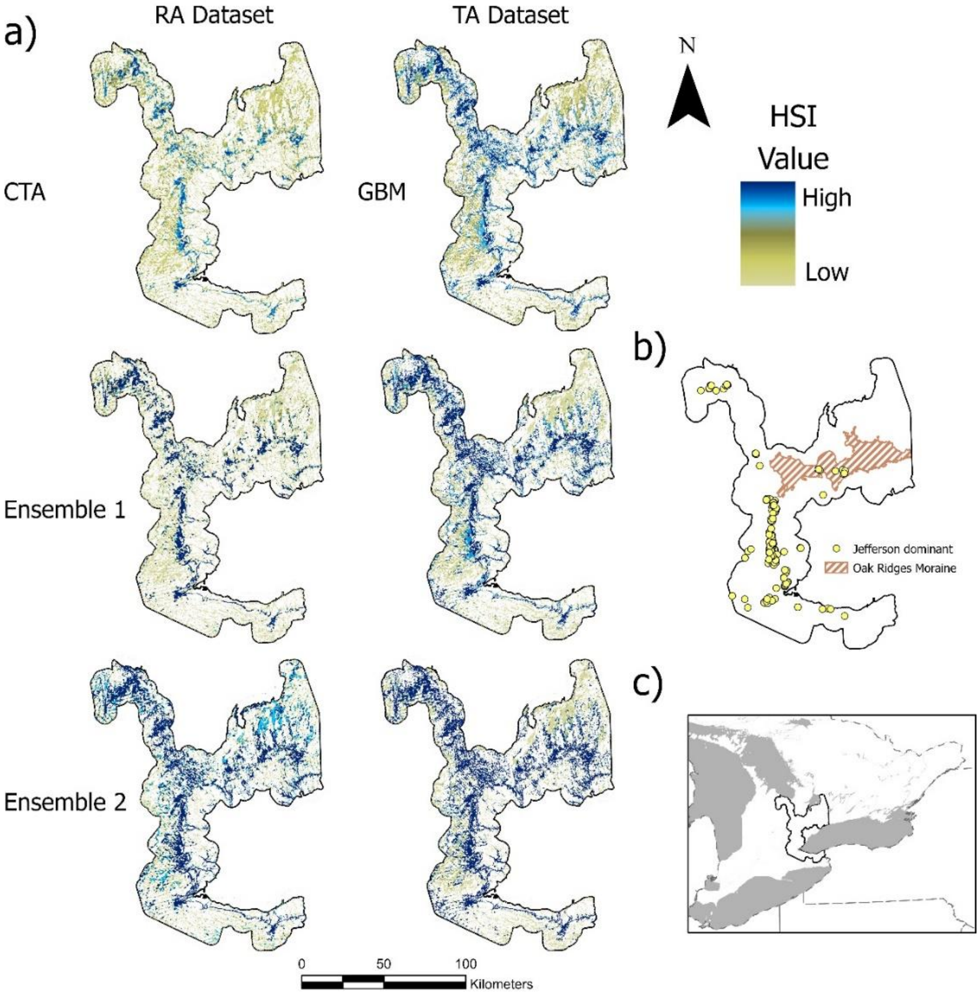

Figure 4.4: a) Habitat suitability maps for Jefferson salamanders within Ontario's Greenbelt region for models using target-species pseudo-absences (TA) and random background pseudo-absences (RA). Maps were created using continuous predictions from the top discriminating models (CTA or GBM) or combined binary predictions from multiple models based on two different thresholds (Ensemble 1 and 2). For Ensemble 1, thresholds were designed to maximize the sum of sensitivity and specificity, whereas for Ensemble 2, thresholds were designed to maximize specificity while allowing at most one false absence. b) recorded locations of Jefferson salamanders within the Greenbelt region. c) location of Greenbelt region within Ontario. HSI = habitat suitability index; CTA = Classification Tree Analysis; FDA = Flexible Discriminant Analysis; GBM = Generalized Boosted Model; ME = Maximum Entropy.

CHAPTER 5: OPTIMIZATIONS FOR TIME AND EFFORT IN LONG-TERM MONITORING: A CASE STUDY USING A MULTIDECADAL TERRESTRIAL SALAMANDER MONITORING PROGRAM

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Abstract

Long-term monitoring programs can identify environmental trends or reveal limitations to protocols, as long as their results are analysed appropriately. While monitoring programs are not necessarily hypothesis-driven, their data are important for conservation and can guide improvements to monitoring programs. Here, we present a case study using dynamic occupancy models to guide the optimization of time and effort in a long-term terrestrial salamander monitoring program. To ensure a detailed analysis, we analysed the available long-term data to first identify estimates of occupancy and detection parameters for the salamanders. Using these estimates, we created simulations to identify the optimal number of years for monitoring and the optimal allocation of spatial and temporal survey replicates. Our data support previous claims that monitoring programs should be allowed to run for at least a decade. We also found that in order to obtain accurate estimates of species occupancy, programs should consider appropriate partitioning of monitoring effort across spatial and temporal scales. We show how analyses of long-term monitoring datasets are valuable not only for trend detection, but also for the development of templates to guide the design and optimization of similar programs.

Introduction

The growing threats to biodiversity from human activities, including habitat alterations and climate change, have been accompanied by an increased demand for long-term environmental monitoring (Urban et al., 2016; McMahon et al., 2011). Long-term monitoring is needed to understand complex population dynamics, so that conclusions can be made about the state of a particular system. Monitoring is often used for two reasons: to determine the effectiveness of conservation management and to inform future management through identifying and understanding the reasons for ecosystem degradation. Some monitoring programs have acquired decades worth of data (Lindenmayer et al., 2012), and these data not only provide information about the system in question but are also as a valuable resource to guide future monitoring. While large studies looking at multiple programs have been imperative to understanding reoccurring patterns in monitoring programs (White, 2019; Rhodes and Jonzen, 2011; MacKenzie and Royle, 2005), case-specific analyses of monitoring data can provide a more detailed understanding of how best to monitor similar systems in the future.

Researchers have argued for more rigorous decision-making processes during the design of monitoring programs (Lindenmayer and Likens, 2009; Caughlan and Oakley, 2001). In many cases, monitoring design has focussed on data collection, giving less consideration to the analysis and presentation of the data (Field et al., 2007; Lindenmayer and Likens, 2009). Proper statistical consideration can ensure time and money are spent on data that can be interpreted in the context of the goals of the monitoring program. While the choice of statistical analysis is intrinsically related to the type of data collected, program-specific considerations of statistical power and survey optimization are also important to the success and cost effectiveness of the program. In particular, White (2019) found that the number of years required for sufficient statistical power varies greatly between monitoring systems, reasoning against the use of conventional rules-of-thumb. Similarly, with regards to the precision of trend estimates, the optimal allocation of sampling effort over time and space has been shown to depend on the dynamics of the chosen system (Rhodes and Jonzen, 2011).

Even with careful consideration of all aspects of the chosen monitoring system, program designs often need to be updated once the data reveal more about the specific nature of the system. Adaptive monitoring sees aspects of monitoring as an iterative process, where information gained through monitoring guides the evolution of new questions, designs, and analyses (Lindenmayer and Likens, 2009). These principles are particularly relevant to long-term monitoring programs, where information on indicator species have been collected for many years in exactly the same fashion, and which can provide a more complete picture of species dynamics and factors that may influence population fluctuations (Kéry et al., 2009; Magurran et al., 2010).

In this paper, we present a case study using data from a terrestrial salamander monitoring program (1999-2016) to show how detailed, program-specific analyses can improve how we approach the design and management of monitoring programs. Our main objective was to identify ways to optimize the time and effort required to accomplish monitoring goals. We identified long-term trends and predictors of short-term change in the salamander populations in order to construct realistic models for optimization. We investigated i) the effect of the number of monitoring years on the reliability of trend detection, and ii) the effect of the number and allocation of survey replicates on the accuracy and precision of model estimates. The terrestrial salamander monitoring program used in this study uses a replicate survey design to account for imperfect detection. Surveys with imperfect detection are likely to be biased over time in their estimates of population parameters. As such, our approach to optimization expands upon previous studies by using estimates of detection probability to adjust final optimizations. We show how analyses of valuable long-term monitoring datasets, spanning almost two decades of continuous monitoring, can be used to improve the efficiency of the monitoring program, and to develop a template for other monitoring programs using similar replicate survey designs.

