LANDSCAPE-LEVEL CONSERVATION OF FRESHWATER TURTLES

# LANDSCAPE-LEVEL STRATEGIES FOR CONSERVATION OF IMPERILED FRESHWATER TURTLES

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McMaster University

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#### Lay Abstract

Turtles are one of the most endangered taxonomic groups, with as many as 1 in 2 species at risk of extinction. Although turtles are one of the oldest groups of animals in the world, anthropogenic impacts causing habitat loss and degradation have led to population declines. The goals of this thesis were to develop and assess landscape-level conservation strategies for imperiled freshwater turtles. I analyzed habitat selection by an imperiled freshwater turtle and determined suitable thermal characteristics of critical overwintering habitat. At the landscape-scale, I modeled habitat suitability and occupancy for at-risk turtles. My research reveals the negative effects of invasive species and the ineffectiveness of partial fencing as a strategy to mitigate reptile road mortality. Lastly, I discuss the implications of habitat succession on reptile habitat and the need for management when protection of land alone does not equate to protection of sensitive species. My research advances our understanding of landscape requirements for turtles and will inform future conservation strategies for imperiled freshwater turtles.

#### Abstract

In Ontario, 7 of the 8 native species of freshwater turtles are listed as at-risk. Protection of species-at-risk requires delineation and identification of their critical habitat to enable the proposal of conservation strategies. Because of pre-existing development and limited resources for conservation, it is difficult to protect land required for species' survival especially when faced with multiple threats such as habitat loss, degradation, and fragmentation, invasive species, and a changing climate. To improve conservation strategies, we must understand how turtle populations use a variety of different landscapes at a range of spatial scales.

The goals of this thesis were to develop and assess landscape-level conservation strategies for imperiled freshwater turtles. I first determined that Blanding's turtles (*Emydoidea blandingii*) require inland and coastal wetlands, a suitable upland matrix and connecting corridors. Since turtles overwinter for majority of the year, I determined suitable thermal parameters of occupied sites, revealing suitable water temperatures range from 0.44° C to 3.68° C and slowly decline throughout the overwintering period. Using a landscape-level approach, my models support that habitat in Georgian Bay is in good condition; 64% of evaluated islands support habitat for Blanding's turtles and majority of surveyed coastal wetlands with higher proportions of forest cover, lower densities of roads, buildings, and docks, and more variable bathymetric slopes provide more suitable habitat. In addition to anthropogenic stressors, invasive species pose a threat to turtles and their habitat. I provided the first quantitative evidence of the negative consequences

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of *Phragmites australis* invasion on availability of habitat for turtles. Since roads are a ubiquitous feature in our landscape, I evaluated strategies aimed at limiting their negative effects on at-risk reptiles. A decade-long dataset of road mortality reveals the ineffectiveness of partial fencing as a mitigation strategy. I recommend emphasis on fence integrity and continuity, limiting impact of edge effects, and conducting a comprehensive monitoring program. Finally, long-term changes in a protected landscape are evaluated and results indicate habitat succession and invasive species can negatively affect habitat suitability, and protection of land alone does not necessarily equate to protection of sensitive herpetofauna. My research integrates geographic information systems and remote sensing to advance our understanding of landscape requirements for turtles and will inform future conservation strategies aimed at mitigating threats to imperiled freshwater turtles.

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# List of all Abbreviations and Symbols

AIC <sub>c</sub>	Akaike Information Criterion
$AIC\omega_i$	Akaike Information Criterion weight for site i
AUC	Area under the curve
AW	Area-weighted
ψc	Conditional Occupancy
CI	Confidence Interval
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
CSR	Complete Spatial Randomness
° C	Degrees Celsius
df	Degrees of Freedom
DHR	Dynamic Home Range
DHR+S	Dynamic Home Range Plus Saturation
EDA	Euclidean Distance Analysis
EPHEMERAL	Vernal pool
ESA	Endangered Species Act
ESRI	Environmental Systems Research Institute

F	Female
FHR	Fixed Home Range
GIS	Geographic Information System
GPS	Global Positioning System
HSD	Honest Significant Difference
KDE	Kernel Density Estimation
KIA	Kappa Index of Agreement
LSCV	Least-squares Cross Validation
М	Male
МСР	Minimum Convex Polygon
MNRF	Ministry of Natural Resources and Forestry
NN	Nearest Neighbor
NOAA	National Oceanic and Atmospheric Administration
NWA	National Wildlife Area
Ψ	Occupancy
OWES	Ontario Wetland Evaluation System
PCA	Principal Component Analysis

PC	Principal Component
PIT	Passive Integrated Transponder
PPNP	Point Pelee National Park
PVC	Polyvinyl Chloride
ROC	Receiver Operating Characteristic Curve
SAV	Submergent Aquatic Vegetation
SARA	Species At Risk Act
SWOOP	South Western Ontario Orthophotography Project
UAV	Unmanned Aerial Vehicle
UNESCO	United Nations Educational, Scientific and Cultural Organization
WSCA	Wildlife Scientific Collectors Authorization

#### **Declaration of Academic Achievement**

This thesis is organized in the "sandwich thesis" format and comprises 9 chapters and 2 appendices. Chapter 1 provides a general introduction to the thesis. Chapters 2, 3, 4, 7 are manuscripts that have been published, chapters 6 and 8 are under peer-review in scientific journals, and chapter 5 is being prepared for submission. Chapter 9 provides a general conclusion. Appendix A is a co-authored chapter drawing from work completed in chapters 2, 3, and 4, and is accepted for publication in a textbook. Appendix B is a co-authored publication resulting from data collected for chapters 3, 6, and 7.

## **Chapter 1: General Introduction**

Author: Chantel E. Markle

# Chapter 2: Habitat Selection by the Blanding's Turtle (*Emydoidea blandingii*) on a Protected Island in Georgian Bay, Lake Huron

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# Chapter 3: Thermal characteristics of overwintering habitats for the Blanding's turtle (*Emydoidea blandingii*) across three study areas in Ontario, Canada

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# Chapter 4: An integrative approach to regional mapping of suitable habitat for the Blanding's turtle (*Emydoidea blandingii*) on islands in Georgian Bay,

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# Chapter 7: The true cost of partial fencing: Evaluating strategies to reduce reptile road mortality

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# Chapter 8: Long-term habitat changes in a protected area: Implications for herpetofauna habitat management and restoration.

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	under the supervision of P.C-F. G.E.M provided edits and
	comments on the manuscript.

### **Chapter 9: General Conclusion**

Author: Chantel E. Markle

# Chapter 10 / Appendix A: Georgian Bay, Lake Huron: freshwater turtles and their habitat in a changing landscape
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	C.E.M and J.V.M collected and analyzed the data, C.E.M and

J.V.M wrote and edited the manuscript under the supervision of P.C-F.

#### **Chapter 1: General Introduction**

#### **Freshwater Turtles**

Turtles are one of the most endangered taxonomic groups, with as many as 1 in 2 species at risk of extinction (IUCN 2013). Although turtles are one of the oldest species in the world and have survived ice ages and mass extinctions, anthropogenic impacts causing habitat loss and degradation have led to their decline (Ernst and Lovich 2009). In Ontario, 7 of the 8 native species of freshwater turtles are listed as 'at-risk' (COSEWIC 2005; Government of Canada 2009). Species designated as Endangered, Threatened, or Extirpated are currently protected under the Endangered Species Act (2007) in Ontario and on federal lands under the Species at Risk Act (2002). Despite these designations, changes to the Endangered Species Act (2007) in July 2013 allows for existing and new projects to obtain exemptions for activities such as mining, logging, and road and municipal development in areas where species-at-risk inhabit. In light of these changes and limited resources for conservation, it can be difficult or undesirable to protect the large expanses of land often suggested as required for species' survival.

Protection for species at risk is primarily focused on protecting habitat required for their survival or recovery, known as critical habitat. A species' critical habitat is defined as habitats used for reproduction, rearing, overwintering, migration or feeding (Government of Canada 2009). The delineation of such critical habitats is one of the first steps needed to enable the proposal of conservation strategies; however, critical habitat is only partially identified for many at-risk turtles.

When identifying critical habitat, it is important to consider both habitat occupancy and habitat suitability. Field campaigns and research funds can be more efficiently targeted once habitats have been prioritized based on their suitability or likelihood to support species at risk. Once classified as suitable, occupancy or presence of species at risk confirms the importance of a landscape to a species' recovery. While identification, classification, and delineation of critical habitat is vital to a species recovery, the implementation of effective management strategies is the next key component to ensuring species declines are stopped or reversed.

## Spatial and Landscape Ecology

We must advance our understanding of how turtles use a landscape to ensure we design effective conservation strategies and implement adequate recovery strategies. To do this, we require geographic information systems (GIS), knowledge of spatial habitat linkages, movement patterns, habitat use, and high-resolution spatial data. In this thesis, we use and apply spatial and landscape ecology principles to develop and assess conservation strategies for freshwater turtles.

The field of landscape ecology became prominent in the 1980's when large-scale environmental issues gained attention and geographic information system capabilities improved (Turner et al. 2001). The landscape ecology approach is primarily interested in determining why a phenomenon occurs at a specific spatial and temporal scale (Turner 1989). Because of the tight coupling of pattern analysis in landscape ecology, it is inextricably linked with spatial ecology. Taken together, this field of research examines the effects of landscape patterns on ecological processes, where a 'landscape' can be

defined from many points of view and ecological processes can occur at many different spatial and temporal scales (Turner 1989).

A landscape can be characterized by its structure and includes information regarding habitat composition and configuration. Habitat composition refers to the type of habitat found in the landscape and the amount of each type. Habitat configuration can be quantified using a wide variety of metrics (see McGarigal et al. 2012), but generally describes how habitats are arranged in the landscape. Although a landscape itself can vary in size, landscape-scale studies don't have to occur over a large area. Instead, a landscape-scale study is one that studies several different landscapes and can occur at the individual, community, population, or ecosystem level (King 1999; Fahrig 2016).

When examining the effects of spatial scale, certain processes may only be evident at a specific scale. For instance, habitat selection may occur at the individual scale (3<sup>rd</sup> order), home range scale (2<sup>nd</sup> order) or population level (1st order; Johnson 1980). The effects of anthropogenic disturbances (chapter 5) or invasive species (chapter 6) may influence the presence of species in wetlands or alter the way they use habitats at different spatial scales. In some cases, we may be interested in a unique population or a specific area or region. For example, we can determine habitat use for a specific population of turtles (chapter 2) or map the distribution of turtle habitat across an entire lakeshore (chapter 4).

It is also important to know what spatial scale findings are transferable too. Certain habitat requirements may be region specific, like nesting habitat in Georgian Bay

(chapter 2, chapter 4), whereas thermal conditions for suitable overwintering habitats appear to be transferable across southwestern and central Ontario (chapter 3). We can also use a single landscape as a case study to inform management strategies (chapter 7); analyzing a long-term dataset from a spatial perspective can reveal insights into best practices.

Another important factor to consider is the temporal scale at which ecological processes occur. First, habitat use and selection can vary within a year (chapter 2). When we integrate turtle behaviour into our analyses, different habitats may be required to complete seasonal activities such as nesting, mating, or overwintering. Habitat selection can also change when the availability and/or distribution of habitat types change (chapter 2). Lastly, landscape structure can also change on a yearly basis, by either natural or anthropogenic forces, changing the quality and availability for sensitive groups of species (chapter 8).

#### Focal Species

Our primary focus is on the Blanding's turtle (*Emydoidea blandingii*), a semiaquatic turtle that requires both upland and aquatic habitats, and makes long upland migrations seasonally (Ernst and Lovich 2009; Edge et al. 2010). These traits result in Blanding's turtles using a mosaic of habitat types within a landscape and make them very vulnerable to the negative impacts of habitat degradation and fragmentation. For example, long migrations can increase their chances of encountering roads and an increase in road mortality can lead to population declines because of low juvenile recruitment rates, delayed sexual maturity, and long lifespan (Congdon et al. 1993;

Marchand and Litvaitis 2004; Steen and Gibbs 2004). Almost 20% of the Blanding's turtles' range is located in Ontario, Canada, where the species is designated as Threatened in Ontario and the Great Lakes population was recently uplisted to Endangered in Canada (Ontario Government 2007; COSEWIC 2016). Their status indicates that the Blanding's turtle is facing imminent extirpation from Canada and is at risk of extirpation from Ontario if limiting factors are not reversed (Ontario Government 2007; COSEWIC 2016), demanding urgent conservation actions.

From a habitat modelling and protection perspective, the Blanding's turtle is an ideal model species. Their sensitivity to mortality and long lifespan requires long-term strategies that will likely benefit other turtles. The Blanding's turtle is known for their long migrations, traveling up to 6 km to nest (Edge et al. 2010); therefore, strategies designed to protect habitat for the Blanding's turtle will encompass habitat used by other turtle species. Additionally, because Blanding's turtles are semi-aquatic they depend on the unique interface between aquatic and terrestrial habitat, effectively linking these habitats that are often treated as distinct from a mangement standpoint (see Ontario Government 2014).

## **Thesis Objectives**

The objectives of my thesis are to develop and assess landscape-level conservation strategies for imperiled freshwater turtles (Figure 1.1). Our study sites occur throughout southwestern and central Ontario, ranging from 41° to 45° latitude. These study sites provide a variety of land use alterations and levels of human disturbance to allow us to design freshwater turtle recovery strategies for some of the major land use

types in Ontario. For freshwater turtles, existing recovery strategies often recommend that approaches to conservation and protection of travel corridors be made at the landscape level (Environment Canada 2016). However, the development and advancement of management strategies requires high resolution animal movement and remote sensing data. In this thesis, we use recently introduced GPS devices in combination with geographic information systems (GIS) and remote sensing techniques, as well as high-resolution topographic data to aid in the conservation of imperiled freshwater turtles in Ontario.

To achieve the thesis objectives, we begin by identifying and delineating critical habitat. In chapters 2 and 3, we study habitat selection by the Blanding's turtle and define suitable overwintering habitat. In chapters 4 and 5 we use a landscape-level approach to model habitat suitability and occupancy for turtles. The approach and framework we develope in these chapters is applied in Georgian Bay, but transferable to other locations and species. Chapters 2-5 lay the foundation for advancing conservation and recovery strategies by studying habitat selection, habitat suitability, and habitat occupancy.

In chapters 6-8, we focus on habitat management and evaluation of conservation strategies. We start chapter 6 by investigating the effects of Canada's worst invasive plant, *Phragmites australis*, on at-risk turtles. We reveal the negative effects on turtle habitat and discuss implications for habitat mangement. In chapter 7, we evaluate the effectivenss of exclusion fencing to reduce reptile mortality and offer recommendations for mitigating road mortality. Finally, in chapter 8, we evaluate long-term changes to a protected landscape and discuss implications for sensitive species.

We end the thesis with 2 appendices that provide additional context. First, we highlight the uniqueness of Georgian Bay and its susceptibility to encroaching development in light of a changing landscape. The Georgian Bay landscape is the primary focus of the first half of the thesis, and requires forward-thinking conservation strategies to ensure freshwater turtles and their habitat will receive adequate long-term protection. We then discuss the importance and use of unmanned aerial vehicles for landscape ecology, a tool we use throughout the thesis. UAVs provide the temporal and spatial resolution required for our work, and are invaluable for studying wetland ecosystems and the species they support.

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Figure 1.1: Concept map to outline the objectives of the thesis.