Methods

Monitoring Program Design

The terrestrial salamander monitoring dataset used in this study was collected by the Long Point World Biosphere Reserve (LPBR) as part of the Environmental Monitoring and Assessment Network (EMAN). The aim of EMAN is to improve understanding of changes in various Canadian ecosystems through long-term monitoring. The red-backed salamander (*Plethodon cinereus*) was the species of interest for this monitoring program. Plethodontid salamanders have been identified as important indicator species for long-term monitoring due to their high abundance in forests, high detectability, large geographic range, sensitivity to environmental disturbances, and importance in the forest food web (Welsh and Hodgson, 2013).

Two forest tracts within LPBR, Backus Woods and Wilson Forest Tract, were surveyed as part of the program. Backus is an old-growth forest while Wilson has been subject to periodic timber extraction. Both tracts are Maple-dominant Mixed Wood Carolinian Forests and have remained relatively unimpacted over the course of the monitoring program. Single square plots (10 km^2) within the interior of each forest were used for the coverboard monitoring project (Figure 5.1). Along the perimeter of each square plot, 160 artificial coverboards were placed in two parallel rows of 80 boards each. The square plots were originally set up to act as reference plots for a tree health monitoring program. Unlike the tree health monitoring program, we did not assume that salamander abundances in the square plots were representative of the entire forest tract. Coverboards were surveyed for the presence of salamanders no more than once weekly (to minimize biases due to repeated disturbance (Marsh and Goicochea, 2003)) in the spring (March to June) and fall (August to October) from 1999 to 2016 inclusive. Corresponding daily and annual precipitation and temperature data were obtained from a weather station located approximately 25 km northeast of the forest plots and gap-filled using an approach similar to Wei and McGuinness (1973).

Trend Analysis

To analyse trends in the monitoring data, we used dynamic occupancy models developed by Mackenzie et al. (2003). False negatives are common in coverboard surveys because salamanders may not be found under the coverboard at the time of the survey (temporarily buried underground or away from the coverboard foraging) even though they normally reside there. Dynamic occupancy models estimate annual occupancy using repeated measures to account for biases from imperfect detection. They allow for estimates of λ_{ij} (probability of salamander occupancy at board i in year j), p_{ijk} (probability of detection at board i, in survey k of year j, given salamanders are present), γ_{ij} (probability that unoccupied board i in year j is colonized in year j+1), and ε_{ij} (probability that occupied board i in year j becomes unoccupied in year j+1). It is important to note that multiple salamanders are often found under individual boards. For models where multiple individuals inhabit a single board, occupancy refers to the probability that at least one salamander is present during a sampling period while detection refers to the probability that at least one of the salamanders occupying the board is present at the time of the survey.

Dynamic occupancy models assume closure for board occupancy within survey periods. Since plethodontids have small home ranges and do not move more than a few meters throughout the active season (Petranka, 1998), it is unlikely that the assumption of closure would be violated during the survey period. In exceptionally wet springs, some coverboards were flooded during the start of the survey period. Flooded boards are unable to be occupied by the terrestrial salamanders, but observations of instances of flooding were originally unforeseen and not fully recorded in the dataset. Consequently, we needed to account for artificial decreases in occupancy estimates from spring flooding and developed separate models for spring and fall monitoring periods.

We used covariates in our models to reduce the number of required parameter estimates and to determine if there were any relationships between meteorological conditions and plethodontid occupancy dynamics. In dynamic occupancy models, parameter estimates of detection, initial occupancy, colonization, and local extinction are functions of covariates on the logit scale. Dynamic occupancy models allow for the inclusion of three types of covariates: site-specific covariates, survey-specific covariates, and year-specific covariates. For all parameters, we included the site-specific covariate of forest plot. For detection probability (p_{ijk}) we also included the year-specific covariate of monitoring year, and the survey-specific covariates of the presence of rain 24 hours prior to the survey, and the linear and quadratic terms for average daily temperature. For the occupancy dynamics of colonization (γ_{ij}) and local extinction (ε_{ij}), we included the year-specific covariates of annual rainfall (April – October), spring rainfall (March – June), average summer temperature (June – September), average winter temperature (December – March), and the year that monitoring was conducted (monitoring year). The spring rainfall covariate was used exclusively in the spring model as an index of flood intensity during the survey period. We ran all models using the program PRESENCE V12.10 (Hines, 2006).

For each season, the top three models based on Quasi-Akaike's Information Criterion (QAIC) values were used to create weighted averages of the parameter estimates. Since board occupancy cannot be directly estimated from the model, we used the smoothing method described by Weir, Fiske, and Royle (2009) to derive occupancy estimates for each year. The delta method was used to derive standard errors for all parameter estimates. To analyse trends in board occupancy across years, we fitted linear models for the plot-averaged occupancy estimates for each season. For each model, we included covariates for year and plot. To account for temporal autocorrelation in the occupancy estimates, we fitted our models using generalized least squares and specified a first-order correlation structure with year as the grouping variable. We used a similar strategy to analyse trends in detection probability, this time combining estimates from spring and fall into a single linear model and using year and season as covariates. For all analyses, we used an 95% level of significance to test for the importance of covariates.

Effect of Monitoring Length on Trend Detection

We simulated detection/non-detection data based on model averages to investigate the ability to detect declines in occupancy for different lengths of monitoring programs. For all simulation analyses, we used model averages from the fall data in order to mitigate any biases related to undocumented coverboard flooding in the spring. We ran simulations for declines ranging from 5% to 25% every five years and for monitoring periods ranging from 5 to 25 years. After the percent decline in occupancy was applied to each year, we allowed for additional fluctuations across years based on average year-to-year fluctuations observed in the monitoring results. We assumed that probability of colonization (γ_{ij}) and persistence ($1 - \varepsilon_{ij}$) were equal, to minimize the number of parameter estimates and to simplify calculations. Using the occupancy estimates from the simulated data, we fitted linear models with year as the covariate and recorded whether the trend estimate was significantly less than zero. We plotted the number of simulations with significant decreasing trend estimates.

Effect of Survey Replicate Allocation on Occupancy Estimates

To investigate the accuracy and precision of single-season occupancy estimates, we completed additional simulations using average detection and occupancy estimates from our models. For these simulations, we investigated the effect of the number of surveys, boards, and survey plots on the accuracy and precision of occupancy estimates. We ran simulations for a moderate degree of within-forest variability in board occupancy (similar to the variability observed in this study) and for a high degree of variability. We used 1000 simulations for each combination and plotted the average occupancy estimate and confidence bars that contained 95% of the occupancy estimates. We qualitatively compared the average estimates to the occupancy value used to create the simulated data. In all cases, we ran simulations in R (R Core Team, 2017) using the unmarked package (Fiske and Chandler, 2009).

Results

Trend Analysis

During the 18 years of monitoring used for this analysis, a total of 369 surveys were completed: 212 (mean of 11.8) in the spring and 157 (mean of 9.2) in the fall. There were 4066 salamanders identified under coverboards during this period. On average, 117 (ranging from 45 to 213) salamanders were identified under coverboards in spring each year, and 115 (ranging from 56 to 197) during the fall. Daily temperatures for the Long

Point area averaged 12°C during the survey periods (spring and fall), 19°C during the summer, and -3°C during the winter.

Detection probabilities associated with the best models (i.e. with lowest AIC scores) covaried with sampling year, forest plot, occurrence of rain in the previous day, and the linear and quadratic terms for average daily temperature. Estimates of the dispersion parameter (c-hat) for the full models were 3.5 for spring and 1.7 for fall, indicating the presence of overdispersion. Using the c-hat estimates for model selections, initial occupancy for the best spring model (i.e. with the lowest QAIC score) covaried with site. The probability of colonization covaried with amount of annual precipitation in the previous year, estimated occupancy in the previous year, and amount of spring precipitation during the survey period; the probability of extinction covaried with amount of annual precipitation in the previous year and average temperature during the previous summer (Table 5.1). By comparison, probability of colonization for the best fall model covaried with amount of annual precipitation corresponding to the current year as well as estimated occupancy in the previous year, while probability of extinction covaried with the amount of annual precipitation in the current year and the average temperature during the previous winter (Table 5.1).

Averages for the smoothed occupancy estimates across years were 0.55 ±0.02 (± SE) for the spring and 0.66 ±0.01 (± SE) for the fall. There was large variation in occupancy estimates for individual boards. For spring data, 10% of the coverboards were estimated to be occupied < 32% of the time while another 10% were estimated to be occupied > 80% of the time. Similarly, for the fall, 10% of the coverboards were estimated to be occupied < 43% of the time, while another 10% was estimated to be occupied > 90% of the time. Interannual variations in occupancy estimates were greater for spring than for fall, with average annual changes of 16% for spring versus only 6% for the fall (Figure 5.2). There were no significant trends for occupancy estimates during the spring (slope = -0.002, p = 0.88), or fall (slope = 0.011, p = 0.15).

Estimates of detection probability averaged 0.15 ±0.01 (± SE) across years for the spring and 0.16 ±0.01 (± SE) for the fall. Despite large fluctuations, mean annual estimates generally increased across years (slope = 0.046, p < 0.001; Figure 5.3). Mean estimates (B_o) and slopes (B_1) for detection probability did not significantly differ between fall and spring ($\Delta B_o = -0.10$, p = 0.41; $\Delta B_1 = 0.013$, p = 0.58). Both models appeared to show an initial increase in detection probability, followed by a decrease, and ending with another increase (Figure 5.3).

Effect of Monitoring Length on Trend Detection

Results of our analysis for trend detection indicated that a survey conducted at 5-year intervals over a period of 10 years would have sufficient power (>0.9) to detect a decline in occupancy of 20%; a survey conducted at 5-year intervals over a 15-year period would have sufficient power to determine a 10% decline, whereas a survey conducted at 5-year intervals over a 25-year period would be required to detect a 5% decline in occupancy (Figure 5.4). These correspond to declines in occupancy of 36% over 10 years, 27% over 15 years, and 23% over 25 years. A decline approaching 60% would have been required to permit detection over a short survey period of only 5 years.

Effect of Survey Replicate Allocation on Occupancy Estimates

Accuracy assessments indicated that accurate estimates of occupancy occur when survey designs include at least 10 survey sites and a combination of either 80 boards and 12 surveys or 160 boards and 8 surveys (Figure 5.5). Increasing the number of boards or surveys past those values tends to have small effects on the precision of occupancy estimates. On the other hand, increases in the number of survey sites tended to have a more pronounced effect on the precision of occupancy estimates. Low numbers of boards, surveys, or sites, regardless of the magnitude of the other two parameters, tended to result in occupancy estimates with low accuracy and precision. Changes in the variability of occupancy estimates within the forest had little impact on the precision of occupancy estimates, and while accuracy was slightly different between treatments, this difference may be more related to the difficulty in estimating the central tendency of occupancy data.

Discussion

While occupancy fluctuated across years, our results did not show any significant decrease or increase in abundance through time, suggesting that populations in both plots have been stable over the monitoring period. Short-term fluctuations in occupancy appeared to be influenced by changes in annual meteorological conditions, supporting the role of moisture in plethodontid abundance (Warren and Bradford, 2010; Grover, 1998). The original EMAN protocol for using salamander abundance as an index of environmental change recommended that "relative changes in abundance….be determined after a few years of sampling" (Zorn, Blazeski, and Craig, 2004; Environment Canada, 2003). Given the observed large annual fluctuations in occupancy in a relatively undisturbed population, it would be unrealistic to expect abundances monitored over a few years to yield meaningful trends. For example, in our simulations a program with fewer than five years of monitoring data would not be able to reliably detect the presence of a declining trend unless the population had been reduced by an unrealistically high rate of 60%. More realistic loss rates of 5 to 10% would require 15 to 25 years of monitoring. For this program and similar programs, we advise against conducting trend analysis with datasets <10 years, since results may lead to incorrect conclusions on the status of the population.

Such a lack of statistical power in short-term trend analysis has been identified for other types of monitoring programs and indicator species (Erb et al., 2015; Nielsen et al., 2009; Helander, Bignert and Asplund, 2008; Meyer et al., 2010). At short timescales, the natural variation in the population parameters of interest tends to mask other, long-term changes that the system is experiencing. This has been shown more generally by White (2019) in their analysis of vertebrate data from 822 different populations. They found that roughly three quarters of the populations they studied required 10 years of continuous monitoring data for trends to be reliably detected. Our results provide further justification for the need for >10 years of monitoring and demonstrate the ability to determine program-specific thresholds for trend detection. Despite the pattern of unreliability for trend detection in short-term monitoring programs, there is considerable variation in the minimum time required for trend detection across monitoring programs (White, 2019). As such, being able to conduct power analyses based on the specific characteristics of the monitoring system and chosen statistical analyses can provide more accurate estimates of the minimum number of years, thus saving time and resources.

The quality of occupancy estimates needs to be considered alongside the number of monitoring years in order to conduct reliable trend analyses. In this study, large fluctuations in detection probability across years and surveys highlight the importance of a replicate survey design for adjusting occupancy estimates. We show that moderate numbers of spatial replicates (coverboards and survey sites) and temporal replicates (surveys) are required to accurately and precisely estimate occupancy. For example, a minimum of 160 boards split among 10 survey sites with 8 surveys per season is recommended for the monitoring program used in this case study. Low numbers of boards, surveys, or sites tend to result in estimates with low accuracy and precision regardless of the magnitude of the other two replicates. Our results suggest that effort should be partitioned across the monitoring parameters of surveys, boards, and sites to avoid the disproportionate effects associated with having a small number of any of these replicates.

Consideration must also be given to the limitations of the monitoring program when optimizing for design. For example, the number of surveys in any given year for the salamander monitoring program is limited by the number of weeks when salamanders are active. Since the number of appropriate weeks can change from year to year, a conservative approach should be taken when planning how many surveys will be conducted. Similarly, though distributing boards across a greater number of survey sites can improve the precision of occupancy estimates, it comes at a cost of increased sampling time.

How one partitions survey effort across temporal and spatial replicates is dependent on the dynamics of the populations used in the monitoring system, the environmental variation and the magnitude of observation error (Rhodes and Jonzen, 2011). Estimates of population parameters for populations with proportionally high spatial correlation will benefit from program designs with a larger emphasis on temporal replicates, and vice versa for populations with proportionally higher temporal correlation (Rhodes and Jonzen, 2011). Due to the dependency of plethodontid salamanders on specific microhabitats (Petranka, 1998), the monitoring program in this case study benefits more from high spatial replication of surveys. However, sufficient temporal replicates are necessary to offset low detection probabilities and minimize the errors associated with uncorrected occupancy estimates (Field, Tyre and Possingham, 2005).

Short-term data, in particular, is important for determining the dynamics of monitoring systems. As more information is gained on the monitoring system, program designs can be adjusted for high or low spatial and temporal correlation in populations. Even if the species of interest is well known, populations in different geographic regions can have different dynamics leading to different optimal survey designs (Petranka, 1998). These adjustments are a key aspect of adaptive monitoring (Lindenmayer and Likens, 2009) and, together with the refinement of program goals and questions, will be a necessary part of monitoring program development going forward.

Our research continues to address important questions related to how monitoring effort should be allocated to optimize the accuracy of parameter estimates and reliability of trend analyses. Researchers have pointed to the importance of considering monitoring program cost and effort in the context of program goals (McDonald-Madden et al., 2010; Reynolds, Thompson and Russell, 2011; Caughlan and Oakley, 2001). Given the small amount of resources allocated to conservation programs, optimizations for program design are increasingly being recognized as integral to the development and continued improvement of monitoring programs (McDonald-Madden et al., 2010). In particular, we have highlighted optimizations in the context of dynamic occupancy models, which represent a relatively recent development for long-term monitoring programs. We have shown that using dynamic occupancy models to account for detection probabilities in replicate survey designs can be important not only for trend analysis, but for the improvement of monitoring program design. We believe that a similar framework to the one used in this study can be applied to other monitoring programs that use replicate survey designs. There is considerable potential for future monitoring based on the infrastructure that has been created through long-term monitoring programs. Using these suggestions, monitoring programs can continue to provide the scientific community with extensive datasets that would otherwise be difficult to collect for researchers limited by short-term funding.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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References

Caughlan L and Oakley KL. (2001). Cost considerations for long-term ecological monitoring. *Ecological Indicators*, 1(2), 123–134.

Environment Canada. (2003) EMAN: monitoring biodiversity in Canadian forests. Resource document. Environment Canada. http://publications.gc.ca/site/eng/462829/publication.html

Erb LA, Willey LL, Johnson LM, Hines JE, Cook RP. (2015). Detecting long-term population trends for an elusive reptile species. *J Wildl Manage*, 79(7), 1062–1071.

Field SA, O'Connor PJ, Tyre AJ, and Possingham HP. (2007). Making monitoring meaningful. *Austral Ecology*, 32(5), 485–491.

Field SA, Tyre AJ, and Possingham HP. (2005). Optimizing allocation of monitoring effort under economic and observational constraints. *Journal of Wildlife Management*, 69(2), 473–482.

Fiske IJ, Chandler R. (2009). unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. *Journal of Statistical Software*, 43(10), 23.

Grover MC. (1998). Influence of cover and moisture on abundances of the terrestrial salamanders *Plethodon cinereus* and *Plethodon glutinosus*. *J Herpetol*, 32(4), 489–497.