## Chapter 2: Habitat selection by the Blanding's turtle (*Emydoidea blandingii*) on a protected island in Georgian Bay, Lake Huron

By

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## Abstract

A key step in generating effective recovery strategies for species at risk is to identify habitat used under a variety of geographic settings. Due in part to habitat loss and degradation, the Blanding's turtle (*Emydoidea blandingii*) is considered at risk across most of its range. Because little information for this species exists for the many islands of Georgian Bay, the world's largest freshwater archipelago, we conducted an intensive study on the habitat use of 12 turtles (6 males, 6 females) on a protected island. We used a combination of radio-tracking and GPS loggers to determine habitat use during the active seasons of 2011 and 2012. We used aerial imagery to quantify available habitat and used compositional analyses to determine habitat selection. Both sexes utilized vernal pools and wet forest to move between habitat patches. Females used inland wetlands early in the year and coastal wetlands during the nesting season, whereas males maintained extensive use of inland wetlands during the entire active season. An effective conservation strategy for Blanding's turtles in Georgian Bay must include protection of inland and coastal wetlands, in addition to the surrounding upland matrix and connecting corridors.

## Introduction

Effective conservation strategies for species at risk rely on accurate identification of critical habitat, such as areas that individuals use for reproduction, feeding, and hibernation. For a semi-aquatic species such as the Blanding's turtle (*Emydoidea blandingii*), this task is particularly important due to their extensive use of both aquatic and terrestrial habitat (Ernst and Lovich 2009). For instance, females are known to migrate long distances to their upland nesting sites (Ernst and Lovich 2009; Edge et al. 2010). These migrations can increase the risk of road mortality, which can lead to population declines because of low juvenile recruitment rates, delayed sexual maturity, and long lifespan (Congdon et al. 1993; Marchand and Litvaitis 2004; Steen and Gibbs 2004; Dowling et al. 2010). Overall, habitat loss, degradation, and fragmentation have led to Blanding's turtles being designated as a species at risk in 17 of the 18 provincial or state jurisdictions throughout their range (NatureServe 2009). In order to effectively conserve this species at risk, habitat use studies are required to identify and protect habitat from further alterations.

Blanding's turtles are ectotherms and regulate their metabolic needs through their behavior, which may require use of diverse habitats throughout the active season (Congdon 1989; Huey 1991; Beaudry et al. 2009). The active season can be divided into "behavioral seasons" (pre-nesting, nesting, and post-nesting) and it is important to determine habitat use during each season, as they are associated with unique behaviors or activities that require different habitats (Rasmussen and Litzgus 2010). According to the reproductive-strategies hypothesis (Morreale et al. 1984; Gibbons et al. 1990), males are

expected to be more active during the pre-nesting season while searching for mates, while females are expected to be more active during the nesting season while making nesting migrations. The difference in activity patterns between males and females may lead to differences in habitat use. Additionally, it may be necessary to examine habitat selection at multiple spatial scales to account for the biology of a species and an individual's arbitrary use of habitats (Johnson 1980). Habitat selection can occur at three scales: firstorder selection can be defined as selection of the population range, second-order selection is defined as the individual's home range, and third-order selection is defined as an individual's location (Johnson 1980). Therefore, it is important to determine habitat selection by both males and females during all behavioral seasons and at multiple scales, in order to fully identify habitat requirements for this species.

Within Canada, there are two isolated Blanding's turtle populations which encompass 20% of their global range, one centered on the Great Lakes and the other in Nova Scotia (Government of Canada 2009). Within the Great Lakes region, Georgian Bay, Lake Huron, is recognized as the largest freshwater archipelago in the world and most of its habitats are still in relatively pristine condition (Cvetkovic and Chow-Fraser 2011). The Georgian Bay archipelago is designated a World Biosphere Reserve by UNESCO and contains over 30,000 islands. However, this area is under increasing threat as road expansion and cottage and residential development along the shoreline are expected to increase (Walton and Villeneuve 1999; Niemi et al. 2007). With increasing human development, there is a pressing need to identify sensitive areas and ensure minimal habitat degradation and fragmentation (Walton and Villeneuve 1999). Even

though this area is of great ecological significance, the subpopulation of Blanding's turtles living in this region has not yet been studied, which is an important information gap when developing conservation strategies. We cannot simply extrapolate information from previous studies to the Georgian Bay region because habitat selection differs among populations of Blanding's turtles in geographically distinct areas partly due to the variation in available habitat types. For example, a study in Maine, U.S.A, identified a population of Blanding's turtles that used wetlands within deciduous forest and with a high cover of sphagnum moss (Beaudry et al. 2009), whereas turtles in a study in New York used wetlands with shallow water depths and dense vegetation (Hartwig and Kiviat 2007). Millar and Blouin-Demers (2011) studied habitat use by Blanding's turtles in the St. Lawrence islands, which are within the Great Lakes basin, but extrapolating results to the Georgian Bay islands may be inappropriate because of different topography, and thus a difference in available habitat types, that originates from disparate bedrock type (Perera et al. 2000). The Georgian Bay islands have bedrock material of Canadian Shield (granitic rock with only a very thin layer of soil; Parks Canada 2010), whereas islands in the St. Lawrence are underlain by sedimentary rock (sandstone and limestone). Therefore, extrapolating information across geographic regions to determine Blanding's turtle habitat is often difficult and should not be done to develop effective management plans to protect the Blanding's turtles in the Georgian Bay archipelago.

The purpose of our study was to identify habitat selection and use by the subpopulation of Blanding's turtles living on a protected island in southeastern Georgian Bay. Based on the reproductive-strategies hypothesis, we predicted that (1) males and

females would select different habitat types. We hypothesized that (2) habitat selection would differ between the sexes across pre-nesting, nesting, and post-nesting seasons due to differing requirements. Our study identified habitats required to sustain the study population and will enable the development of effective strategies for the islands of Georgian Bay to ensure that Blanding's turtles are adequately protected in an area under threat of development. Furthermore, we make general recommendations to enhance conservation of the Blanding's turtle in this region and identify areas for future research.

#### Methods

#### Study site

Our study was carried out on a protected island in Georgian Bay, Lake Huron. The island contains 11 km<sup>2</sup> of pristine habitat characterized by Canadian Shield landscape and a mix of coniferous and hardwood forest (Fig. 2.1). We collected the majority of habitat data *in situ* when each turtle was individually radio-tracked and collected additional data at locations identified by GPS loggers. We identified eight habitat types and classified wetlands using the Canadian National Wetlands Classification System (Warner and Rubec 1997) and created additional classes to include all habitat types in the study area (Table 2.1). We used ArcGIS 10 (ESRI, Redlands, California, USA) to digitize habitat types in orthophotos taken in the spring of 2008 (30 cm resolution) and ground-truthed these aerial images. We used our resulting maps to calculate habitat areas. We also digitized and ground-truthed *Sphagnum* mats and determined bathymetry of two bogs to characterize differences between these habitats.

We calculated the number of *Sphagnum* mats and surface area of each mat in ArcGIS 10. We recorded depth measurements from a boat with a meter stick and collected associated GPS coordinates to input into ArcGIS 10 to create bathymetric maps. We used our resulting *Sphagnum* mat and bathymetric maps to determine percent total mat coverage, average mat size, and average depth of bog 1 and bog 2.

## Turtle movements

Our study was carried out according to the *guide to the care and use of* experimental animals (Canadian Council on Animal Care 1993). All of the turtles in this study were captured initially and radio-tagged between 26 April and 31 May 2011. Six male and six female Blanding's turtles were caught opportunistically by hand, dip net, or in baited hoop nets. We identified the sex of each turtle using secondary morphological characteristics such as concavity of the plastron and position of the cloacal opening (Hamernick 2000; Innes et al. 2008). We weighed each turtle (Starfrit Digital Scale, acc.  $\pm 1$ g) to ensure they were sufficiently large to carry the weight of the radio-transmitter or the radio-transmitter and GPS logger combination. The attachments were <5% of the turtle's body mass. We notched the scutes of each turtle with a unique code for later identification (Cagle 1939). Once the rear marginal scutes were cleaned, we attached AI-2F radio-transmitters (Holohil Systems Ltd., Carp, Ontario, Canada, 19 g) with quick dry epoxy and plumber's epoxy. Additionally, three females were outfitted with GPS loggers in 2011, as were two females and two males in 2012 (Lotek Wireless, Newmarket, Ontario, Canada, 10 g; Telemetry Solutions, California, USA, 30 g). After tagging, we returned turtles within 24 hours to the same locations where they had been caught.

We radio-tracked turtles at least once per week from 31 May to 1 September in 2011 and from 1 May to 25 July in 2012. We used a 3-element Yagi antenna (Wildlife Materials International, Murphysboro, Illinois, USA) and a Lotek Biotracker Receiver (Lotek Wireless, Newmarket, Ontario, Canada) to locate their positions during these weekly surveys. We conducted nesting surveys starting on 23 May 2012 until all tagged females were no longer gravid. Surveys commenced at 1700 hrs and each gravid female was radio tracked. If females remained in the same location, the survey ended at 2400 hrs. If females remained active, we used a red light (to minimize disturbance) to identify a nesting site. When we located a turtle, the date, time of day, GPS location (Handheld Garmin, accuracy to within 6 m) and habitat type were recorded. We recaptured turtles with GPS loggers several times throughout the active season to download data (accuracy to within ~10 m) and to recharge the devices. We also tracked during November 2011, February 2012, and February 2013 to determine hibernation locations.

## Statistical analyses

We used compositional analyses to test for habitat selection (disproportionate habitat use) over the active season (Aebischer et al. 1993). Disproportionate use or habitat selection is defined as the use of a habitat type in greater proportion than its availability (Johnson 1980). This approach has been used in recent studies (Schmid et al. 2003; Rasmussen and Litzgus 2010) and provides three statistical advantages over previous habitat analyses such as the Chi-square method (Carrière and Blouin-Demers 2010). First, the sample size is equal to the number of tagged turtles and not the number of radio locations; this avoids pseudoreplication and does not inflate the degrees of

freedom which would increase the chance for type 1 errors (Aebischer et al. 1993). Second, use of log ratios in compositional analyses avoids the unit sum constraint which can lead to inappropriate conclusions because proportions will sum to one and habitats that are avoided will lead to an apparent selection for the remaining habitats. Lastly, habitat selection can be determined for different groups of individuals. Even with this statistical approach and considering habitat selection at different spatial scales, the problem of arbitrary boundary selection still remains. This problem occurs because population range size and home range size must be calculated when using compositional analyses in order to determine habitat selection. Although this fundamental problem exists for all range size estimation methods, we used the minimum convex polygon (MCP) method (Mohr 1947). This method is common for determining turtle ranges (Litzgus et al. 2004; Row and Blouin-Demers 2006; Rasmussen and Litzgus 2010; Millar and Blouin-Demers 2011), is an accurate estimator for reptile home ranges, and reduces the requirement for arbitrary choices involved in methods such as kernel estimation and selection of a smoothing factor (Row and Blouin-Demers 2006). Drawbacks to MCP include sensitivity to additional data points (i.e., as the number of location points increases, the estimated home range size increases) and inclusion of large unused areas (Harris et al. 1990; White and Garrott 1990). These drawbacks were addressed by collecting the number of locations required for home range stabilization (Harris et al. 1990) and combining the MCPs of all individual turtles to obtain the population range. In our study, we operationally defined  $2^{nd}$ -order habitat availability as the population range and 2<sup>nd</sup>-order habitat use as the individual home range. We defined 3<sup>rd</sup>-order habitat

availability as the MCP for individual turtles and 3<sup>rd</sup>-order habitat use as the individual's locations.

We used two-way ANOVAs with sex and season as factors to determine effects on habitat selection, as well as the interaction between sex and season. We divided the active season into three behavioral seasons: pre-nesting (between the first sighting of a basking turtle and the first observed female to begin the nesting migration), nesting (from the first observed female to begin the nesting migration to the last female returning from nesting), and post-nesting (from the last female returning from nesting and the end of our field season; Table 2.2).

Weather differences between the two years provided a natural opportunity to examine changes in habitat selection. Temperature, rainfall, and snowmelt are important factors when considering habitat selection since they impact the drying of vernal pools. An early spring with little precipitation can indicate early drying of vernal pools which may impact secondary productivity (Brooks 2004). Therefore, we calculated habitat selection during 2011 and 2012 separately to examine differences between years. We used weather data from Environment Canada's (2012) national climate data and information archive.

All statistical analyses were carried out in JMP version 10 (SAS Institute Inc., Toronto, Ontario, Canada) and significance of tests was accepted at  $\alpha = 0.05$ . Although compositional analyses allow for the ranking of all possible habitat types according to selection, we only report the top one or two habitat types selected because results

associated with lower rankings do not necessarily lead to ecologically meaningful interpretations.

#### **Results**

Through ground-truthing, we determined that there were eight main habitat types used by the tagged Blanding's turtles on the protected island: bog 1, bog 2, dry forest, wet forest (swamp), shallow-water wetlands (beaver pools and vernal pools), rock, lake, and marsh (Table 2.1). Although the presence of Blanding's turtles was also confirmed in bog 3 (Fig. 2.1c), no tagged turtles used this habitat. The two bogs were determined to be ecologically different and were thus treated as two separate habitat types. Bog 1 was further along in ecological succession and had *Sphagnum* mats with a significantly larger surface area than bog 2 (281 m<sup>2</sup> vs. 43 m<sup>2</sup>; Mann-Whitney *U*-test, *Z* = 3.16, *p* = 0.0016) and provided more total coverage (38% vs. 30%; Fig. 2.2). Additionally, bog 1 was significantly shallower than bog 2 (54.5 cm vs. 85.8 cm; Mann-Whitney *U*-test, *Z* = 6.46, *p* < 0.0001), with a maximum depth of 139 cm compared to 147 cm. These differences were deemed to be ecologically relevant because water depth and vegetation structure are variables that may affect the amount of time turtles spend swimming, basking, and feeding in a wetland (Sexton 1995; Black 2000 as cited in Marchand and Litvaitis 2004).

The amount of snow covering the ground was lower in 2012 than in 2011 in January (8.00 cm vs. 3.65 cm) and February (8.90 cm vs. 1.10 cm; Table 2.3). Coincident with lower snowfall, temperatures were also consistently warmer in 2012 than in 2011 (Table 2.3).

## General use of habitat

Blanding's turtles spent the majority of time during the pre-nesting and postnesting seasons in their resident wetlands. We define a turtle's resident wetland as the wetland habitat where the majority of the active season is spent. In our study, bogs 1 and 2 served as resident wetlands and hibernacula (Fig. 2.1c). Three males and 4 females used bog 1 as their resident wetland, whereas 3 males and 2 females used bog 2 as their resident wetland. When travelling between resident wetlands, turtles primarily utilized beaver ponds or shallow-water wetlands. During the pre-nesting season, females appeared to remain in their resident wetlands while some males left their resident wetlands to utilize vernal pools. During the nesting season, females utilized forest, wet forest, and vernal pools to travel to and from the staging area (Long Bay; Fig. 2.1c) and nesting area. During this time, two males remained in their resident wetland, three males utilized both bogs, and one male utilized the coastal marsh in Turtle Bay (Fig. 2.1c). During post-nesting, all turtles returned and remained in their resident wetlands until hibernation. Only two females hibernated in bog 2 compared to the majority of turtles that hibernated in bog 1.