Helander B, Bignert A, and Asplund L. (2008). Using Raptors as Environmental Sentinels: Monitoring the White-tailed Sea Eagle Haliaeetus albicilla in Sweden. *AMBIO: A Journal of the Human Environment*, 37(6), 425–431.

Hines JE. (2006). PRESENCE- Software to estimate patch occupancy and related parameters. Version 12.10 [software]. USGS-PWRC. http://www.mbr-pwrc.usgs.gov/software/presence.html.

Kéry M, Dorazio RM, Soldaat L, Van Strien A, Zuiderwijk A, Royle JA. (2009). Trend estimation in populations with imperfect detection. *J Appl Ecol*, 46(6), 1163–1172.

Lindenmayer DB, Likens GE. (2009). Adaptive monitoring: a new paradigm for long-term research and monitoring. *Trends Ecol Evol*, 24(9), 482–486.

Lindenmayer DB, Likens GE, Andersen A, Bowman D, Bull CM, Burns E, Dickman CR, Hoffmann AA, Keith DA, Liddell MJ, Lowe AJ, Metcalfe DJ, Phinn SR, Russell-Smith J, Thurgate N, And Wardle GM. (2012). Value of long-term ecological studies. *Austral Ecology*, 37(7), 745–757.

MacKenzie DI, Nichols JD, Hines JE, Knutson MG, Franklin AB. (2003). Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology*, 84(8), 2200–2207.

MacKenzie DI and Royle JA. (2005). Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology*, 42(6), 1105–1114.

Magurran AE, Baillie SR, Buckland ST, Dick JM, Elston DA, Scott EM, et al. (2010). Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. *Trends Ecol Evol*, 25(10), 574–582.

Marsh DM, Goicochea MA. (2003). Monitoring terrestrial salamanders: biases caused by intense sampling and choice of cover objects. *J Herpetol*, 37(3), 460–466.

McDonald-Madden E, Baxter PWJ, Fuller RA, Martin TG, Game ET, Montambault J, and Possingham HP. (2010). Monitoring does not always count. *Trends in Ecology & Evolution*, 25(10), 547–550.

McMahon SM, Harrison SP, Armbruster WS, Bartlein PJ, Beale CM, Edwards ME, Kattge J, Midgley G, Morin X, and Prentice IC. (2011). Improving assessment and modelling of climate change impacts on global terrestrial biodiversity. *Trends in Ecology & Evolution*, 26(5), 249–259.

Meyer CFJ, Aguiar LMS, Aguirre LF, Baumgarten J, Clarke FM, Cosson J-F, Villegas SE, Fahr J, Faria D, Furey N, Henry M, Hodgkison R, Jenkins RKB, Jung KG, Kingston T, Kunz TH, MacSwiney Gonzalez MC, Moya I, Pons J-M, Racey PA, Rex K, Sampaio EM, Stoner KE, Voigt CC, Staden D von, Weise CD, and Kalko EKV..

(2010). Long-term monitoring of tropical bats for anthropogenic impact assessment: Gauging the statistical power to detect population change. *Biological Conservation*, 143(11), 2797–2807.

Nielsen SE, Haughland DL, Bayne E, Schieck J. (2009). Capacity of large-scale, long-term biodiversity monitoring programmes to detect trends in species prevalence. *Biodivers Conserv*, 18(11), 2961–2978.

Petranka, JW. (1998). *Salamanders of the United States and Canada.* Washington (DC): Smithsonian Institution Press.

R Core Team. (2017). R: A language and environment for statistical computing [software]. R Foundation for Statistical Computing. https://www.R-project.org/.

Reynolds JH, Thompson WL, and Russell B. (2011). Planning for success: Identifying effective and efficient survey designs for monitoring. *Biological Conservation*, 144(5), 1278–1284.

Rhodes JR and Jonzén N. (2011). Monitoring temporal trends in spatially structured populations: how should sampling effort be allocated between space and time? *Ecography*, 34(6), 1040–1048.

Urban MC, Bocedi G, Hendry AP, Mihoub J.-B., Pe'er G, Singer A, Bridle JR, Crozier LG, De Meester L, Godsoe W, Gonzalez A, Hellmann JJ, Holt RD, Huth A, Johst K, Krug CB, Leadley PW, Palmer SCF, Pantel JH, Schmitz A, Zollner PA and Travis JMJ (2016). Improving the forecast for biodiversity under climate change. *Science*, 353(6304), aad8466.

Warren II RJ, Bradford MA. (2010). Seasonal climate trends, the North Atlantic Oscillation, and salamander abundance in the southern Appalachian Mountain region. *J Appl Meteorol Climatol*, 49, 1597–1603.

Wei TC and McGuinness JL. (1973). Reciprocal Distance Squared Method, A Computer Technique for Estimating Area Precipitation. Technical Report ARS-Nc-8. US Agricultural Research Service, North Central Region, Ohio. https://archive.org/stream/reciprocaldistan08weit/reciprocaldistan08weit_djvu.txt

Weir L, Fiske IJ, Royle JA. (2009). Trends in anuran occupancy from northeastern states of the North American Amphibian Monitoring Program. *Herpetol Conserv Biol*, 4(3), 389–402.

Welsh HH, Hodgson GR. (2013). Woodland salamanders as metrics of forest ecosystem recovery: a case study from California's redwoods. *Ecosphere*, 4(5), 25.

White ER. (2019). Minimum Time Required to Detect Population Trends: The Need for Long-Term Monitoring Programs. *BioScience*, 69(1), 40–46.

Zorn P, Blazeski V, Craig B. (2004). Joint EMAN/Parks Canada national monitoring program for Plethodontid salamanders. Resource document. Environment Canada. http://publications.gc.ca/site/eng/462270/publication.html

Table 5.1: Quasi-Akaike's Information Criterion values (QAIC) for the top three ranked models, the full model, and the null model for each season. QAIC values are calculated using the dispersion parameter (c-hat) from the full model. The top three models are used to calculate weighted averages of the model parameters. The covariates for initial occupancy (ψ), colonization (γ), extinction (ε), and detection probability (*p*) are listed in brackets. (w) model weights; (K) number of parameters

Spring			
Model	**QAIC**	**w**	**K**
ψ(site), γ(annual precip., previous occ., spring precip.), ε(annual precip., summer temp.), *p*(year, site, rain, temp., temp.^{2})	3386.97	0.45	31
ψ(site), γ(annual precip., winter temp., previous occ.), ε(annual precip., summer temp.), *p*(year, site, rain, temp., temp.^{2})	3387.66	0.31	31
ψ(site), γ(annual precip., previous occ.), ε(annual precip., summer temp.), *p*(year, site, rain, temp., temp.^{2})	3388.19	0.24	32
ψ(site), γ(year), ε(year), *p*(*model*)	3431.19	0	58
ψ(), γ(), ε(),*p*(year, site, rain, temp., temp.^{2})	3389.60	0	25
Fall			
Model	**QAIC**	**w**	**K**
ψ(), γ(annual precip., previous occ.), ε(annual precip., winter temp.), *p*(year, site, rain, temp., temp.^{2})	6319.17	0.42	28
ψ(), γ(annual precip., previous occ.), ε(annual precip.), *p*(year, site, rain, temp., temp.^{2})	6319.87	0.38	27
ψ(site), γ(previous occ.), ε(annual precip., winter temp.), *p*(year, site, rain, temp., temp.^{2})	6321.20	0.20	27
ψ(site), γ(year), ε(year), *p*(year, site, rain, temp., temp.^{2})	6358.22	0	55
ψ(), γ(), ε(),*p*(year, site, rain, temp., temp.^{2})	6335.99	0	24

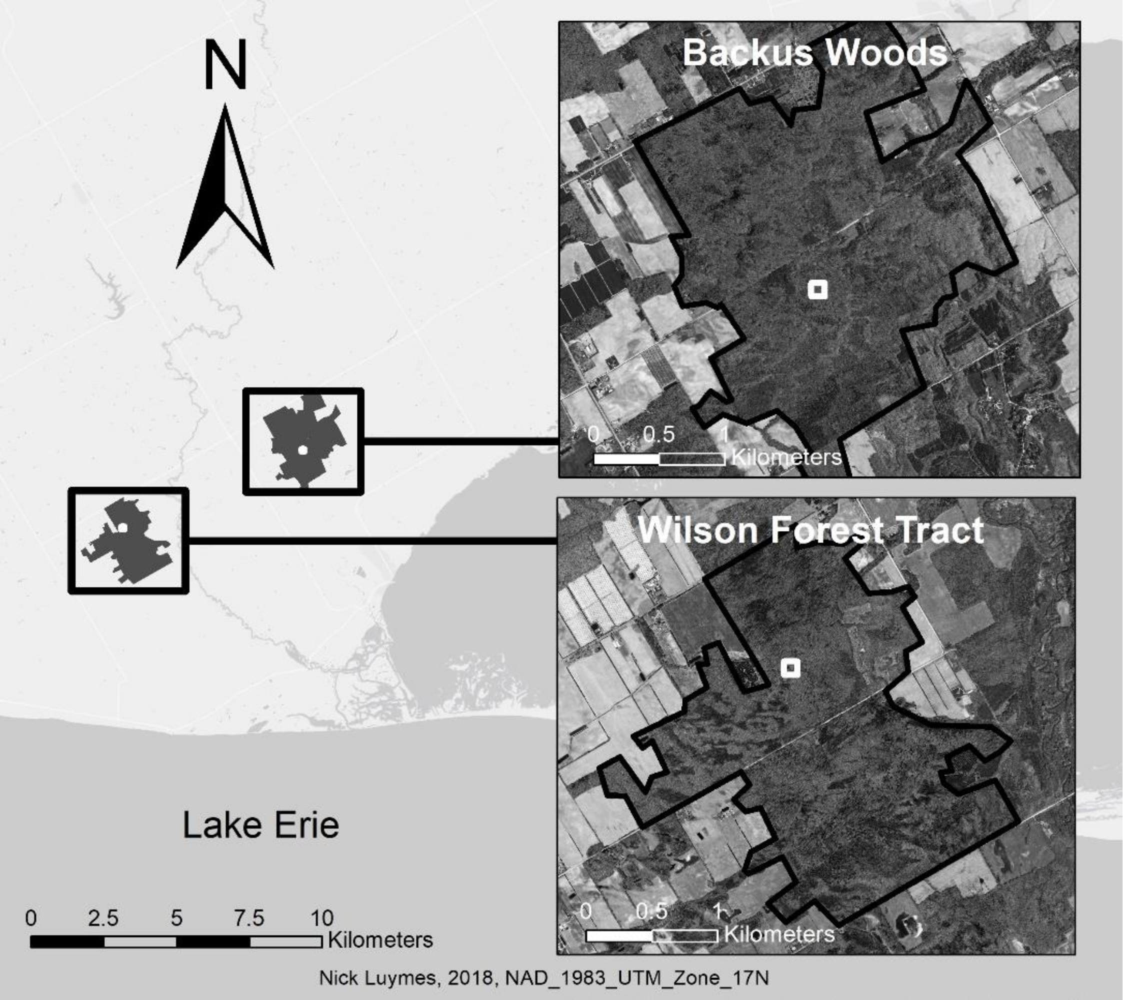

Figure 5.1: Location of the EMAN plots. White squares represent the locations of EMAN plots used for the salamander monitoring program within Backus Woods and Wilson Forest Tract. Satellite imagery source: Ontario Ministry of Natural Resources and Forestry, South Western Orthophotography Project. Basemap source: © OpenStreetMap contributors

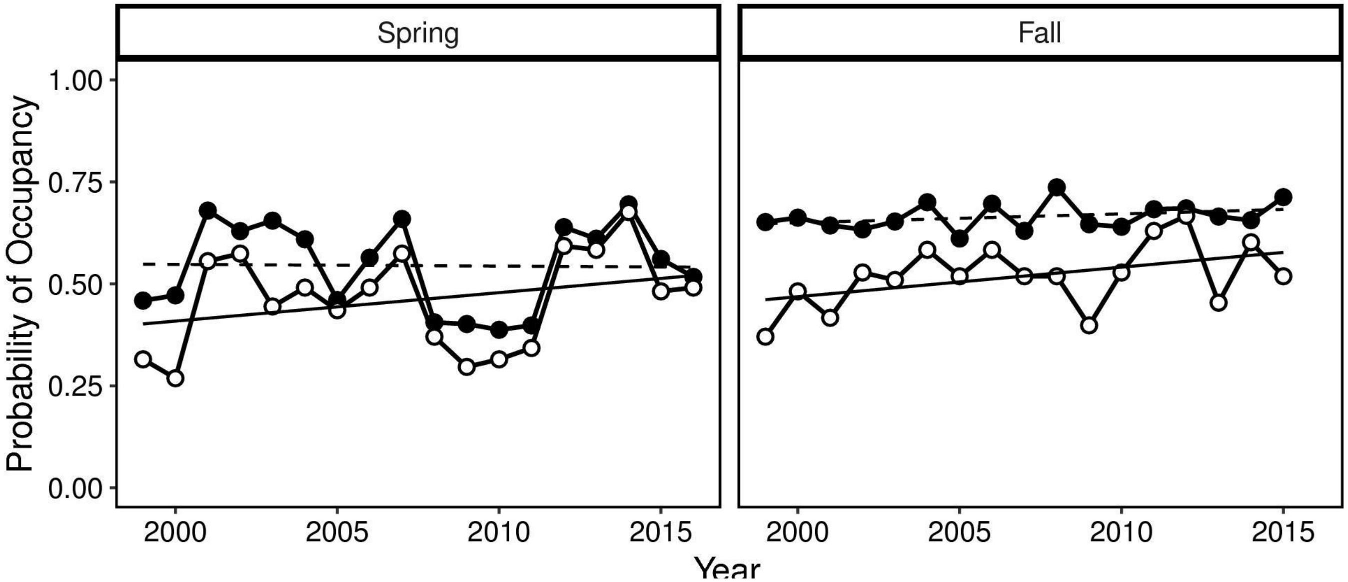

Figure 5.2: Smoothed model estimates of the probability of occupancy for each season. Seasonal occupancy estimates for modelled (closed circles) and raw (open circles) estimates of both sites combined. Naïve estimates are based off the raw data and do not account for detection probabilities. Linear trendlines for modelled (solid lines) and raw (dashed lines) estimates are included for to highlight the absence of a long-term trend in occupancy

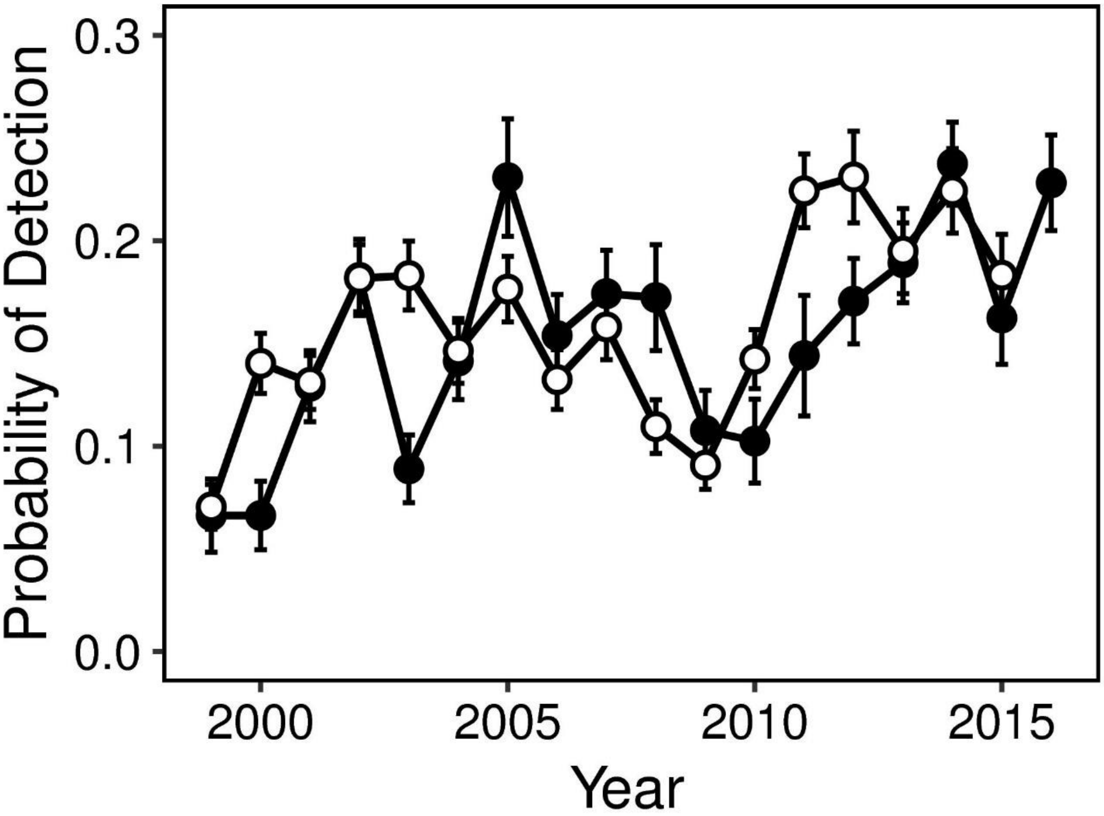

Figure 5.3: Estimated probability of detection for spring (closed circles) and fall (open circles) of each year of the monitoring program