## Second-order habitat selection

During pre-nesting seasons, females selected bog 1 (Fig. 2.3a, f) in both years and shallow-water wetlands in 2012 (Fig. 2.3i, j). By comparison, males selected bog 2 during the pre-nesting season in both years (Fig. 2.4b, h) and shallow-water wetlands in 2011 (Fig. 2.4c to f). Although we observed males using shallow-water wetlands (vernal pools) in 2012, these did not appear to be selected.

There were some year-to-year differences with respect to habitat selection by females during the nesting seasons. In 2011, females selected shallow-water wetlands (Fig. 2.3b, d, e) while in 2012 (23 May to 30 June), they selected both wet forest and dry forest (Fig. 2.3g, h). Although wet forests were selected in both years (Fig. 2.3c, g), only dry forest was selected only in 2012 (Fig. 2.3g) when weather conditions were exceptionally dry. Overall, males appeared to use resident wetlands primarily. Analysis of variance indicated a significant effect of season for selection of marsh ( $F_{5,25} = 34.8$ , p < 0.0001) and wet forest ( $F_{5,25} = 8.3$ , p = 0.0017) during 2011, and a significant interaction between sex and season for selection of marsh ( $F_{5,25} = 14.3$ , p < 0.0001) and wet forest ( $F_{5,25} = 4.9$ , p = 0.0154). A Tukey HSD post hoc test indicated that females selected marsh (p < 0.0001), rock (p < 0.0001), and wet forest (p = 0.0273) significantly more often during the nesting season compared to the pre-nesting season; they also selected marsh (p < 0.0001), rock (p < 0.0001), and wet forests (p = 0.0193) significantly more often during the nesting season compared to the post-nesting season. Additionally, females selected marsh (p < 0.0005) and rock (p < 0.0016) significantly more often during the nesting season than males. During 2012, females also selected rock during the nesting season compared to the pre-nesting (p = 0.0082) and post-nesting (p = 0.0036) seasons. Based on these habitat selections, we suspected that nest sites would be found in marsh, wet forest, or rock habitats. The GPS logger located a female at 2100 hrs on an upland rocky outcrop on 14 June 2011, approximately 570 m from her resident wetland. In 2012, this female was observed successfully nesting 60 m from the 2011 location. The clutch was laid in soil that had accumulated in a crack in the bedrock. The GPS logger

captured another female on an upland rocky outcrop on 10 June 2012 from 2200 hrs until 2400 hrs. Based on these observations, we confirmed females are using this rocky habitat for nesting.

During the post-nesting season, males selected bog 1 in both years (Fig. 2.4a, b, g, h). Males selected shallow-water wetlands and forest habitat in 2012 (Fig. 2.4i, j, k). Females selected bog 1 (Fig. 2.3a, f) and shallow-water wetlands (Fig. 2.3i, j). By November 2011, all turtles were found in their respective hibernation wetlands.

#### Third-order habitat selection

Third-order analyses determined habitats selected at the individual scale. Our data revealed that both sexes selected either bog 1 or bog 2 throughout the entire active season. For both males and females, use of the other habitat types were in proportion to their availability at this scale and not selected.

## Discussion

This is the first study to determine habitat selection by Blanding's turtles in the Georgian Bay archipelago. Consistent with our first hypothesis, we found that habitat selection differed for males and females. Supporting our second hypothesis, we found that males utilized different habitat in the pre-nesting season compared to the remainder of the active season, while females traveled to access a variety of habitats during nesting. We also identified year-to-year differences in habitat selection by females during the nesting season that we attribute to differences in weather (amount of precipitation in the spring) and its effect on availability of wet forests.

In both years, males were found to initially use bog 2 prior to using bog 1 for most of the active season. This switch in usage may be due to several factors: (1) presence of females, (2) competition from other species of turtles, or (3) use of shallow water in the late summer. Selection of the shallower bog in this study differs from that in previous studies in Maine and the St. Lawrence Islands (Beaudry et al. 2009; Millar and Blouin-Demers 2011), but is consistent with the preference for shallow, warm water by turtles in New York (Hartwig and Kiviat 2007). As expected, females remained in their resident wetland during the pre-nesting season, presumably to conserve energy in preparation for the nesting season (Congdon 1989; Millar and Blouin-Demers 2011). This finding was similar to that of Millar and Blouin-Demers (2011), who found that female Blanding's turtles did not make long-distance movements in spring and were found basking more often than males and non-gravid females, potentially because gravid females have higher energetic needs (Congdon 1989). Overall, selection of bog habitat by males and females during the pre-nesting season was similar to that of Blanding's turtles studied in Maine (Beaudry et al. 2009), Nova Scotia (Newton and Herman 2009), and a few turtles in Illinois (Rowe and Moll 1991; Table 2.4). Contrary to our results however, Blanding's turtles in New York were found to be associated with wetlands with buttonbush cover (Hartwig and Kiviat 2007), while Blanding's turtles in Wisconsin were found to prefer ponds (Ross and Anderson 1990; Table 2.4). These variations in Blanding's turtle habitat selections across their geographic range highlight the importance of site-specific habitat studies, as results may not be transferable among locations.

We confirmed fidelity to resident bogs and nesting sites between years. All tagged turtles caught in either bog 1 or bog 2 during 2011 were found to emerge from the same bog in spring 2012. In addition, none of the turtles in our study used a third bog that was located only 300 m from bog 1 and 475 m from bog 2 (see Fig. 2.1). We confirmed lichen-filled cracks in bedrock as nesting sites and observed fidelity to this location. One female nested in 2011 and 2012 with both nests within a 30-m radius. A second female was also confirmed to have nested in similar habitat in 2012. Similar nesting habitat has been confirmed in Georgian Bay for *Clemmys guttata* (Litzgus and Brooks 2000) and *Sternotherus odoratus* (Edmonds and Brooks 1996). Other studies on Blanding's turtles have found nests in grasslands (Ross and Anderson 1990) as well as beaches and along roadways (Standing et al. 1999; Table 2.4). Using GPS loggers in combination with radio-tracking allowed us to obtain more detailed information on Blanding's turtle habitat use than radio-tracking alone (Christensen and Chow-Fraser 2014) and proved important in obtaining locations late at night when turtles were nesting.

Vernal pools are important temporary habitats and can provide a source of food, hydration, and shelter for turtles. Access to these pools by turtles may vary from year to year, however, depending on the amount and timing of precipitation, because pool depths respond quickly to precipitation (Brooks 2004). For example, in 2012, usage of these vernal pools during early May stopped when the pools dried up by 21 May. By comparison, the much wetter spring in 2011 provided access to vernal pools throughout the month of May. Similar findings of weather impacting the amount of wet habitat available to turtles occurred in Maine (Joyal et al. 2001). We also found females using

dry forest during the nesting season, presumably because the wet habitats had been severely reduced or had become difficult to access in 2012. Inter-annual differences in weather patterns (e.g., reduced snowmelt and warmer winter temperatures in 2012) may therefore influence usage of wet and dry habitats and should be investigated further, especially in light of predicted changes associated with global climate change in the region.

Differences in weather may also affect the timing of nesting migrations for Blanding's turtles. Our field observations suggest that females use wet forest, vernal pools, and beaver pools as travel corridors to access the staging area in Long Bay (see Fig. 2.1). These temporary wet habitats can also be important for providing food, hydration, and shelter (Grgurovic and Sievert 2005), although our data did not allow for confirmation of their importance. Female Blanding's turtles spent a few days to a few weeks in the staging area (Long Bay) before making migrations to nest sites in upland areas. Although they used Long Bay as their staging area in both years, the nesting season started 8 days earlier and ended 12 days earlier in 2012 than in 2011. Warmer temperatures between January and March in 2012 may have accelerated female emergence from hibernation and led to earlier nesting migrations. This shift in timing may have consequences for the long-term viability of Blanding's turtle populations on this protected island since other freshwater turtles have been shown to be negatively affected by climate change because of the association of nesting with weather-related cues (Bowen et al. 2005).

After returning from nesting, females selected bog 1 during the post-nesting season. Similarly, males also selected bog 1 during the post-nesting season. Contrary to this, Blanding's turtles in Maine used forested swamps prior to hibernation, despite having access to more permanent pools (Joyal et al. 2001; Table 2.4). By November 2011, all tagged Blanding's turtles were found in their hibernation wetlands. Only 2 of 12 turtles hibernated in bog 1 compared to 10 of 12 turtles hibernating in bog 2. Hibernation in permanent wetlands was also confirmed for Blanding's turtles in Wisconsin (Ross and Anderson 1990) and Maine (Joyal et al. 2001), while hibernation habitat varied in Nova Scotia (Standing et al. 1999; Newton and Herman 2009; Table 2.4). Determining hibernation sites is important for conservation planning and research on microhabitat may determine key features in hibernacula.

It is noteworthy that one of the tagged males spent the majority of the active season in the lake on the west side of the island, approximately 900 m from the resident wetland (Turtle Bay; see Fig. 2.1). We recorded this behavior in both years and recorded the presence of two untagged Blanding's turtles in the same area. This behavior may aid in gene dispersal if a male mates with females from different resident wetlands. It is important to be aware of the turtles that travel long distances as they could be important for sustaining the population and an effort should be made to identify and protect the habitat used as travel corridors.

Studying habitat selection is essential for conservation because it provides data for the design of effective management and conservation strategies. From previous habitat use studies across North America, it is evident that discrete populations of

Blanding's turtles are using a variety of habitats. Research regarding the habitat selection of the Blanding's turtle is vital for the Georgian Bay population since there have been no previous studies conducted in this unique geographic region. Our results demonstrate the extent and differences in type of habitat that are necessary for this population of Blanding's turtles to carry out its life processes. Critical habitat types for both males and females included: 1) upland and coastal wetlands for annual use, 2) vernal pools, beaver ponds, and wet forest to access and travel between wetlands, and 3) rocky outcrops for nesting sites.

Overall, we have identified differential habitat selection throughout the active season and between years, which has important implications for conservation of Blanding's turtles throughout their range. First, conservation plans should emphasize the protection of nesting habitats and identify and protect the common habitats used to travel throughout the landscape. Second, future research should focus on differences in precipitation from year to year and how they may affect the timing of migration and the use of temporary habitats. Third, we recommend using remote sensing and GIS techniques to create regional models of habitat suitability so critical habitat for Blanding's turtles can be delineated and protected from future human disturbance. In conclusion, habitat types identified in our study can be used to guide the protection of other subpopulations of Blanding's turtles in this freshwater archipelago.

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**Table 2.1:** Definitions of habitat types, following the Canadian National WetlandsClassification System (Warner and Rubec 1997) and additional classes toinclude all habitat types in the study area.

Habitat Type	Brief Description
Bog 1	An older bog that is at a more advanced stage of ecological succession. Dominated by <i>Sphagnum</i> mosses with shrubs and young trees. The presence of many pitcher ( <i>Sarracenia purpurea</i> ) and sundew ( <i>Drosera</i> spp.) plants are indicative of the acidic water from decomposed peat and nitrogen limitation. The main source of water is through precipitation and snowmelt.
Bog 2	A younger bog in an earlier stage of ecological succession. A thin layer of peat is present, with many dead trees still standing to indicate it has been recently flooded. Only a few areas with pitcher plants ( <i>Sarracenia</i> <i>purpurea</i> ) indicate that the water may not be sufficiently acidic to support additional carnivorous plants. The main source of water is through precipitation and snowmelt.
Dry Forest	Coniferous forest is dominated by needleleaf species such as white pine ( <i>Pinus strobus</i> ) and hemlock ( <i>Tsuga</i> spp.). Hardwood forest is dominated by broadleaf species such as sugar maple ( <i>Acer saccharum</i> ) and beech ( <i>Fagus</i> spp.).
Wet Forest	A tree- or shrub-dominated wetland with highly decomposed peat that is not as wet as bogs or marshes. Also known as swamp.
Shallow- water Wetlands	Transitional wetlands between bogs, fens, marshes, and swamps. They contain deep water and are beaver and vernal pools in this study area.
Rock	Rocky outcrops characteristic of the Canadian Shield.
Lake	Large body of water where the maximum depth is >5 m. Surface vegetation is confined to bays.
Marsh	Dominated by rushes, reeds, grasses, and sedges. Typically has shallow water which can fluctuate daily, seasonally, or annually.

Year	Pre-nesting	Nesting	Post-nesting
2011	27 April – 31 May	1 June – 12 July	13 July – 1 September
2012	1 May – 22 May	23 May – 30 June	1 July – 19 September

**Table 2.2:** Dates of pre-nesting, nesting, and post-nesting behavioral seasons.

**Table 2.3:** Comparison of temperature, total rain, and snow cover from January to Marchin 2011 and 2012. All data obtained from Environment Canada's (2012)national climate data and information archive for the closest station (~5 km) toour study site (Midland Water Pollution Control Plant).

		Ter	nperature (°C	C)		Total
Month	Year	Maximum	Mean	Minimum	Total rain (mm)	snow cover (cm)
January	2011 2012 <i>p</i> - value <sup>a</sup>	-4.28 0.68 0.0105	-8.72 -3.59 0.0087	-13.17 -7.85 0.0154	0.00 0.23 NS	8.00 3.65 NS
February	2011 2012 <i>p</i> - value <sup>a</sup>	-1.50 2.60 NS	-6.06 -1.80 0.0712	-10.63 -6.20 0.0313	0.00 0.00 —	8.90 1.10 NS
March	2011 2012 <i>p</i> - value <sup>a</sup>	2.41 12.57 0.0050	-1.99 6.94 0.0011	-6.39 1.29 0.0003	1.22 1.21 NS	  

<sup>a</sup> *p*-values correspond to paired *t*-tests comparing 2011 and 2012 data; NS = not significant (*p* > 0.05). Due to non-normality of data, total rain and total snow cover for January were analyzed with a Wilcoxon matched-pairs signed-rank test.

at use by Blanding's turtles in various locations during pre-nesting, nesting, and post-nesting nation.	Study Location Habitat characteristics	udry et al. (2009) Maine Bogs with deciduous forest high sun exposure and a
ıbitat use by Blaı əernation.	Study	Beaudry et al. (20
Table 2.4: Habita season and hiberna	Season	Pre-Nesting

Season	Study	Location	Habitat characteristics
Pre-Nesting	Beaudry et al. (2009)	Maine	Bogs with deciduous forest, high sun exposure and a high abundance of wood frog egg masses
	Hartwig and Kiviat (2007)	New York	Wetlands with buttonbush cover
	Newton and Herman (2009) Nova Scotia	Nova Scotia	Wetlands dominated by sedges, sweet gale, and leatherleaf
	Ross and Anderson (1990)	Central Wisconsin	Preference for ponds compared to all other habitat types
	Rowe and Moll (1991)	Illinois	Majority of time spent in marsh and fen habitats
	Current study	Ontario	Males: Bog 2 (2011, 2012) and shallow-water wetlands
			(2011)
			Females: Bog 1 (2011, 2012) and shallow-water wetlands (2012)
Nesting	Standing et al. (1999)	Nova Scotia	Beaches and roadways
	Ross and Anderson (1990)	Central Wisconsin	Grasslands
	Current study	Ontario	Rocky outcrops (2011, 2012)
Post-Nesting Joyal et	Joyal et al. (2001)	Southern Maine	Forested swamps
	Current study	Ontario	Males: Bog 1 (2011, 2012), shallow- water wetlands
			(2012) and forest (2012)
			Females: Bog 1 (2011, 2012) and shallow-water
			wetlands (2011, 2012)
Hibernation	Ross and Anderson (1990)	Central Wisconsin	Deep ponds
	Joyal et al. (2001)	Southern Maine	70% of a population in Maine used permanent pools
	Standing et al. (1999);	Nova Scotia	Backwaters, streams, seasonally isolated ponds, small but
	Newton and Herman (2009)		deep pools in a mixed forest, fens, and bogs
	Current study	Ontario	Males: Bog 1 (2011-2012, 2012-2013)
			Females: Bogs 1 & 2 (2011–2012, 2012–2013)



**Figure 2.1:** Map of the Great Lakes indicating the approximate location of our study site (A). Our study site is located on a protected island in southeastern Georgian Bay (B and C).



Figure 2.2: Comparison of size and cover of *Sphagnum* spp. mats in bogs 1 and 2.



**Figure 2.3:** Results of compositional analyses for female Blanding's turtles in 2011 (panels a to e) and 2012 (panels f to j) for pre-nesting (hollow), nesting (black), and post-nesting (gray) seasons. Two-tailed one-sample t-tests were used to determine significant differences in habitat usage. Only significant results are depicted ( $\alpha < 0.05$ ). A positive *t*-value indicates significant selection for the corresponding habitat category along the *y*-axis, whereas a negative *t*-value indicates significant selection for the bottom right of each panel. Habitat categories include: SW = shallow water, L = lake, R = rock, WF = wet forest, M = marsh, DF = dry forest, B2 = bog 2, and B1 = bog 1.



**Figure 2.4:** Results of compositional analyses for male Blanding's turtles in 2011 (panels a to f) and 2012 (panels g to k) for pre-nesting (hollow), nesting (black), and post-nesting (gray) seasons. See Fig. 2 for description.

Ph.D. Thesis – C. Markle; McMaster University – Biology

# Chapter 3: Thermal characteristics of overwintering habitats for the Blanding's turtle (*Emydoidea blandingii*) across three study areas in Ontario, Canada

By

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# Abstract

Habitat restoration is a necessary strategy to protect populations of Blanding's Turtles (Emydoidea blandingii) living in settled areas. Relatively little is known about thermal tolerances and requirements of this species in situ during the overwintering period, except that these turtles must find water bodies that do not freeze completely and that are sufficiently cool to allow them to stay dormant throughout the winter. We used water temperature data associated with Blanding's Turtle populations in a northern, central, and southern study area within Ontario, Canada to determine thermal characteristics of occupied overwintering habitats. From fall through spring from 2012 to 2014, we measured water temperature of 20 potential overwintering habitats within the three study areas. We also radio tracked 48 adult turtles to determine which habitats they occupied during winter. Water temperatures of all occupied habitats ranged from 0.44° C to 3.68° C, with a mean of  $1.77^{\circ}$  C ( $\pm 0.03^{\circ}$  C), and showed slow steady declines throughout the overwintering period. Regardless of location, average water temperatures at all confirmed overwintering habitats remained above the freezing point of turtle body fluids ( $-0.6^{\circ}$  C). Average water temperature at five of the six confirmed overwintering habitats never dropped below 0° C, but dropped to -0.33° C for eight days at the sixth overwintering habitat. Determining thermal parameters of overwintering sites can provide knowledge useful for habitat restoration and creation to ensure habitats provide suitable overwintering conditions in the face of global climate change.

# Introduction

The Blanding's Turtle (Emydoidea blandingii) is a northern species of freshwater turtle with populations concentrated around the Great Lakes, and isolated populations in New York, Massachusetts, New Hampshire, Maine, Pennsylvania, USA, and Nova Scotia, Canada (Congdon et al. 2008). Almost 20% of the Blanding's Turtles' range is located in Ontario, Canada, where the species is designated as Threatened (Ontario Government. 2007. Endangered Species Act. Available from http://www.ontario.ca/laws/statute/07e06 [Accessed 27 May 2016]). Throughout their range, adult Blanding's Turtles have been known to overwinter in a variety of aquatic habitat types including marshes (Kofron and Schreiber 1985; Rowe and Moll 1991; Joyal et al. 2001; Edge et al. 2009), swamps (Seburn 2010), bogs (Herman et al. 2003; Edge et al. 2009; Newton and Herman 2009), fens (Edge et al. 2009; Newton and Herman 2009), vernal pools (Joyal et al. 2001; Seburn 2010), streams (Ross and Anderson 1990; Herman et al. 2003; Newton and Herman 2009) and permanent ponds (Graham and Butler 1993; Joyal et al. 2001; Beaudry et al. 2009; Refsnider and Linck 2012). Because turtles must maintain a cool body temperature to slow metabolism and conserve energy until spring (Edge et al. 2009), characteristics of suitable overwintering sites may include dissolved oxygen content (Dinkelacker et al. 2005; Ultsch 2006; Edge et al. 2009; Jackson and Ultsch 2010), substrate type (Greaves and Litzgus 2007), vegetation cover (Millar and Blouin-Demers 2011), and/or water temperature (Edge et al. 2009).

Given the many types of habitats used across the range of Blanding's Turtles, the only generalization that can be made is that individuals tend to overwinter in wetlands or

areas of standing water. Not all wetlands are suitable overwintering habitats, however, and there is little field information that points to critical environmental requirements that must be included to make the habitat suitable beyond identification of occupied habitat types (Kofron and Schreiber 1985; Kiviat 1997; Hartwig and Kiviat 2007; Millar and Blouin-Demers 2011). Some conservation plans require confirmation of overwintering sites before sites can be designated as a sensitive area with low tolerance to alterations (Ontario Ministry of Natural Resources and Forestry 2013). Otherwise, sites that may be suitable but that do not have confirmed occupancy could be exposed to harmful alterations or activities. Furthermore, without more detailed knowledge regarding suitable overwintering habitats, recovery strategies that rely on creation or restoration of overwintering habitats do not have the specific criteria necessary to ensure habitats are suitable.

Even though little is known about the specific tolerance ranges of adult Blanding's Turtles *in situ*, oxic and thermal parameters of the water body are likely the largest contributors to overwintering site suitability (Ultsch 2006; Edge et al. 2009). Blanding's Turtles are considered anoxia tolerant because they often share overwintering sites with the Painted Turtle (*Chrysemys picta*) and Snapping Turtle (*Chelydra serpentina*), species known to be anoxia tolerant (Dinkelacker et al. 2005; Ultsch 2006). Under anoxic conditions, energy is produced through anaerobic respiration that can lead to the accumulation of lactic acid, but which can be buffered by release of carbonates stored in the shell (Dinkelacker et al. 2005; Jackson and Ultsch 2010). Nevertheless, excessive accumulation of lactic acid can result in metabolic acidosis and death when

turtles are exposed to low oxygen conditions for an extended period of time (Dinkelacker et al. 2004; Jackson and Ultsch 2010). Given that Blanding's Turtles have been found to tolerate anoxic conditions (Edge et al. 2009; Newton and Herman 2009), temperature is likely more important than the level of dissolved oxygen in determining the suitability of a site. Temperatures, however, do not have to be above freezing consistently because the equilibrium freezing point for turtle body fluids is approximately -0.6° C (Costanzo et al. 2006). An ideal overwintering site should therefore have sufficiently cool temperature that will reduce the metabolism of the turtle but not so cold that the entire water column freezes.

With the large number of aquatic bodies throughout different landscapes in Ontario, Canada, and considering competing land uses, it would be beneficial for managers to identify and protect the most critical wetlands within their jurisidictions. Rather than focusing on the type of aquatic habitat per se, the goal of our study is to determine the thermal characteristics of occupied overwintering habitats within three populations of Blanding's Turtles in Ontario, Canada. Our primary objective is to use *insitu* water temperature data to characterize suitable temperatures at occupied overwintering habitats. Determining thermal parameters suitable for overwintering is one step towards more thoroughly understanding where turtles overwinter and may provide a cost-effective framework to filter out unsuitable areas so that occupied habitats can be further evaluated with field surveys.

## Materials and Methods

## Study sites

Our study was conducted in southern Ontario, Canada, with study areas located along the southeastern shore of Georgian Bay (northern study area), the northern shore of Lake Erie (southern study area), and a central study area located south of Georgian Bay on Lake Huron (Fig. 3.1). We selected these three study areas because they encompass a variety of different aquatic habitat types occupied by Blanding's Turtles, and reflect the potential range of thermal regimes experienced across southern Ontario (study areas range between  $42.2^{\circ}$  and  $45.2^{\circ}$  latitude). Within each study area, we monitored aquatic habitat types found within the core range of the local population of Blanding's Turtles. In the northern study area, we monitored four main aquatic habitat types including a coastal marsh, two bogs (east and west), and a vernal pool (Fig. 3.1). There are other vernal pools located in this landscape, but the particular pool we monitored was frequented by Blanding's Turtles in the springtime. It was also one of the few vernal pools to remain flooded during the winter study period, allowing us to monitor changes in water temperature. We analyzed the two bogs separately because they are distinct water bodies with unique physical attributes that may result in differences in water temperatures relevant to overwintering. For example, the East bog was significantly shallower with greater Sphagnum mat coverage compared to the West bog (Markle and Chow-Fraser 2014). In our southern study area, we monitored four aquatic habitat types: an open pool within a cattail marsh (herein referred to as south marsh pool), an open-water channel (herein referred to as open water), and two impounded wetlands (North and South; Fig.

3.1). The impounded wetlands were treated as separate habitat types because of differences in depth and vegetation cover and structure that may impact winter water temperature. The South impoundment is shallower, has more vegetation cover, and is dominated by dense cattails; whereas, the North impoundment has more open water and is dominated by graminoids. At our central study area, we monitored five aquatic habitat types, which included a string fen, thicket swamp, deciduous swamp, coniferous swamp, and a river (Fig. 3.1).

#### Blanding's turtle locations

We radio tracked adult Blanding's Turtles to identify occupied overwintering habitats. Any habitat that was occupied by one of the tagged turtles was considered confirmed overwintering habitat, while all others were considered unconfirmed. We used these designations solely to determine if sites occupied by radio-tagged Blanding's Turtles have thermal characteristics that are different from those of unconfirmed sites. In our northern study area, we radio tracked 12 individuals (six males, six females) in 2011 and an additional three turtles (two males, one female) in 2012 as part of another study (15 total; See Markle and Chow-Fraser 2014 for detailed tagging methods). Turtles were radio tracked on three dates during the winter season (19 November 2011, 24 February 2012, and 19 February 2013; Fig. 3.1). In the southern study area, we radio tracked 30 Blanding's Turtles (15 males, 15 females) and identified their overwintering sites on 6 November 2014 (Fig. 3.1) and confirmed sites in April 2014 and 2015. The central study area was very difficult to sample because of the rugged terrain; therefore, we radio tracked only three females and no males. On 31 October 2014 and 3 April 2015, we tracked the females in the central study area to identify overwintering sites (Fig. 3.1).

## Temperature data

We used Tidbit v2 temperature loggers (HOBO Onset, Bourne, Massachusetts, USA) to monitor water temperatures of 13 potential overwintering habitat types across our three study areas (Fig. 3.1). We randomly selected sites for aquatic logger deployment using ArcGIS 10.2 (Esri, Redlands, California, USA) and set loggers to record water temperature every four hours. In some instances, water depth (> 1m) and access prevented us from deploying loggers in the previously selected sites; therefore, we placed loggers as close to their randomly selected site as possible. We also installed three loggers within each study area to measure winter air temperatures (nine air sites total). We placed three loggers in 12 of the aquatic habitat types, and 10 loggers in the large continuous string fen complex in the central study area because of the large variety of suspected microhabitats. In total, we deployed temperature loggers at 46 aquatic sites (see logger sites in Fig. 3.1). We deployed temperature loggers from 21 September 2012 until 27 May 2013 at our northern site, from 7 November 2014 until 9 April 2015 at our southern site and from 30 October 2014 until 3 April 2015 at our central site.

Across all study areas, we mounted loggers used to monitor water temperature on rebar and placed them 7 cm above the substrate surface to approximate the location of an overwintering turtle, as outlined by Edge et al. (2009). We mounted loggers used to monitor air temperature on trees, 150 cm above the ground and shaded. Exclusive to the northern site, we set-up a Bushnell Trophy Camera XLT (Bushnell Corporation,

Overland Park, Kansas, USA, model 119466CN) at the East bog to capture daily photographs to monitor the timing of onset and breakdown of ice cover during the 2012/2013 season.

#### Analyses

We used a Spearman's correlation test (JMP v12; SAS Institute Inc., Cary, North Carolina, USA) to determine significant correlations among temperature datasets collected from loggers deployed within the same habitat type and study area. We pooled data from loggers deployed within the same habitat type and study area if  $\rho > 0.6$ . If temperature data were not correlated, we treated each independently to represent a unique thermal microhabitat within the larger aquatic habitat type (Table 3.1). We compared differences in mean water temperatures between confirmed and unconfirmed overwintering habitats using either non-parametric (Mann-Whitney U) or parametric (ttests) tests as appropriate. We also tested for differences among air temperatures at our northern, southern, and central study areas using a Kruskal-Wallis one-way ANOVA and Steel-Dwass post-hoc tests. Lastly, we conducted a break-point analysis in R 3.2.1 to define periods of change in the water temperature data (R Core Team 2015). Because data for the three study areas had been collected during different winters (northern in 2012/2013, and the central and southern in 2013/2014), we used a break-point analysis to statistically delineate pre-overwintering, overwintering and post-overwintering periods rather than use calendar dates to do this because we did not know if there were interannual differences in onset of freezing and thawing. For all statistical tests,  $\alpha = 0.05$ .

We used water temperature data associated with occupied habitat types to characterize suitable overwintering temperatures in two ways. First, we calculated mean water temperature during the pre-overwintering and overwintering periods. Second, we calculated the rate of water temperature change during the overwintering period to quantify the pattern of cooling or warming. To define the suitable range for each criterion, we calculated 95% confidence intervals of the mean for each occupied habitat type. We evaluated each habitat type and selected the lowest and highest temperatures estimated from the 95% confidence intervals. We used the selected temperatures to form a conservative definition of suitability for each criterion.

# <u>Results</u>

# **Overwintering sites**

Turtles in our northern study area overwintered in the two large bogs, and were not found in the coastal marsh or vernal pool. Individuals in the southern site occupied both impoundments and the southern marsh pool during the overwintering period, and none were found overwintering in the open water channel. All three female Blanding's Turtles in our central study area overwintered in the deciduous swamp.

In the northern and southern study areas, we found no evidence of males and females using different overwintering habitats. Exclusive to the northern site, however, Blanding's Turtles overwintered in groups of two or three individuals where turtles were within 10 m of each other (11 groups during the 2011/2012 winter and seven groups during the 2012/2013 winter). Because we radio tracked 12 turtles in the northern study area for two consecutive winters (the additional three turtles were only tracked for one

season), we were able to confirm site fidelity; 11 of the 12 individuals overwintered in the same habitat type, within 2–25 m of the location from the previous year. Remarkably, 11 of the 15 turtles overwintered in the East bog and experienced 99 d of continuous ice cover (21 December 2012 until 29 March 2013).

# Temperature data

Our three study areas included 13 different aquatic habitat types (Table 3.1). For 10 habitat types, temperature data from the three loggers within each habitat type were significantly correlated and were pooled. Except for the open-water habitat in the southern study area, one of the three loggers deviated from the other two and was treated as a different microhabitat type (Open Water A and B; Table 3.1). About a third of the loggers placed in the central study area yielded temperature data that were statistically unique and were treated as microhabitats (Fen A-E; Coniferous Swamp A-C; Table 3.1). The string fen spans over 3.5 km at its maximum length and provides both open water and densely vegetated microhabitats. After accounting for temperature correlations among logger sites, we were left with 20 potential overwintering habitat types that provided a unique thermal regime.