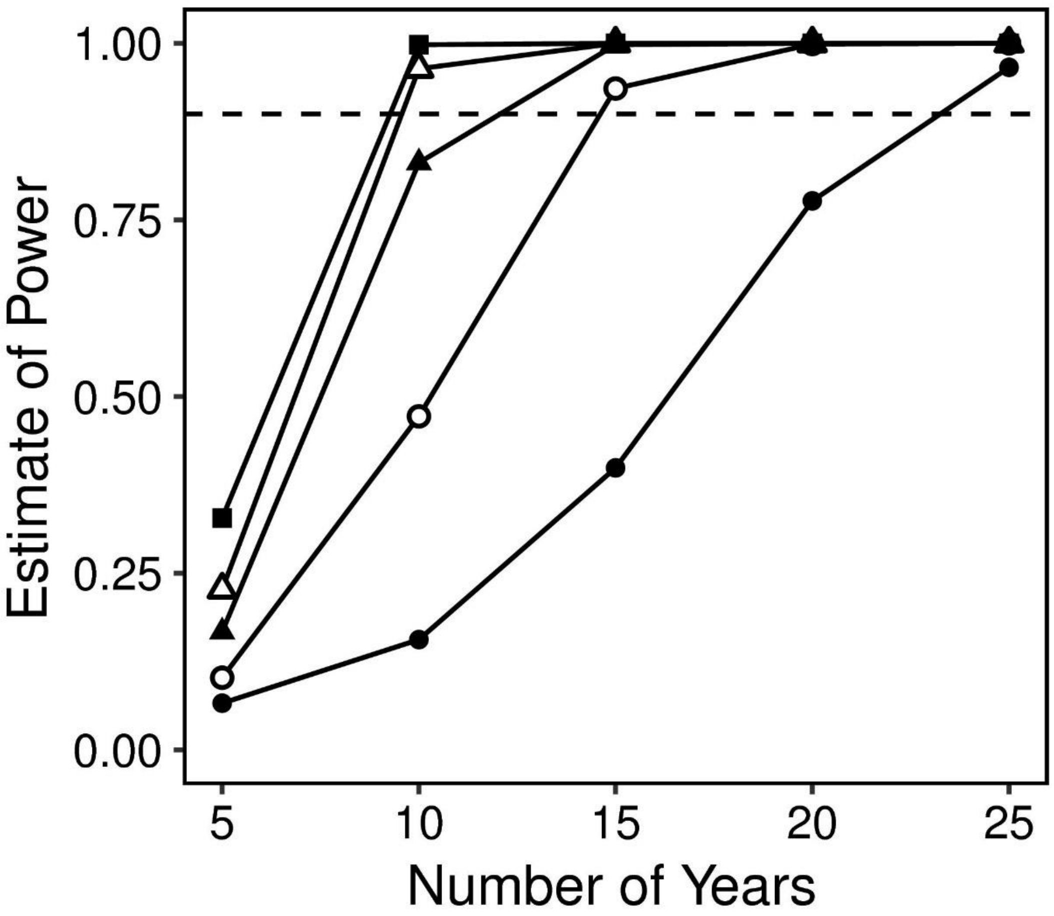

Figure 5.4: Estimated power to detect declines in coverboard occupancy. Simulations for declines of 0.05 (closed circles), 0.10 (open circles), 0.15 (closed triangles), 0.20 (open triangles), and 0.25 (squares) every 5 years. Significance of the estimated slopes was assessed using an alpha of 0.05. The dotted horizontal line represents the chosen threshold for sufficient power (≥0.9). 1000 simulations were run for each unique combination.

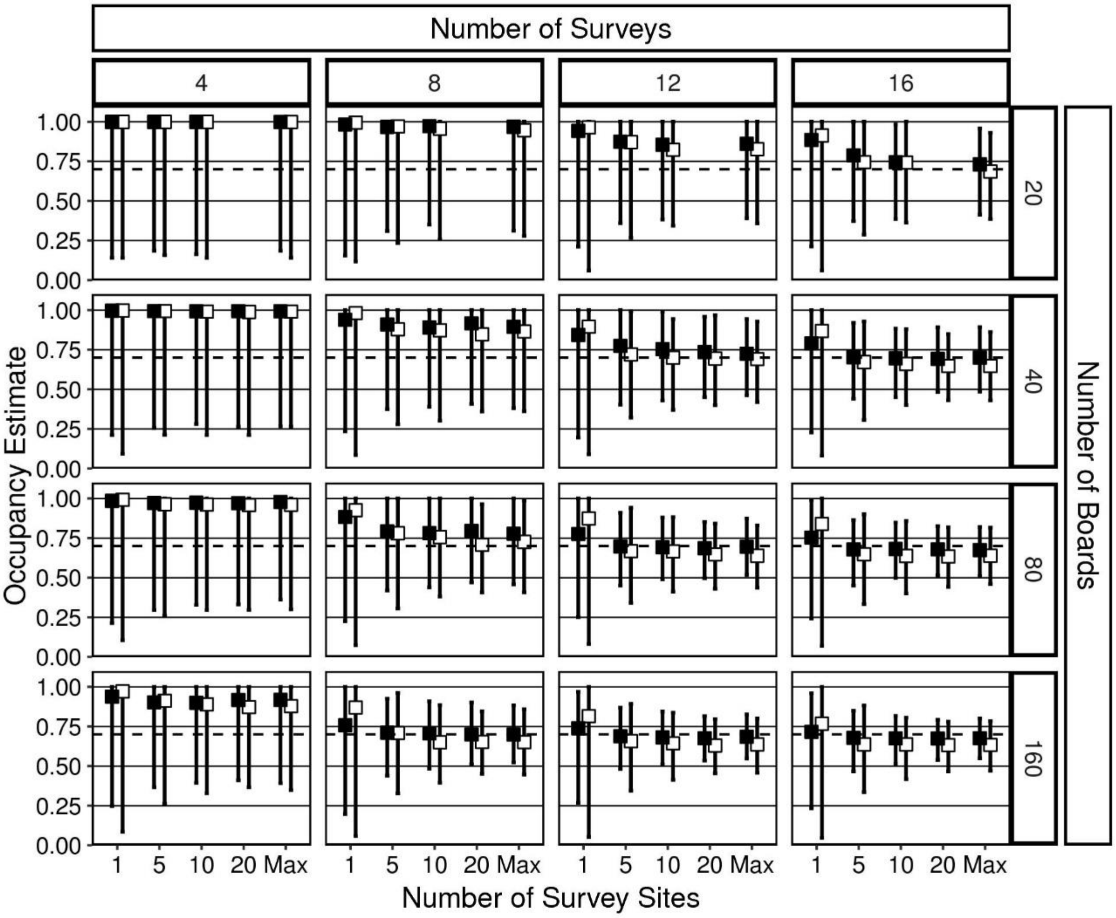

Figure 5.5: Mean and 95% CI of single-year occupancy estimates based on simulated coverboard occupancy data. Simulations for a range of boards, surveys, and survey sites. The closed squares represent averages for simulations run with a moderate degree of within-forest variability in occupancy, whereas the open squares represent averages for simulations run with a high degree of variability. The maximum number of survey sites condition refers to simulations run with a survey site for every board. We omitted simulations with 20 boards and 20 survey sites to avoid repetition. 1000 simulations were run for each unique board-survey-site combination. The dotted line represents the true average occupancy value (0.7) for the forest.

CHAPTER 6: GENERAL DISCUSSION

The primary goals of this thesis were to improve the understanding of amphibian habitat use in Ontario forests and develop tools and techniques to aid in the identification of habitat and monitoring of species. These goals were formulated with the intent to address the conservation needs for amphibians in different forested regions of Ontario, particularly in the face of regional changes in land-use and climate. In Chapter 2, we created and implemented a technique to map undocumented vernal pool breeding habitat in eastern Georgian Bay, and in Chapter 3, we evaluated the species-habitat relationships for amphibian communities in these pools. In Chapter 4 and Chapter 5, we explored the utility of large, but biased, species occurrence datasets for addressing conservation issues related to the distribution of critical amphibian habitat and the effectiveness of long-term monitoring.