Onset and duration of the overwintering period were consistent among sites, beginning in November and ending in April, lasting between 133 and 147 d (Table 3.2; Fig. 3.2). There were among-site differences in air temperatures during the overwintering season (H = 72.12, P < 0.001); mean air temperature at the northern (-2.78° C) and southern (-2.19° C) study areas were not significantly different (Z = -1.17, P = 0.471), but they were both warmer than that monitored at the central (-5.52° C) study area (Z = -6.64, P < 0.001 for south vs. central; Z = -7.97, P < 0.001 for north vs. central). During the overwintering period, air temperature ranges were fairly equivalent among sites (northern site, 18° C to -29° C; southern site, 19° C to -30° C; central site, 19.5° C to -37° C).

We analyzed water temperatures separately for each of the study areas to elucidate differences between confirmed and unconfirmed overwintering habitats (Fig. 3.3). In the northern study area, water temperature of confirmed (2.68° C) overwintering habitats was warmer than that of unconfirmed (-0.07° C) habitats (Z = -43.3, P < 0.001; Fig. 3.3). A similar result was observed in the central study area (confirmed  $[1.00^{\circ} \text{ C}]$  vs. unconfirmed  $[0.15^{\circ} \text{ C}]$ ; Z = 22.9, P < 0.001; Fig. 3.3). The opposite was true, however, for the southern study area; mean water temperature of confirmed  $(0.54^{\circ} \text{ C})$ overwintering habitats were cooler than that of unconfirmed (4.08° C) habitats (Z = -36.6, P < 0.001; Fig. 3.3). Average water temperature of five of the six confirmed overwintering habitats never dropped below 0° C during the overwintering period (Table 3.1). The only confirmed overwintering habitat with average water temperature below  $0^{\circ}$ C was the deciduous swamp in the central study area (-0.33° C for 8 d). Furthermore, the lowest water temperature recorded by an individual logger in occupied habitat was in the East bog (northern study area) where water temperature was -0.62° C for fewer than 8 h. Also in the East bog, on two additional occasions, we recorded water temperatures below 0°C, both events lasting fewer than 8 h.

To assess differences between confirmed and unconfirmed overwintering habitat types at the landscape scale, we pooled temperature data across all three study areas. During the pre-overwintering period, mean water temperature at occupied habitats was slightly cooler (4.54° C) than that at unconfirmed habitats (4.77° C; Z = 5.35, P < 0.001; Fig. 3.4). On the contrary, during the overwintering period, mean water temperature at occupied habitats (1.77° C) was warmer than that at unconfirmed habitats (0.58° C; Z =50.27, P < 0.001; Fig. 3.4). We tested for significant differences in rate of change in water temperature (pattern of cooling) during the pre-overwintering and overwintering period. We saw no difference in slopes between confirmed (-0.02) and unconfirmed (-0.08) habitat types during the pre-overwintering period (Z = 1.36, P = 0.17). During the overwintering period, we found that water temperature decreased by  $0.50^{\circ}$  C every six weeks; whereas, at unconfirmed habitats, water temperature increased by 0.25° C every six weeks (t = 2.44, df = 8.47, P = 0.019). Because rate of change in temperature (slope) is a single value per habitat type, we used the smallest and largest slopes to define the suitable range. Based on rate of temperature change at each study area, water temperatures in suitable habitats decline at a slow steady rate of -0.25° C to -1.3° C every six weeks.

We determined that suitable water temperature during the pre-overwintering period can range between  $2.33^{\circ}$  C and  $10.22^{\circ}$  C and between  $0.44^{\circ}$  C and  $3.68^{\circ}$  C during the overwintering period (Table 3.3). Additionally, we calculated the variance and range in suitable temperature during the pre-overwintering and overwintering periods. For occupied habitats, temperature variance was higher in the pre-overwintering ( $12.6^{\circ}$  C) season than in the overwintering ( $2.25^{\circ}$  C) season; similarly, the range in temperature was higher during the pre-overwintering ( $20.83^{\circ}$  C) than in the overwintering ( $7.60^{\circ}$  C)

period (Table 3.3). Occupied habitats provided stable water temperatures during the overwintering periods, despite air temperatures dropping below -29° C.

# Discussion

Our study used *in-situ* water temperature data from confirmed and unconfirmed overwintering habitats to characterize thermal suitability of overwintering habitat for the Blanding's Turtle. For purposes of habitat protection and restoration, field data are most appropriate but are not always available or easily attainable. Across our three study sites, Blanding's Turtles overwintered in water bodies with an average water temperature of  $1.77^{\circ}$  C (± [SE]  $0.03^{\circ}$  C), which was significantly warmer than that of unoccupied sites  $(0.58^{\circ} \text{ C} \pm 0.01^{\circ} \text{ C})$ . This finding is opposite to that by Edge et al. (2009) where Blanding's Turtles in Algonquin Park were found occupying sites with water temperature cooler than what was generally available. That said, based on temperature data measured at occupied habitat types across all study areas, suitable overwintering water temperatures ranged between  $0.44^{\circ}$  C to  $3.68^{\circ}$  C, similar to the range between  $1.0^{\circ}$  C to  $3.0^{\circ}$  C reported by Edge et al. (2009). Taken together, it appears that Blanding's Turtles overwinter in habitat types with a specific water temperature regardless of whether warmer or colder water temperatures are available. Water temperature data collected at additional overwintering habitats in Ontario would be useful in confirming these findings because a population of Blanding's Turtles in Nova Scotia overwintered in water temperatures that had a much larger range  $(0.3^{\circ} \text{ C to } 7.6^{\circ} \text{ C}; \text{ Newton and Herman 2009})$ , and there was no evidence that turtles selected overwintering sites based on water temperature (Newton and Herman 2009).

Consistent with other studies (Litzgus et al. 1999; Newton and Herman 2009), we confirmed that overwintering water temperatures were stable (variance of 2.25° C), even though air temperatures were highly variable and dropped as low as -37° C. Our final in*situ* thermal characteristic, rate of temperature change, differed significantly among habitats with confirmed and unconfirmed occupancy. In all occupied habitats, water temperature slowly decreased throughout the overwintering period ( $-0.5^{\circ}$  C every six weeks). The steady decline in water temperatures may be energetically favourable and permit turtles to maintain an optimally low and stable body temperature as opposed to water temperatures in unoccupied habitats that slowly increased throughout the overwintering period (0.25° C every six weeks). Field studies in Ontario, Canada, have shown that a stable body temperature of just above  $0^{\circ}$  C is maintained by overwintering Blanding's Turtles (Edge et al. 2009), Spotted Turtles (*Clemmys guttata*; Litzgus et al. 1999; Rasmussen and Litzgus 2010) and Wood Turtles (*Glyptemys insculpta*; Greaves and Litzgus 2007). Although we did not measure the shell temperature of tagged Blanding's Turtles, Rasmussen and Litzgus (2010) found that Spotted Turtle shell temperature was not significantly different from water temperature recorded in occupied overwintering habitats. Therefore, our water temperature data are likely a close approximation to turtle shell temperature.

Considering the overwintering duration of the Blanding's Turtles in Algonquin Park was between 101 and 136 d (Edge et al. 2009) and ours was between 133 to 147 d, this species appears to overwinter for 3.5–5 mo in Ontario, Canada. The Blanding's Turtles that overwintered in the East bog (northern study area) were under continuous ice

cover for 99 d (confirmed through time-lapse photography), which is longer than the 87 d that Blanding's Turtles spent in anoxic conditions in Wisconsin (Thiel and Wilder 2010). Together, these data strongly support the hypothesis that Blanding's Turtles are anoxia tolerant, as suggested by Ultsch (2006), Edge et al. (2009), and Newton and Herman (2009) because ice cover would require turtles to remain submerged underwater without access to aerial oxygen. Blanding's Turtle's ability to survive prolonged periods of hypoxic or anoxic conditions suggest that dissolved oxygen conditions are not a major selection criterion of overwintering habitats, and indicates that water temperature is the primary selection criterion for the Blanding's Turtle.

At our northern site, both bogs and the vernal pool maintained sufficiently warm water temperatures above the freezing point of turtle body fluids ( $-0.6^{\circ}$  C); but, only the bogs were used for overwintering. The lowest temperature recorded by an individual aquatic logger was  $-0.62^{\circ}$  C in the East bog, a habitat that was used by majority of our tagged turtles in two consecutive winters. Although this sub-zero temperature lasted fewer than 8 h, these data lend support that adult Blanding's Turtles can survive in water temperatures of  $-0.6^{\circ}$  C at least temporarily. It is possible that other untagged Blanding's Turtles overwintered in vernal pools because water temperature was sufficiently warm; however, this population is small and was extensively surveyed. Therefore, it is more likely that the habitat was unsuitable based on other criteria. For example, in all study areas, sites used for overwintering were characterized by organic muck, deep enough to allow complete burial of the turtle. Although we were unable to confirm the overwintering position of our tracked turtles, the ability to bury in substrate may provide

protection from predators and temporary escape from freezing; however, prolonged burial inhibits access to dissolved oxygen (Ultsch 2006; Greaves and Litzgus 2007; Edge et al. 2009). The trade-off between requiring shelter and dissolved oxygen may explain why Blanding's Turtles have been found either partially (Ross and Anderson 1990) or fully (Kofron and Schreiber 1985; Sajwaj and Lang 2000) buried in mud, or just on top of the substrate (Graham and Butler 1993). Deep substrate was not available in the vernal pool, which was characterized by a shallow leaf-litter bottom and would not permit easy burial or concealment; potentially limiting suitable overwintering sites.

In the southern study area, all habitat types had water temperatures above 0° C, deep, organic muck substrate, and vegetation cover; however, we did not confirm occupancy in the open-water channel. In this situation, it is highly plausible that untagged turtles were overwintering in at least some of the sites deemed suitable because the population of Blanding's Turtles in the southern area is very large (Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2005). At the central site, however, 10 of the 11 aquatic habitats had water temperatures ranging from -0.01° C to -10.62° C with sub-zero temperatures lasting upwards of 90 d (Table 3.1). Water temperatures in many of these habitats are well below the freezing point of body fluids (-0.6° C) and likely do not provide sufficient overwintering habitat. The large string fen complex, however, had been suspected to provide overwintering habitat for Blanding's Turtles. While two fen sites (fen A, D) never dropped below -0.6° C, all sites in the string fen had a mean overwintering temperature that was below the range of temperatures we monitored at occupied sites. Instead, we identified the deciduous

swamp as overwintering habitat, a habitat type that has been declining in this wetland complex (Rootham and Featherstone 2014). Although the river site was thermally suitable, we did not confirm its use by Blanding's Turtles. In this case, we suspect that there are untagged turtles overwintering in habitat types that we did not monitor, but given the expansive and difficult terrain of this study area, it would have been extremely difficult to locate and track additional turtles.

Blanding's Turtles, among other freshwater turtles, have been found overwintering in groups (Ross and Anderson 1990; Newton and Herman 2009) possibly to increase access to mates or due to lack of suitable overwintering sites (Gregory 1982). Communal overwintering was observed in Algonquin Park (Edge et al. 2009), Nova Scotia (Newton and Herman 2009), and Wisconsin (Ross and Anderson 1990), similar to our observation at the northern study area. Greater availability of thermally suitable overwintering sites within the southern study area may contribute to the observed lack of communal overwintering. Limited communal overwintering in southern turtle populations is common (Gregory 1982; Brown and Brooks 1994) except for Wood Turtles, where communal overwintering is more apparent in southern compared to northern populations (Greaves and Litzgus 2008). Although Blanding's Turtles usually do not wander in excess of 5 m from their overwintering site, longer movements appear to be correlated with warmer water temperature and vice versa (Newton and Herman 2009). During the 2011/2012 overwintering season, only one female Blanding's Turtle in the northern study area made winter movements. Between her tracked locations on 19 November 2011 and 24 February 2012, she moved 25 m. Additionally, one other

individual was seen on 24 February 2012 with its head above the surface of the water, indicative of the ability of turtles to respond to stimuli (temperature, light, limited ice cover) during the overwintering season (Madsen et al. 2013)

To survive until spring time, turtles in Ontario must select overwintering sites that balance the risk of freezing, metabolic acidosis, and predation. Aquatic overwintering poses many risks to turtle survival, and increasing air temperatures and decreasing precipitation associated with global climate change may have negative ramifications on the suitability of overwintering sites. The predicted warmer winters and drier conditions will result in wetlands with warmer water temperatures, reduced ice cover and lower water levels (Flato and Boer 2001; Colombo et al. 2007). These factors can all impact turtle overwintering, especially for populations that exhibit site fidelity (e.g., Innes et al. 2008; Edge et al. 2009; Newton and Herman 2009; Thiel and Wilder 2010). If commonly used sites become unsuitable over time, and individuals are unable to locate new sites, large numbers of turtles can be lost from the population at once (Brooks et al. 1991). Some studies suggest that turtles may be able to shift to new overwintering sites, although the mechanism triggering this shift is largely unknown (McNeil et al. 2000; Herman et al. 2003).

Turtles living at the northern extent of their range already experience more variable and extreme climates compared to those living in more southern areas. When we analyzed the northern, central and southern water temperature data separately, Blanding's Turtles used sites differently compared to available temperatures. In the northern and central areas, Blanding's Turtles selected for warmer habitat compared to

unconfirmed habitats (Fig. 3.3), whereas those in the southern population selected for cooler habitat compared to what was available (Fig. 3.3). Newton and Herman (2009) suggested that turtles living in more northern areas would be better able to adapt to changing climates; however, existing habitat loss combined with climate change provides a unique set of challenges for species with a long generation time, and may render them unable to adapt to sudden environmental changes (Heller and Zavaleta 2009). Future research should investigate changes in phenology attributed to climate change because there is the potential for dissociation between times when turtles emerge and when resources become available. With warmer winter temperatures and reduced ice cover, turtles may emerge multiple times throughout the winter season. Additional energy reserves would be required to allow turtles to move into and out of overwintering sites, but these may not be forthcoming if food resources are unavailable during the winter months. Another risk is the possibility that the turtles become stranded on land when air temperatures suddenly plummet and ice forms over the surface, leaving the turtle to freeze (Neill 1948).

The impacts of climate change on turtles are largely unknown, but we can improve population resilience with targeted wetland conservation, restoration, and creation. Managers and planners can record wetland water temperatures during the winter months as a cost-effective framework to determine and monitor suitable overwintering sites. Our study provides thermal characteristics which define suitable overwintering sites in Ontario, Canada. Based on these characteristics, we can begin to determine the structural requirements of the site necessary to provide suitable water

temperatures. Parameters outlining suitable temperatures and additional research on the physical structure of suitable overwintering habitats can then help improve wetland restoration and creation projects to ensure they provide necessary overwintering refugia. As additional field data become available, temperature thresholds determined from *in-situ* research will have the greatest applicability for habitat conservation purposes, especially in the face of a changing climate. Determining thermal parameters suitable for overwintering is one step towards more thoroughly understanding where turtles overwinter and why, and can provide knowledge useful for habitat creation and ensuring current habitats continue to provide suitable overwintering conditions.

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<b>Table 3.1:</b> Lowest average water temperature experienced during the Blanding's Turtle
(Emydoidea blandingii) overwintering period in each habitat type monitored
in Ontario, Canada. Logger Unit # identifies the specific loggers averaged
for the analysis. Longest duration refers to the number of hours that the
temperature at a site dropped below 0° C.

Study Area	Aquatic Habitat Type	Logger Unit #	Lowest Average Temp (° C)	Longest Duration (h)	BLTU Occupancy
Northern	East Bog	1, 2, 3	2.39		Confirmed
	West Bog	1, 2, 3	0.05		Confirmed
	Marsh	1, 2, 3	-10.03	8	Unconfirmed
	Vernal Pool	1, 2, 3	-0.07	20	Unconfirmed
Southern	Open Water A	1	3.20		Unconfirmed
	Open Water B	2, 3	1.74		Unconfirmed
	South Impoundment	1, 2, 3	0.50		Confirmed
	North Impoundment	1, 2, 3	0.38		Confirmed
	South Marsh Pool	1, 2, 3	0.35		Confirmed
Central	Fen A	1, 5, 9	-0.01	12	Unconfirmed
	Fen B	2, 6, 7, 8	-2.04	1840	Unconfirmed
	Fen C	3	-1.27	40	Unconfirmed
	Fen D	4	-0.03	116	Unconfirmed
	Fen E	10	-1.79	2148	Unconfirmed
	Coniferous Swamp A	1	-1.67	1208	Unconfirmed
	Coniferous Swamp B	2	0.00		Unconfirmed
	Coniferous Swamp C	3	-10.62	1820	Unconfirmed
	River	1, 2, 3	-0.58	16	Unconfirmed
	Thicket Swamp	1, 2, 3	-1.83	1184	Unconfirmed
	Deciduous Swamp	1, 2, 3	-0.33	196	Confirmed

**Table 3.2:** Comparison of dates marking the beginning and end of overwintering periods<br/>for three populations of Blanding's Turtles (*Emydoidea blandingii*) in Ontario,<br/>Canada. Duration of consistent ice cover was ascertained from daily<br/>photographs taken of the east bog.

Study Area	Overwintering Start Date	Overwintering End Date	Duration of Overwintering Period	Duration of Consistent Ice Cover
Northern	9 November 2012	3 April 2013	147 d	99 d
Southern	14 November 2014	3 April 2015	140 d	
Central	23 November 2014	9 April 2015	133 d	

**Table 3.3:** Mean pre-overwintering and overwintering water temperatures (Temp; ° C) calculated for each of the habitat types where Blanding's Turtles (*Emydoidea blandingii*) occupancy was confirmed in Ontario, Canada. For each parameter, the most conservative confidence limit values were chosen to define the lower and upper boundaries of mean suitable temperature. We also indicate variance and range of suitable water temperature (° C) during the pre-overwintering and overwintering periods.

Parameter	Study Area	Aquatic Habitat Type	Mean Temp	Lower Mean Confidence Limit (95%)	Upper Mean Confidence Limit (95%)	Mean Suitable Temp	Suitable Variance	Suitable Range
Pre-	Northern	East Bog	9.80	9.37	10.22			
Overwintering Water Temp	<b>a</b> 1	West Bog	8.62	8.28	8.97	0.00		
water remp	Southern	S. Impoundment	3.02	2.92	3.11	2.33	10 - 50	
		N. Impoundment	3.11	2.96	3.26	to	12.60	20.83
		S. Marsh Pool	2.46	2.33	2.59	10.22		
	Central	Deciduous Swamp	5.98	5.47	6.48			
Overwintering	Northern	E. Bog	3.62	3.55	3.68			
Water Temp		W. Bog	1.58	1.52	1.65	<u> </u>		
-	Southern	S. Impoundment	0.70	0.69	0.71	0.44	2.25	7.60
		N. Impoundment	0.45	0.44	0.45	to	2.25	7.60
		S. Marsh Pool	0.48	0.47	0.49	3.68		
	Central	Deciduous Swamp	1.00	0.90	1.11			



Figure 3.1: The southern, central, and northern study areas for Blanding's Turtles (*Emydoidea blandingii*) are distributed between 42.2° and 45.2° latitude in Ontario, Canada. Locations of our aquatic temperature logger sites (closed circles), overwintering Blanding's Turtles (triangles), and habitat types are shown for each study area. We monitored four aquatic habitat types (E. Bog, W. Bog, Marsh, Vernal Pool; 12 logger sites) in our northern study area, four aquatic habitat types (Open Water, S. Marsh Pool, S. Impoundment, N. Impoundment; 12 logger sites) in our southern study area, and five aquatic habitat types (River, Fen, Coniferous Swamp (SWC), Deciduous Swamp (SWD), and Thicket Swamp (SWT); 22 logger sites) in our central study area.



**Figure 3.2:** Example of typical changes in water temperatures (logged every 4 h) through the fall and winter months corresponding to habitats with (a) confirmed and (b) unconfirmed occupancy of Blanding's Turtles (*Emydoidea blandingii*) in Ontario, Canada. Best fit lines through the same temperature data after performing a break-point analysis identify the pre-overwintering, overwintering and post-overwintering periods and change in temperature for habitats with (c) confirmed and (d) unconfirmed occupancy.



**Figure 3.3:** Mean water temperatures during the overwintering period in our central, northern, southern study areas for habitats with confirmed or unconfirmed occupancy of Blanding's Turtles (*Emydoidea blandingii*) in Ontario, Canada. Box plots are the central 50% of the data. The three horizontal lines of the box plots represent quantiles (25%, 50%, and 75% of the distribution). The closed circle is the mean value.



**Figure 3.4:** Mean water temperatures in confirmed and unconfirmed overwintering habitats during the pre-overwintering and overwintering periods of Blanding's Turtles (*Emydoidea blandingii*) in Ontario, Canada. Box plots are the central 50% of the data. The three horizontal lines of the box plots represent quantiles (25%, 50%, and 75% of the distribution). The closed circle is the mean value.

Ph.D. Thesis – C.E. Markle; McMaster University - Biology

Chapter 4: An integrative approach to regional mapping of suitable habitat for the Blanding's turtle (*Emydoidea blandingii*) on islands in Georgian Bay, Lake Huron

By

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### <u>Abstract</u>

Mapping suitable habitat for a species at risk is one of the first steps in a conservation plan. Creating habitat suitability maps can be very challenging when the area of interest is large and located in remote areas where field excursions can be difficult to implement. Such is the case for the Blanding's turtle, a threatened species in Ontario, that live on the Georgian Bay archipelago. With increasing anthropogenic pressures, maps indicating suitable habitat can aid management decisions and prioritize areas for protection. We apply an interdisciplinary approach using traditional field data and generalized linear models to produce high resolution, regional maps which identify suitable habitat for Blanding's turtles throughout the archipelago. We assessed the accuracy of our models using an independent survey dataset of 16 island sites distributed throughout the archipelago, and evaluated models using a reference island as a threshold for determining suitability of survey sites. Islands with higher proportions of wetlands and vernal pools were generally considered to be suitable for Blanding's turtles compared to those with lower proportions. Our findings highlight the importance of both permanent and temporary wet habitats for Blanding's turtles. Based on our final model, approximately 64% of evaluated islands support habitat for Blanding's turtles. Our study is the first to produce detailed habitat suitability maps for Blanding's turtles on the Georgian Bay archipelago. We recommend an integrative approach be applied to create habitat suitability maps for other species at risk in Georgian Bay.

### Introduction

Identifying potential or suitable habitat for species at risk can provide useful information when developing conservation strategies. Habitat suitability models based on environmental variables and habitat classes can be created to predict distribution of important habitats or species occurrence (Ottaviani et al. 2004). Resulting models can guide management plans, identify gaps in distribution, reveal areas with previously undetected populations, and predict distribution changes in response to climate change or land-use alterations (Manel et al. 2001). Development of effective habitat suitability models relies on availability of accurate and up-to-date information on the target species but such information is often limited. In the case of the Blanding's turtle (*Emydoidea blandingii*), conservations plans are empirically derived (The Blanding's Turtle Recovery Team 2002) and, in Canada, are available for areas where extensive research has previously been conducted (e.g., Nova Scotia and Quebec). For Ontario, development of a recovery strategy has been identified as a priority but knowledge gaps exist and additional research is required (Government of Canada 2015).

Across the species' range, the Blanding's turtle is known to use aquatic habitats such as vernal pools, bogs, marshes, and fens (Rowe & Moll 1991; Hartwig & Kiviat 2007; Edge et al. 2010; Markle & Chow-Fraser 2014), and terrestrial habitats throughout the active season (Ernst & Lovich 2009). During spring, Blanding's turtles emerge from overwintering habitats such as permanent pools (Ross & Anderson 1990; Graham & Butler 1993; Joyal et al. 2001), streams (Ross & Anderson 1990; Newton & Herman 2009), marshes (Kofron & Schreiber 1985; Rowe & Moll 1991; Edge et al. 2009; Seburn

2010), and a variety of upland wetlands (Joyal et al. 2001; Edge et al. 2009; Newton & Herman 2009; Seburn 2010). During the reminder of the active season, Blanding's turtles have been found to display site fidelity to residence wetlands (Congdon et al. 2011) but utilize a mosaic of aquatic and terrestrial habitats to move among wetlands and access nesting sites (e.g., Standing et al. 1999; Hartwig & Kiviat 2007; Beaudry et al. 2009; Markle & Chow-Fraser 2014). In addition to diverse habitat use, male and female Blanding's turtles may make long distance terrestrial movements (Ross & Anderson 1990; Rowe & Moll 1991), suggested to be an important vector for increased gene flow (McGuire et al. 2013); studies have reported males travelling 900 m in early summer (Markle & Chow-Fraser 2014) and females migrating over 6 km to nest (Edge et al. 2010). Extensive upland movements in combination with varied habitat use requires conservation plans which understand Blanding's turtle response to landscape composition. With the development of habitat suitability models, we can provide a landscape-level perspective on habitat requirements.

In Canada, the Great Lakes/St. Lawrence population of Blanding's turtles is listed as both federally and provincially threatened (COSEWIC 2005; Government of Canada 2009). Within the Great Lakes, a population of Blanding's turtles exists on the Georgian Bay archipelago, located in the eastern arm of Lake Huron and designated a world biosphere reserve (UNESCO 2014). Because Georgian Bay is only 2 hours north of Toronto, it is easily accessible to many weekend users and contains the busiest recreational waterway in Canada (Walton & Villeneuve 1999). Although the archipelago consists of mostly pristine habitat (Cvetkovic & Chow-Fraser 2011), increasing

development pressures threaten species and habitats (Walton & Villeneuve 1999). Limited data exist because the remote location and large number of islands make it difficult to conduct intensive field studies in the archipelago. Comparison of two Blanding's turtle populations on Canadian Shield, one on an island (protected island, Markle & Chow-Fraser 2014) and the other on mainland (Algonquin Park, Edge et al. 2010), revealed differences in habitat use and home range size. Selection of ephemeral wetlands was more pronounced in the island population, and average home range sizes were smaller compared to the mainland population (female: 20.5 ha vs 61 ha; male: 15 vs 57 ha, respectively; Edge et al. 2010; Christensen & Chow-Fraser 2012). Such a comparison of populations living in different parts of Ontario highlights difficulties that may arise when managers develop conservation strategies with data derived elsewhere when no relevant information exists for the system of interest (Hubert & Rahel 1989). In addition to differences in turtle home range size and habitat use, Georgian Bay is also recognized as the northern range limit for Blanding's turtles (Ontario Government 2014), and this may have implications for ectotherms that must adapt to cooler temperatures. Therefore, it is important that we develop a habitat suitability model using parameters appropriate to the Georgian Bay landscape, based on data collected only from the Georgian Bay archipelago.

To date, three models have been published for the Blanding's turtle, those of Poynter (2011), Barker and King (2012) and Millar and Blouin-Demers (2012). Millar and Blouin-Demers (2012) used two modeling approaches (boosted regression trees and maximum entropy modelling) to predict habitat suitability for southern Ontario. In their

resulting models, Millar and Blouin-Demers (2012) determined that habitat suitability increased with increasing air temperature and wetland area, and decreased with increasing cropland area. Given that cropland is limited only to the southern portion of Georgian Bay, the southern Ontario model may be unable to discriminate between suitable and unsuitable habitat in most of eastern Georgian Bay. Results obtained at a broad provincial scale are particularly useful for evaluating species distribution patterns, but are usually difficult to incorporate into specific conservation or recovery strategies that agencies aim to develop for specific parcels of land. Barker and King (2012) developed a parcel-specific model for the Gatineau Park, Quebec. They identified the suitability of individual wetlands for Blanding's turtles; however, transferability of their model to Georgian Bay is limited by inclusion of habitat features that they identified as being important to Gatineau Park, but which do not correspond with features in the Shield landscape of Georgian Bay (Edge et al. 2010; Markle & Chow-Fraser 2014). A similar approach was used to identify potential Blanding's turtle habitat in Ohio (Poynter 2011), although vegetation categories used were too coarse to be applied to the Georgian Bay context. Overall, it appears that the published models of habitat suitability are not directly applicable or transferable to the Georgian Bay archipelago.

The primary objective of our study is to develop a habitat suitability model for the Blanding's turtle specifically for the Georgian Bay archipelago, so that suitable habitat can be identified and marked for protection in conservation plans before habitat is degraded or developed. We assume that radio tracking data for a population of Blanding's turtles on a protected island can be used to indicate suitable habitat.

Therefore, we use landscape composition of the reference island to map habitat suitability of other islands within the archipelago. Secondly, we investigate changes in model accuracy when habitat data are extracted with different buffers (i.e. circular or grid). Specifically, we hypothesize that the approach which more specifically quantifies habitat used by radio-tracked turtles (circular buffer centered on locational point) will be more accurate in determining important landscape components compared to a more general approach (grid overlaid on the study area). The resulting model can produce maps at the regional scale for use in conservation and management strategies. We use an interdisciplinary approach that combines field data, remote sensing, and statistical modeling to produce spatially explicit statistical models to identify and map key habitats for the Blanding's turtle over a large region, and should advance efforts to develop effective management plans for Blanding's turtles throughout the biosphere reserve.

#### Methods

### Study area

Our area of interest includes all islands spanning the eastern shoreline of Georgian Bay from the French River to Severn Sound (Figure 4.1). Specifically, the study area encompasses island habitat in the Parry Sound Ecodistrict, which is found in the Georgian Bay Ecoregion in the southern portion of the Ontario Shield Ecozone (Crins et al. 2009). Restricting the model to an ecodistrict eliminates major landscape, habitat and geological differences which influence vegetation (Ontario Government 2007) and may result in differences in habitat use by turtles. The Parry Sound Ecodistrict currently supports relatively high biodiversity, including 11 reptile species at risk; due to increased

cottage development and recreational boating, some habitats are being threatened (Bywater 2013), although not to the same extent as are wetlands and natural habitats south of the Canadian Shield, that receive much greater negative impact from urbanization and agricultural development (Environment Canada 2013).