Vernal pools serve as key breeding habitat for a number of amphibian species, but little is known of their distribution in Ontario, particularly for relatively intact forested regions. For Chapter 2, we combined common machine learning techniques with object-based image analysis to map potential vernal pools in the intact forests of southeastern Georgian Bay. Despite difficulty differentiating between small pools and dark shadows, our approach provided a relatively accurate and fast strategy for mapping vernal pools when compared to conventional strategies, such as manual photointerpretation. Our use of readily available remote sensing datasets, including provincial orthoimagery and digital elevation models, should allow for this technique to be calibrated to a variety of forested regions across Ontario. When used on a regional scale, these models can be combined with more in-depth field studies to verify and evaluate the presence of particular amphibian communities.

The mixed forests of central Ontario support a unique combination of Ontario's amphibians, but little is known about the environmental characteristics that drive differences in habitat use across the landscape, particularly for species that use vernal pools as breeding habitat. In Chapter 3, we surveyed amphibian larvae and collected observations of habitat qualities for vernal pools in the eastern Georgian Bay region to investigate drivers of changes in amphibian communities and species abundances. We found that amphibian community composition was influenced by differences in hydroperiod length and canopy openness, and that these differences were driven by increases in the abundance of spring peepers (*Pseudocris crucifer*) in pools with long hydroperiods and high canopy openness. For early-breeding vernal pool amphibians, including *Ambystoma* salamanders and wood frogs (*Lithobates sylvaticus*), abundance was primarily influenced by the availability of vegetation communities. These relationships are important for beginning to understand the diversity and functions of vernal pools in eastern Georgian Bay and can be used to guide species- and community-specific habitat management for a region under considerable development pressure.

The identification of critical habitat is an important management goal for at-risk amphibians in Ontario. In Chapter 4, we mapped suitable habitat for the endangered Jefferson salamander (*Ambystoma jeffersonianum*) by applying species distribution models to a long-term occurrence dataset. We demonstrated the use of a variety of techniques to account for inherent sampling biases in the occurrence data, including data thinning, subsampling, and pseudo-absence selection. We found that suitable habitat for Jefferson salamanders consisted of large deciduous/mixed forests with complex topographies, potentially due to the ability of these forests to support overwintering habitat in forest uplands and breeding wetland habitat in landscape depressions. Habitat suitability maps based on our models can help identify new populations of Jefferson salamanders in Ontario, especially in regions that are targeted as part of the provincial recovery strategies.

Monitoring is an important component of many conservation initiatives, both for evaluating the success of management programs and assessing the stability of populations. In Chapter 5, we used dynamic occupancy models to evaluate trends and suggest improvements for a long-term terrestrial salamander monitoring program. We identified several key areas for enhancing the monitoring program design, including revised expectations for the minimum number of years required for trend analysis and improved partitioning of effort across spatial and temporal scales. We also stressed the importance of hypothesis-driven short-term monitoring goals for contributing to other aspects of amphibian conservation. The optimization techniques used in this study can be generalized to other amphibian monitoring programs and can contribute to more efficient use of available resources and more focussed monitoring goals.

In summary, this thesis addresses gaps in our knowledge of amphibian-habitat relationships in Ontario and provides techniques to guide amphibian conservation efforts, including tools for mapping critical habitat and strategies to improve the ways we design and implement monitoring programs. Given widespread declines of amphibians and the important contributions they make to forest ecology, medicine, and culture (Hocking and Babbitt, 2014), the protection of these species is vital, especially as changes in climate and land-use continue to negatively affect the availability of their habitat. The recommendations and suggested future research described herein expand the current scope of amphibian management in Ontario and provide direction for continued improvement to our understanding of the requirements of these species across the province.

Recommendations

1. Ontario's wetland distribution data are spread across multiple datasets and do not contain comprehensive information about wetland type or function. Vernal pools are also poorly represented in these datasets, notably for central Ontario but also for many forests in southern Ontario. In order to better understand the amount of available

wetland resources in the province, we should prioritize the consolidation of existing information and the identification of undocumented wetlands. This could involve creating a centralized location to gather information from groups using citizen science to map wetlands, such as Ontario Nature; from conservation organizations and municipalities with detailed inventories of wetland resources on their lands; or from mapping projects, such as the one presented in this thesis.

2. Under Ontario's current system for wetland evaluation, it is exceedingly unlikely for vernal pools to be considered provincially significant wetlands. Their small size, homogenous vegetation communities, and ephemeral nature contribute to low evaluation scores (Ontario Ministry of Natural Resources and Forestry, 2014). Historically, the definition of "wetland" in the Conservation Authorities Act (CAA) has omitted vernal pools that lack hydric soils, water tolerant plants, and connected watercourses. Throughout our research, we have encountered many vernal pools that do not meet this definition but still provide habitat for amphibians. As of the latest revision to the CAA, this definition is now at the discretion of the Minister, meaning there is even greater confusion over what is considered a wetland. We strongly advise that wetland legislation be updated to address the value of vernal pools. In particular, we need a more inclusive definition of wetlands. This definition should focus more on the ecosystem functions that are shared among wetlands, including the provision of unique habitat and the diversion, collection, and storage of water. We also believe that

the current wetland evaluation system should be revised to incorporate the importance of groups of wetlands. For example, vernal pools may not make large individual contributions to the local success of amphibians, but the contributions of multiple neighbouring vernal pools and wetlands can be substantial. This example can be extended to other wetland functions including energy production and transfer and water diversion and storage. Future conservation efforts may benefit from a strategy that focusses on the delineation and protection of provincially significant wetland groups or regions.

3. Sustainable forestry practices have become an important part of forest management, but strategies should consider the influence that forest thinning (or lack-thereof) can have on canopy openness and the composition of vernal pool communities (deMaynadier and Hunter, 1995). Similarly, if vernal pools (or groups of pools) are granted protection from development, strategies to conserve amphibian communities in these pools should consider preservation of the pool basin, the surrounding upland forest habitat, the amount of light penetration, and drainage basin hydrology.
4. Despite the widespread and accessible nature of vernal pools, public awareness is relatively low compared to larger waterbodies. In addition, public perception is often limited to their notoriety as mosquito breeding grounds. Municipalities and conservation groups should work to improve public perception of these wetlands,

especially for pools that are frequently encountered on trails or forest edges. Interactive signs that educate the public on the benefits of vernal pools and warn of their sensitivity to disturbance would be an important first step. If shoreline disturbance can be kept to a minimum, we also recommend the use of vernal pools as educational resources. The large diversity and density of invertebrate, amphibian, and plant life rivals that of many larger wetlands and can be easily viewed from pool edges. In addition, many important topics from biology and environmental science are clearly represented in vernal pools, including metamorphosis, disturbance regimes, food webs, photosynthesis, and water balance. Stewardship initiatives, similar to those used for urban streams and marshes, could also be applied to vernal pools to increase public support and awareness. Stewardship activities could involve collecting water chemistry measures, carrying out shoreline surveys of egg mass abundance, and measuring changes in pool size throughout the spring and summer.

5. The habitat suitability models from this thesis should not be the final verdict as to whether a forest is considered critical habitat for Jefferson salamanders. We recommend the use of surveys to identify new populations of Jefferson salamanders within the Greenbelt region using our habitat suitability maps. These surveys should focus on forests that have high suitability estimates and exhibit signs of wetland availability. A better understanding of the distribution of Jefferson salamanders will

ensure more habitat is protected and will improve our knowledge of the status of this endangered species across the province.

6. Existing amphibian monitoring programs in the province should be regularly re-examined to identify areas where efficiency can be improved and to assess whether monitoring goals can be achieved under current designs. We also recommend the practice of adapting monitoring designs when necessary. Given the considerable lengths of time that are often required for accurate trend detection, we encourage the incorporation of hypothesis-driven research questions into monitoring designs to make use of short-term data.
7. Coverboards and call surveys are commonly used for amphibian monitoring in Ontario but are less applicable for vernal pool amphibians. We recommend the use of egg mass surveys as a non-invasive, easy to implement monitoring technique for vernal pool breeding amphibians (i.e. *Ambystoma* salamanders and wood frogs; Grant et al., 2005). These surveys can be carried out annually by volunteers to estimate breeding population size. Data from these surveys can complement existing amphibian monitoring programs to provide a more complete picture of amphibian population trends and spatial patterns.