# Habitat classification

To map suitable habitat, we require both input data (spatial layers of different habitat types) and a suite of spatial and statistical tools (see Figure 4.2). We created habitat layers prior to model development and included all available habitat types in Georgian Bay as predictors in our models: forest, wetland, vernal pool, rock, and open water (Table 4.1; Markle & Chow-Fraser 2014). We decided to keep wetland as a broad category rather than sub-dividing since Blanding's turtles use a variety of wetlands at the home range scale (Markle & Chow-Fraser 2014) and use of a particular wetland type may depend on its availability within an island. Other than vernal pools, all habitat types could be classified from satellite image data; the vernal pools, however, could not be classified from satellite image data and required manual delineation in ArcGIS 10.1 (ESRI, California, USA). We used a combination of 2008 spring orthophotos (30 cm resolution), Google Earth (Digital Globe) and ground truthing to map all vernal pools on the islands. We digitized a feature as a vernal pool if we identified a small temporary pool (usually isolated within a forest matrix) typically visible only in images acquired during springtime. Temporary pools were often located around permanent upland wetlands or in forested areas.

To create the layer of forest, wetland, rock and open water, we used IKONOS imagery (Geoeye, Dulles, VA, USA), acquired during 2002 (22 scenes), July 2003 (19 scenes), July and August 2005 (3 scenes) and July 2008 (1 scene). All images were cloud-free, multispectral (red, green, blue and near infrared), pan-sharpened and radiometrically corrected with a resolution of 1 m. We classified IKONOS images in eCognition Developer 8.9.1 (Trimble, Munich, Germany) using a nearest neighbour (NN) approach at the image object level. Object-based image classification provides benefits over pixel-based classification such as including object shape and size (Blaschke 2010) and has been used to classify habitat for Blanding's turtles in Quebec, Ontario (Barker & King 2012). The NN approach combines multiresolution segmentation and supervised classification to identify object class based on selected training objects (Wang et al. 2004; Grenier et al. 2007). This approach requires a set of defined features to create a group of training and testing objects. Before training and testing objects were selected, we developed an initial rule set to classify major bodies of water and to separate upland areas for further segmentation. Once upland areas were segmented, we selected the training group to be representative of the range of objects present in the scene which allows for a more accurate classification. Since rule set transferability has been found to vary in its accuracy (Rokitnicki-Wojcik et al. 2011), each scene was individually classified with the NN approach. We randomly selected 10 of the 45 classified scenes to determine habitat classification accuracy. For each scene a stratified random sampling method was implemented similar to that of Grenier et al. (2007). A 1 km x 1km grid was placed over the scene and points were randomly generated in each grid. A total of 50 objects per class

were verified, excluding objects used for training. Verified objects (testing group) were then used to compute error matrices and kappa index of agreement (KIA). The kappa index measures the difference between agreement and agreement by chance (Viera & Garrett 2005). It is measured on a scale from 0 to 1, where 1 is perfect agreement and 0 is the outcome expected by chance.

All habitat classes were exported from eCognition 8.9.1 into ArcGIS 10.1 and individual islands in the archipelago were manually checked for boundary accuracy. All habitat classes were then converted to a 5 m cell size raster.

# Statistical analyses

We used locations of Blanding's turtle collected in 2011 and 2012 (obtained by radio-tracking and GPS devices; see details in Markle and Chow-Fraser 2014) on a protected island (considered our reference site) in southeastern Georgian Bay to quantify suitable island habitat. In total, location data from 15 adult Blanding's turtles (8 males, 7 females, 509 locations) were used to quantify used or suitable habitat. Turtles were radio tracked at least once per week during the active season (April – September) and hibernation locations were collected in November 2011, February 2012 and February 2013. To quantify unused or unsuitable island habitat, we randomly generated 1018 background locations in ArcGIS 10.1 that did not overlap with turtle locations. We included both suitable and unsuitable locations because modeling techniques that used both data sources have been shown to outperform those using presence-only data (Elith et al. 2006; Elith & Graham 2009). To extract habitat variables, we used the circular buffer approach and the grid approach (Figure 4.3). For both methods, data were extracted as

cover percentage in Geospatial Modelling Environment 0.7.2.1 (Beyer 2014). For the circular buffer method, we extracted habitat variables within two circular buffers (24 m and 58 m) surrounding turtle and background locations; distances were chosen to represent the minimum and maximum distances travelled by turtles on a daily basis in our reference site (Markle & Chow-Fraser unpub). For the grid method, we overlaid a grid with cell size equivalent to circular buffer area. We extracted habitat variables from cells containing turtle or background locations.

We used habitat variables (forest, wetland, vernal pool, rock, and open water) as predictors in 8 generalized linear models run in R 3.2.1 (R Core Team 2015) to determine the suitability of other islands in Georgian Bay. In a generalized linear model, the expected value of Y ( $mu_y$ ) is linearly related to the response variables (X<sub>i</sub>) through a link function (f(x)); so that:

$$f(mu_y) = b_0 + b_1X_1 + b_2X_2 + \ldots + b_kX_k$$

We chose generalized linear models because they are easily applied to new data to make predictions (Guisan & Zimmermann 2000; Early et al. 2008), are better for datasets including both detections and non-detections (Guisan et al. 1999; Elith & Graham 2009), and are frequently used in species distribution modeling (Guisan & Theurillat 2000; Randin et al. 2006). Since data for the buffer method were expressed in binary format (0 or 1), we ran generalized linear models (logit link function: f(x) = log(x/1-x)). We ran negative binomial generalized linear models (log link function: f(x) = log(x)) for the grid method since data were expressed as counts (many zeros, various integers). In models

using the buffer method, background data were weighted to have equal prevalence to turtle locations.

Of the 8 models, models (a), (c), (e), and (g) include all predictors for each approach (full model with all predictors), while models (b), (d), (f) and (h) are the models with the lowest corrected Akaike Information Criterion (AICc) for each approach (reduced model; Figure 4.2). We selected an information-theoretic tool as they tend to be preferred to methods such as stepwise regression (Guisan et al. 2002; Bolker et al. 2009) and the corrected AIC since it is advantageous in small-sample applications (Burnham & Anderson 2002).

### Spatial analyses

Our study area contained 16,586 islands, many of which are small and have mostly rocky habitat. Since the smallest Blanding's turtle home range in our reference population was 6.5 ha (Christensen & Chow-Fraser 2012), we eliminated all rocky islands < 6.5 ha from further analyses since this is likely smaller than the minimal area required by the Blanding's turtle on an island in the archipelago. Although Blanding's turtles home ranges have been estimated for other populations (e.g., Hamernick 2000; Piepgras & Lang 2000; Innes et al. 2008; Schuler & Thiel 2008; Edge et al. 2010; Millar & Blouin-Demers 2011), estimates can vary among studies due to sample size, duration of study and most importantly differences in habitat (Cagle 1944; Bury 1979). Therefore, setting the constraint using home range estimates from a population within our study area provides the most comparable estimate. Of the 16,199 excluded islands, majority were below 0.25 ha ( $\mu = 0.3$  ha  $\pm 0.006$ ) and unlikely to support Blanding's turtles. The

remaining 387 islands in the dataset had the best chance of containing potential Blanding's turtle habitat, and we applied a zonal statistics approach to obtain an overall suitability score for each of the islands. The 8 models run in R 3.2.1 (Figure 4.2) yielded statistical outputs that were applied in ArcGIS 10.1 to produce spatial representations of those equations. Since our reference island is known to support Blanding's turtles, the degree of similarity of other islands to our reference site was used to indicate their suitability as Blanding's turtle habitat.

#### Model evaluation

Testing data were required to determine the relative accuracy of each model's ability to determine potential or suitable habitat. Although it is more common to use data partitioning or resampling techniques to derive the testing dataset rather than using an independent dataset, the latter will yield more robust measures (Verbyla & Litvaitis 1989; Fielding & Bell 1997). We therefore conducted field surveys at 10 additional sites and obtained sighting data for 12 sites from local citizens (Figure 4.1). Citizen sighting data were only used if we could confirm species identification with photographs. We are withholding the exact location of specific sightings to protect Blanding's turtles and instead use general survey zones. Sites were chosen in similar fashion to an equal-stratified design (Hirzel & Guisan 2002) where shoreline was divided into regions and we attempted to randomly select sites based on our ability to access selected islands. Surveys were conducted in 2013 and 2014 during the summer months on sunny, calm days when possible. Each site was surveyed either by foot or cance with the aid of binoculars and

was searched for 12 person hours. All species of turtles encountered were recorded as either detected or undetected.

We used a threshold-based evaluation method to assess the appropriateness of using landscape composition of a reference island to map habitat suitability of other islands in the archipelago. For each model, the score assigned to the reference island is used as the threshold value. The threshold value is then used to evaluate whether or not external survey sites should be able to support Blanding's turtle and accuracy of each evaluation is assessed with field information. For example, for each model, we calculated the suitability scores for all external survey sites and our reference island. We then used the score for the reference island as a threshold value. When validating the model with external data, sites with scores that were greater than the threshold value were considered to be suitable and conversely, sites with scores less than threshold value were considered to be unsuitable. Models which correctly classified external survey sites in comparison to the reference island score were retained. Models that failed to correctly classify external survey sites were subsequently eliminated. Although threshold-based model evaluation is often used to classify areas into categories of either suitable or unsuitable habitat (Bean et al. 2012), we show final maps using continuous suitability predictions (from 0 to 1) and use thresholds only for evaluation.

# Results

### Habitat classification

Our habitat classification of the land cover layer had a mean overall accuracy of  $92.3\% \pm 1.68$  with an average kappa index of agreement of  $0.88 \pm 0.0198$ . Therefore, we

were confident in using the resulting classification to conduct the habitat suitability mapping.

# Statistical analyses

We ran 8 different models using a generalized linear model in R 3.2.1. In the full models, rock, forest, and open water were negative predictors of habitat suitability whereas wetland and vernal pools were positive predictors (Table 4.2). In reduced buffer approach models (b and d), forest was dropped at both scales. In reduced grid approach models (f and h), vernal pool was dropped at both scales. At the smaller spatial scale, reduced models that were created with either approach did not include rock as a predictor (d and h). For models using the buffer approach, wetland was the largest positive predictor of habitat suitability, followed by vernal pools. On the other hand, for models using the grid approach, vernal pool was the largest positive predictor of habitat suitability in model (e), and wetland was the primary predictor in models (g) and (h).

For each predictor, we individually plotted estimated marginal means which indicated mean response while holding other variables in the model at a constant value (Fox 2003; Table 4.2). Although we created one set of plots for each dataset, we only show plots using the 58 m buffer, full model dataset (model (a)) because all results were similar (Figure 4.4). Suitability of an island tended to decrease with proportionate increase in forest, open water and rock; on the contrary, suitability increased for islands that had a percentage increase in amount of wetland and vernal pools.

When selecting our reduced models (models b, d, f, h), there were instances where the top models had comparable AICc values. We chose to use AICc to select the reduced model; however, uncertainty exists in any selection process. Although still debated (Burnham et al. 2011; Richards et al. 2011), models with an AICc difference of less than 2 are considered as good as the 'best' model (Symonds & Moussalli 2011). In situations where  $\Delta$ AICc is less than 2, models are sometimes averaged together to create a new 'best' model (e.g., Rice et al. 2013), but due to our compositional dataset, averaging was not a feasible option (See Cade 2015). Instead, we determined our reduced models (models b, d, f, h) as those with the lowest AICc and relied on our external dataset to test the spatial accuracy of all 8 models to determine the final ('best') model for our intended mapping application. Since we do not use our final model to make statistical predictions, but rather a spatial mapping of suitable habitat, our approach should be valid.

### Spatial analyses and model evaluation

We confirmed presence of Blanding's turtles at 7 of our 22 external sites (Table 3). During field surveys, we also encountered additional turtle species such as spotted turtles (*Clemmys guttata*), midland painted turtles (*Chrysemys picta marginata*), snapping turtles (*Chelydra serpentina*), Northern map turtles (*Graptemys geographica*) and musk turtles (*Sternotherus odoratus*; Table 3). Since all surveyed islands were located on the Canadian Shield, have minimal human disturbance, and were distributed throughout the eastern shoreline of Georgian Bay, we are confident in extrapolating our model results to the entire archipelago (Figure 4.1; Hirzel & Guisan 2002; Vaughan & Ormerod 2005).

We applied all models in ArcGIS 10.1 to obtain predicted suitability scores for each island in our study site. Of our 22 external validation sites, 7 were eliminated based on the minimum size constraint (< 6.5 ha) or because they were deemed to be located too close to the mainland to function as an "island". After exclusions, we had 15 sites remaining to assess model accuracy. While the use of field data for model evaluation is considered rigorous (Verbyla & Litvaitis 1989), logistics surrounding island sampling limited our ability to survey each island multiple times, even though that is often desirable. Despite this drawback, our sampling protocol allowed us to detect Blanding's turtles and therefore we deem this to be sufficient for purposes of model evaluation. We used the calculated score for our reference island as the threshold for determining the ability of each model to classify external validation sites. Models (c), (d) and (g) were eliminated because external sites were incorrectly classified based on the corresponding threshold value. Model (e) and (f) successfully classified suitable sites, but incorrectly classified unsuitable sites and were therefore eliminated. Models (a), (b) and (h) (Figure 4.5) all correctly classified suitable sites and the highest number of unsuitable sites, but also classified 4 sites as suitable even though we did not detect Blanding's turtles there during our surveys. Of the models with the highest classification accuracy, model (a) and (b) estimated that 64% of evaluated islands provided suitable habitat for Blanding's turtles, whereas model (h) only estimated 60% of islands to be suitable. Although only 60 -64% of evaluated islands were considered suitable, this comprised 85 - 90% of the total area in this study. Model (a) and (b) identified 90% and 89% of total area evaluated as

suitable habitat for the Blanding's turtles, respectively; whereas, model (h) identified 85% as suitable.

# Discussion

When we visually compared the 3 habitat maps (a, b and h) that correctly classified external survey sites, we observed differences among them (Figure 4.5). We ran models (a) and (b) using data extracted with a 58 m buffer. While model (a) included all predictors, model (b) included only significant predictors (i.e. forest was dropped). Both models (a) and (b) yielded similar scores for islands; however, model (b) discriminated between islands with a smaller percentage of wetlands from those that had a high percentage of wetlands by giving them lower and higher scores, respectively. Therefore, we rank model (b) more highly than we do model (a). By comparison, model (h) included only forest, wetland and open water, but the data were extracted from a grid with size equivalent to the area of a 24-m buffer. Overall, model (h) was very conservative and more likely to make errors of omission where an island is given a low suitability score even though the target species is found there. A conservative model, like model (h), is more likely to omit important islands that support suitable habitat for Blanding's turtles, and lack of sensitivity (with most scores approaching zero) compared to other models make it less desirable for conservation purposes (Figure 4.5). Therefore, we ranked model (h) which only uses percent forest, wetland and open water to assess island suitability lower than model (b) which uses percent wetland, open water, vernal pool, and rock because it more accurately classified island suitability.

Our final habitat suitability model (b) indicates that approximately 64% of the evaluated islands or 89% of the total mapped area in the archipelago is suitable for Blanding's turtles. Our final model is consistent with large-scale modeling efforts of Ontario (Millar & Blouin-Demers 2012), where Georgian Bay was consistently associated with higher habitat suitability scores than were sites in southern Ontario. Since our model is intended for use in conservation, false absences (errors of omission) are more problematic than false presences (errors of commission) especially for the Blanding's turtle, a species at risk. We therefore recommend using a model that is prone to errors of commission (model b) where the model predicts suitable habitat even though the species cannot be detected. While the extent of suitable habitat will always be larger than a species' realized distribution and its overestimation may be preferred, the model should have reasonably good performance so that money and resources are not wasted (Fielding 1999; Zaniewski et al. 2002)

Our models scored the suitability of an island for Blanding's turtles from 0 to 1, based on the similarity of habitat features on the island relative to a reference island (Figure 4.5). We interpret an island with a score of zero to indicate that the island has low probability of having any suitable habitat for Blanding's turtles; conversely, an island with a score of one indicates that the island has very high probability of containing suitable habitat for the Blanding's turtle. We cannot, however, assume habitat suitability scores are proportional to prevalence, which would require model calibration. Our intention is to provide managers a means to identify locations of suitable habitat so they

can conduct proper field studies to survey for Blanding's turtles on islands that have high scores.