Future work

1. LiDAR data have been shown to be effective for vernal pool mapping, particularly through the use of LiDAR-derived digital elevation models (DEMs; Julian et al. 2009; Leonard et al. 2012; Riley et al. 2017; Wu et al. 2014). LiDAR-derived DEMs have higher resolution and precision compared to the DEMs used in our study. Though LiDAR was not widespread in Ontario at the time of our study, the coverage is steadily increasing in the province and future vernal pool mapping efforts should use LiDAR data to delineate the locations of landscape depressions. In addition to providing greater spatial precision and reducing the influence of tree shadows, LiDAR-derived DEMs can provide more information on pool size and shape. LiDAR intensity data can also serve as another indicator of the presence of water on the landscape. The effectiveness of an object-based, machine learning approach to map vernal pools using LiDAR data has not been evaluated in the literature, so this study would advance our knowledge on the utility of these combined technologies and potentially provide an even better approach to detect these elusive wetlands.
2. We focussed on vernal pools for our surveys of amphibian larvae because these wetland communities had been poorly represented in eastern Georgian Bay. As a result of this focused approach, we only identified a subset of the amphibians that are known to breed in forested wetlands. Past studies have found more pronounced

effects of wetland hydroperiod, surface area, and canopy cover when incorporating the full range of forested wetlands (Babbitt, Baber, and Tarr, 2003; Werner et al., 2007; Semlitsch et al., 2015). Future studies in eastern Georgian Bay should incorporate a wider range of wetlands, including beaver ponds, thicket swamps, and marshes, so that differences in amphibian communities can be investigated across larger environmental gradients. Given the large interannual fluctuations in larval abundance and pool hydroperiods, we also suggest that future studies conduct surveys for a greater number of years. Ideally, power analyses should be used to estimate the number of years that will be required to achieve the level of accuracy that is required for the study. The larvae data we have collected can be used to parameterize initial power analyses, and these analyses can be revisited once more data is collected.

3. There are a variety of techniques used to survey populations of pool-breeding amphibians, including eDNA, egg mass, dip-net, bucket-trap, pit-fall trap, drift fence, and call surveys. Though these techniques are used for different reasons, there have been few studies empirically comparing the pros and cons. In order to better understand the applicability of different techniques to different scenarios, future studies should compare techniques across a range of relevant parameters, including required survey time and effort, difficulty of the technique, estimated cost and resources, degree of wetland disturbance, and accuracy and precision of collected

data. This information will be critical for the development of amphibian monitoring programs and will help establish the value of emerging technologies like eDNA.

4. Hydroperiods naturally fluctuate from year to year as a result of fluctuations in precipitation and temperature (Brooks, 2004). Uncharacteristically hot and dry springs can cause pools to dry earlier, while wet and cold springs can lead to standing water being available for a longer period of time. Under current climate change scenarios, temperate forests in northeastern North America are expected to have more intense storms separated by greater periods without rain. Combined with increases in average spring temperatures, these changes are expected to decrease average vernal pool hydroperiods and lead to more variability across years (Brooks, 2009). In order to precisely understand how hydroperiods are going to be affected by changes in climate, we first need better methods of estimating hydroperiods for large datasets of vernal pools. Future studies should make use of the increasing availability of high-resolution remote sensing data to estimate changes in hydroperiods across years. Satellite imagery with high repeat rates (e.g. LANDSAT, Sentinel) can be used to estimate changes in the relative size of wetlands across the spring and summer (e.g. Kordelas et al., 2018; Huang et al., 2018). If enough of these data can be collected across multiple years, the rate of change in pool area can then be compared with precipitation and temperature data to create statistical models for vernal pool hydroperiods.

5. Some historical locations of Jefferson salamander populations have not been revisited in over 30 years. Given the known sensitivity of this species to changes in land-use, confirmation of occupancy at these sites should be a priority. The results from these surveys can be compared with knowledge of land-use changes and other habitat alterations to refine our understanding of the major threats to this species.

6. It would be interesting to compare vernal pools in south and central Ontario because differences in climate, land cover, surficial geology, and forest types may lead to considerable variation in vernal pool conditions and in the composition of amphibian communities. For example, the dominant surficial geology of each region (bedrock vs. glacial deposits) may affect the relative influence of precipitation and groundwater on vernal pool hydroperiods (Rheinhardt and Hollands, 2008). We have also observed differences in water chemistry (e.g. pH, conductivity, total phosphorus), vegetation communities, and pool morphology between pools in eastern Georgian Bay and pools in old growth Carolinian forests of southern Ontario. Comparative studies may tell us more about the adaptability of Ontario's amphibians to a wide range of environmental conditions.

7. The timing and frequency of spring-thaw cycles represents a potential source of variation in our species-habitat relationship models that we were unable to account for in our study. Timings for spring emergence and breeding differ between species and

within populations. Groups that breed early risk freezing temperatures that could kill adults and/or reduce the success of embryos (Hocking, 1980). Unusually cold springs could also force some individuals to skip the breeding season (Woodward, 1982). The threat of freezing in spring could also depend on the characteristics of the vernal pool, such as latitude, elevation, and sunlight exposure. To account for this potential source of variation between pools and years, future studies could incorporate measures of ice-off timing and freeze-thaw occurrence into models using camera setups and temperature loggers. Breeding effort could also be monitored during the spring through the use of repeated egg mass surveys.

References

Babbitt KJ, Baber MJ, and Tarr TL. (2003). Patterns of larval amphibian distribution along a wetland hydroperiod gradient. *Canadian Journal of Zoology*, *81*(9), 1539–1552.

Brooks RT. (2009). Potential impacts of global climate change on the hydrology and ecology of ephemeral freshwater systems of the forests of the northeastern United States. *Climatic Change*, *95*, 469–483.

Brooks RT. (2004). Weather-related effects on woodland vernal pool hydrology and hydroperiod. *The Society of Wetland Scientists*, *24*(1), 104–114.

deMaynadier PG and Hunter ML. (1999). Relationship between forest management and amphibian ecology: a review of the North American literature. *NCASI Technical Bulletin*, *3*(781 I), 129.

Grant EHC, Jung RE, Nichols JD, and Hines JE. (2005). Double-observer approach to estimating egg mass abundance of pool-breeding amphibians. *Wetlands Ecology and Management*, *13*(3), 305–320.

Harris RN. (1980). The consequences of within-year timing of breeding in *Ambystoma maculatum*. *Copeia*, *1980*(4), 719.

Hocking DJ, Babbitt KJ, and Hocking DJ. (2014). Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology*, *9*(1), 1–17.

Huang W, DeVries B, Huang C, Lang MW, Jones JW, Creed IF, and Carroll ML. (2018). Automated extraction of surface water extent from Sentinel-1 data. *Remote Sensing*, *10*(5), 797.

Julian JT, Young JA, Jones JW, Snyder CD, and Wright CW. (2009). The use of local indicators of spatial association to improve LiDAR-derived predictions of potential amphibian breeding ponds. *Journal of Geographical Systems*, *11*(1), 89–106.

Kordelas GA, Manakos I, Aragonés D, Díaz-Delgado R, and Bustamante J. (2018). Fast and automatic data-driven thresholding for inundation mapping with Sentinel-2 data. *Remote Sensing*, *10*(6), 910.

Leonard PB, Baldwin RF, Homyack JA, and Wigley TB. (2012). Remote detection of small wetlands in the Atlantic coastal plain of North America: Local relief models, ground validation, and high-throughput computing. *Forest Ecology and Management*, *284*, 107–115.

Ontario Ministry of Natural Resources and Forestry. (2014). *Ontario Wetland Evaluation System: Soutern Manual 3rd Edition*. https://www.ontario.ca/page/wetlands-evaluation

Rheinhardt RD and Hollands GG. (2008). Classification of Vernal Pools: Geomorphic Setting and Distribution. In *Science and Conservation of Vernal Pools in Northeastern North America* (pp. 11–30).

Riley JW, Calhoun DL, Barichivich WJ, and Walls SC. (2017). Identifying Small Depressional Wetlands and Using a Topographic Position Index to Infer Hydroperiod Regimes for Pond-Breeding Amphibians. *Wetlands*, *37*, 325–338.

Semlitsch RD, Peterman WE, Anderson TL, Drake DL, and Ousterhout BH. (2015). Intermediate pond sizes contain the highest density, richness, and diversity of pond-breeding amphibians. *PLoS ONE*, *10*(4), 20.

Werner EE, Skelly DK, Relyea RA, and Yurewicz KL. (2007). Amphibian species richness across environmental gradients. *Oikos*, *116*(10), 1697–1712.

Woodward BD. (1982). Local intraspecific variation in clutch parameters in the spotted salamander (*Ambystoma maculatum*). *Copeia*, *1982*(1), 157.

Wu Q, Lane C, and Liu H. (2014). An effective method for detecting potential woodland vernal pools using high-resolution LiDAR data and aerial imagery. *Remote Sensing*, *6*(11), 11444–11467.