All models were based on radio tracking data that were pooled from both male and female Blanding's turtles. While our overall goal was to determine suitability of islands based on habitat requirements of both sexes, we also ran models separately for males and females to investigate differences between them. Parameter estimates differed by more than 10%, indicating that males and females do utilize different habitats in their home ranges; however, wetland habitat remained a strong positive predictor of suitability for both sexes. For males, vernal pools were also a positive predictor, highlighting the importance of this habitat feature in the Georgian Bay landscape. We therefore emphasize the need to capture variability in habitat use by males and females when creating overall models of habitat suitability for the Blanding's turtle.

Our models revealed the relative importance of wetlands and vernal pools on islands that are deemed suitable for Blanding's turtles; the higher the amount of wetland and vernal pools, the more suitable the site. Given that the Blanding's turtle is a semiaquatic species, frequent use of wetlands and vernal pools is expected and confirmed in previous studies (e.g., Joyal et al. 2001; Congdon et al. 2011; Millar & Blouin-Demers 2011; Markle & Chow-Fraser 2014). Both Fortin et al. (2012) and Joyal et al. (2001) found that increase in wetland area increased the probability of Blanding's turtle occupancy. Simulations run by Gibbs (1993) found that when small ephemeral wetlands were lost from the landscape, extinction risk for turtles increased, supporting our finding

that vernal pools are relatively important. Our models also suggested that amount of forest had a negative impact on overall island suitability score, or was not significant (Table 4.2). We do not interpret this as evidence that turtles do not require forest habitat, because other studies have found that probability of turtle presence increased with proportion of forest (Fortin et al. 2012; Quesnelle et al. 2013). Instead, Blanding's turtles have been found to use forest as upland travel corridors (Joyal et al. 2001) and for aestivation (Ross & Anderson 1990; Joyal et al. 2001) in some populations. We do not know the reason for reported differences, but we know that overall, landscapes with wetlands that are further from roads with more natural habitat composition (i.e. unmodified landscape) are important for sustaining species at risk (Litvaitis & Tash 2008; Millar & Blouin-Demers 2012). We propose that it is the matrix of natural landscape with wetlands that contribute to the importance of the Georgian Bay archipelago as being primary habitats for Blanding's turtles.

Since our model scores the suitability of an island based on the proportion of habitat types present on the landscape, naturally, some habitats contribute a higher relative proportion in comparison to the other remaining habitats. These type of data are known as compositional data (Aitchison 1982) and can lead to collinearity among predictor variables when used in model development. The nature of compositional data can be seen in modeling applications when signs of predictor coefficients differ among models with differing variables (Cade 2015). For example, in a model without forest as a predictor, rock becomes a positive predictor (Table 4.2, model b). In a similar fashion, in a model without vernal pools, wetland becomes a negative predictor; however, when this

happened in model (f), performance was poor (Table 4.2). Although some degree of collinearity exists in all field datasets, we aimed to limit impacts of collinearity on our model by restricting our predictions to the Parry Sound Ecodistrict which features similar landscape composition (Dormann et al. 2013). Moreover, in model (b) (i.e. our final model), not all predictor variables were retained in the model and, as a result, data were no longer compositional, which reduces collinearity among predictor variables.

Creating habitat suitability models for species at risk can be challenging as data on the target species are often limited. Not only are species-specific data difficult to acquire, but non-contiguous distribution of species-at-risk can affect the accuracy of habitat suitability models and lead to inflated errors of commission. For instance, even though the target species cannot be detected, suitable habitat may nevertheless exist on islands, as is seen in our final model (model b). In the case of the Georgian Bay archipelago, some of the islands with suitable habitat may be located too far to be colonized by the Blanding's turtle. Moreover, different water level regimes could lead to the formation of land bridges that allow dispersal to new islands or create isolated populations. Since our habitat use models were developed during the summers of 2011 and 2012 (Canadian Hydrographic Service 2012), all of our habitats have been mapped and suitability predicted based a relatively long period of low water levels. Given that the structure of vegetation communities in coastal wetlands are significantly affected by inter-annual variation of water-levels (Midwood & Chow-Fraser 2012), our model may be used in a comparison to investigate how changes in wetland habitats affect habitat use by Blanding's turtles under different water-level regimes. Additionally, although an

island may be determined to have suitable habitat, other variables may preclude Blanding's turtle's presence such as predator abundance and quality of nesting, feeding and hibernation sites.

It is common to delineate boundaries around habitat features before assigning habitat suitability scores to them (Store & Kangas 2001), even though these delineated boundaries are artificial and may not necessarily be recognized by wildlife. For instance, Blanding's turtles may be able to make use of several islands within a certain distance of each other on a seasonal basis. Thus, an island without any vernal pools, but that has suitable permanent wetlands, may still be used by Blanding's turtles if it is located within swimming distance of an island with vernal pools. To our knowledge, use of multiple islands has not been reported in Georgian Bay, although there is no reason to believe that multiple island use may not occur. Without data to determine the extent at which Blanding's turtles can swim to access habitats across multiple islands, we choose to evaluate each island separately.

We used high-resolution (5 m per pixel) satellite imagery to classify all habitat types within our study area. Acquisition of satellite imagery occurred during mid-summer (July and August) meaning that we were not able to use these images to map vernal pools. Instead, we used orthophotos acquired during spring, when pools are usually fully inundated and canopy cover is minimal. But, even with the combination of spring imagery and some ground truthing, it is likely that presence of vernal pools had been underestimated. We had neither time nor resources to conduct all the ground surveys to map the full extent of all vernal pools present on the landscape throughout the year, and

this should be acknowledged as a limitation. Although undermapping of vernal pools may have reduced the overall suitability score of some islands, we surmise that the error would have been small given the small proportion of vernal pools compared to wetlands, forests and rocks. Since the magnitude of change for a suitability score is dependent on the proportion of all habitat types on the island, addition or subtraction of a few vernal pools would not have changed the overall suitability of the island. We would need more detailed data on movement patterns before we can tease out how Blanding's turtles respond to variation in size, orientation and distribution of vernal pools throughout the landscape.

In Georgian Bay, the only way to protect wetlands and vernal pools is to get them designated as provincially significant under the Ontario Wetland Evaluation System (OWES; Ontario Ministry of Natural Resources and Forestry 2013b). Typically, wetlands must be larger than 2 ha in order to be eligible for evaluation, but wetlands < 2 ha or those within 750 m of each other may be evaluated if their ecological importance is determined (e.g., presence of species at risk). Midwood et al. (2012) found that 89% of the 3,771 coastal wetlands inventoried in Georgian Bay are < 2 ha in size, with an average wetland size of 1.4 ha, but despite their small size supported many important fish species (Midwood & Chow-Fraser 2014). This inventory suggests that many of the relatively pristine wetlands of Georgian Bay are receiving no formal protection. To receive protection, these wetlands must first be evaluated, and an evaluation is unlikely to be triggered unless nearby development is pending. Given that both wetlands and vernal pools are significant predictors of site suitability in our model results, loss or degradation

of either land-cover types could have negative impacts on Blanding's turtles. The importance of vernal pools for Blanding's turtles (Joyal et al. 2001; Markle & Chow-Fraser 2014) and other species (e.g., amphibians) has been recognized in the literature, but these ephemeral wetlands have yet to receive any formal protection as an independent category. Currently, the only way to protect vernal pools is to have each classified, on a case-by-case basis, as part of a wetland complex through OWES or the Blanding's turtle habitat regulation (Ontario Ministry of Natural Resources and Forestry 2013a, b).

Ensuring valuable habitats are protected is essential for long-term conservation efforts, especially because human development has increased throughout the archipelago in recent years (Bywater 2013). For areas experiencing higher levels of development, such as in Severn Sound and Honey Harbour, availability of habitat suitability maps at the scale of each island can help managers design more detailed and effective management plans.

### Conclusion

We mapped suitable Blanding's turtle habitat on islands in the Georgian Bay archipelago based on landscape composition of wetlands, vernal pools, rock, and open water. The most accurate model used data derived from a circular buffer centered on turtles' locations at the larger of the two scales (58 m vs. 24 m). Habitat models that used data derived from the grid approach or using a 24-m scale resulted in high errors of omission, and predicted that between 18 to 55% of evaluated islands provided suitable habitat. By comparison, our most accurate model indicated that 64% of evaluated islands (89% total area) have suitable habitats for Blanding's turtles. The importance of wetlands

and vernal pools in determining habitat suitability for Blanding's turtles is reflected in the literature, highlighting their high ecological value within the Georgian Bay archipelago. We produced maps using an interdisciplinary approach combining field data, external validation sites, and a spatial representation of statistical models to identify suitable habitats for Blanding's turtles in the Georgian Bay archipelago. Due to the sensitive nature of data regarding species at risk, our maps do not include names of specific island sites but we intend to freely provide our maps to management agencies, municipalities and interested conservation groups.

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**Table 4.1:** Definitions of habitat types following the Canadian National WetlandsClassification System (Warner & Rubec 1997) and Markle and Chow-Fraser(2014).

Habitat type	Brief description
Wetland	Contains water long enough to promote aquatic processes. We classified fens, bogs, swamps, marshes as wetlands in our study area.
Open Water	Large body of open water where the maximum depth is $> 5$ m.
Forest	Coniferous forest with needleleaf species such as white pine ( <i>Pinus strobus</i> ) and hemlock ( <i>Tsuga</i> spp.). Hardwood forest with species such as sugar maple ( <i>Acer saccharum</i> ) and beech ( <i>Fagus</i> spp.).
Rock	Rocky outcrops characteristic of the Canadian Shield.
Vernal Pool	Temporary pools that are only seasonally flooded. Also called ephemeral pools.

				18 1			Habitat Predictor	sr	
Model	Method	Size	Model	Intercept	Rock	Forest	Wetland	Open Water	Vernal Pool
(a)	Buffer	58	Full	0.737	-0.002	-0.026	0.070	-0.039	0.057
					(-0.17,0.17)	(-0.19,0.14)	(-0.10,0.24)	(-0.21,0.13)	(-0.12,0.24)
Q	Buffer	58	Reduced	-1.893	0.024	n/a	0.096	-0.013	0.083
					(0.01, 0.04)		(0.08, 0.11)	(-0.02, -0.00)	(0.01, 0.15)
(c)	Buffer	24	Full	1.092	-0.016	-0.027	0.059	-0.038	0.026
					(-0.14,0.12)	(-0.15,0.10)	(-0.07,0.18)	(-0.16,0.09)	(-0.11,0.16)
(p)	Buffer	24	Reduced	-1.479	n/a	n/a	0.083	-0.011	0.051
							(0.07, 0.09)	(-0.02, -0.00)	(0.00, 0.10)
(e)	Grid	58	Full	7.128	-0.065	-0.098	0.010	-0.111	0.078
					(-0.63.0.50)	(-0.67,0.47)	(-0.56,0.58)	(-0.68,0.46)	(-0.50,0.65)
Ð	Grid	58	Reduced	14.718	-0.140	-0.174	-0.065	-0.187	n/a
					(-0.23, -0.05)	(-0.27, -0.08)	(-0.16,0.03)	(-0.28, -0.09)	
g	Grid	24	Full	-1.907	-0.009	-0.021	0.055	-0.036	0.024
					(-0.21,0.19)	(-0.22,0.18)	(-0.14,0.25)	(-0.23,0.16)	(-0.17,0.22)
(l)	Grid	24	Reduced	-2.631	n/a	-0.013	0.062	-0.030	n/a
						(-0.02,0.00)	(0.05,0.07)	(-0.04, -0.02)	

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		Turtle Species					
Site Number	Zone ID	Blanding's turtle	Spotted turtle	Northern map turtle	Musk turtle	Snapping turtle	Midland painted turtle
1	А					х	
2	А						
3	А						Х
4	А						
5	А	Х					
6	В	Х					
7	С	Х					Х
8	С						
9	D					Х	Х
10	D						
11	Е			Х	Х		
12	Е			х			
13	Е			Х			
14	Е	Х					
15	Е	Х					
16	Е						
17	Е	х					
18	F						
19	F		х				
20	F	х		х			
21	F					Х	Х
22	F			Х	X		Х

<b>Table 4.3:</b> Survey results from each island are reported by corresponding survey zone
(See Figure 4.1). If a turtle species was detected, it is indicated with an 'x'.



**Figure 4.1:** Mapping area for determining suitable Blanding's turtle habitat along the eastern shoreline of Georgian Bay. Distribution and number of external survey zones are labelled A–F.







**Figure 4.3:** The circular buffer approach extracts habitat variables centered on the location of interest (Left panel). The grid approach extracts habitat variables from cells containing locations (Right panel). Notice how the locations in both panels are the same, yet habitat variables extracted differ based on the approach used.



**Figure 4.4:** We plotted estimated marginal means for each predictor individually using the binomial dataset with a 58 m circular buffer (Dataset 2; See Figure 4.2). Plots were created for each predictor while holding other predictor variables at a constant mean value. Response is shown with 95% confidence intervals. The x-axis is the value of the predictor and the y-axis is on the probability scale. The points distributed along 0 and 1 of the y-axis are the distribution of the raw data used to produce plots.



Chapter 5: Factors affecting coastal wetland occupancy for the eastern musk turtle (*Sternotherus odoratus*) in Georgian Bay, Lake Huron

By

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Presented as a manuscript and in preparation for submission.

# <u>Abstract</u>

In many jurisdictions, rare species and their habitats can receive protection if the species is assessed as being at risk of declining. The assessment process requires data on habitat occupancy, as well as identification of threats to species' critical habitat, both of which are difficult to obtain when the species occurs across large spatial scales. Such is the case for the eastern musk turtle (Sternotherus odoratus) that are obligate coastal wetland species in the Laurentian Great Lakes. We use data collected over 7 years between 2003 and 2015, to map occupancy and conditional occupancy for the musk turtle in coastal wetlands of eastern Georgian Bay (Lake Huron) to identify threats to resident wetland habitat. Data collected from a synoptic survey of 58 coastal wetlands were used to create occupancy models, estimate detection probability, and to conduct a sensitivity analysis to determine model robustness. We had a 64–71% probability of detecting a musk turtle whenever present in the wetland, and an AUC (area under curve) value of 0.82 confirmed high model accuracy. Coastal wetlands which supported musk turtles were associated with higher proportions of forest cover, lower densities of roads, buildings, and docks within 1 km of the wetland, and more variable bathymetric slopes. High conditional occupancy across majority of our study area indicates that at present, habitat quality in eastern Georgian Bay is in good condition; however, land-use alterations and development should be limited to ensure continued musk turtle occupancy.

# Introduction

Assessment of the status of species at risk requires key information such as longterm trends detailing the extent of occurrence and area of occupancy of the species, as well as identification of habitat requirements and threats to their critical habitat (e.g., COSEWIC 2012). Ideally, occupancy information and habitat assessments are collected in the field during targeted species surveys. Often, however, the species in question occurs across a large geographic region, in remote areas that are sensitive to human disturbance, or budgets restrict long-term intensive field surveys. Yet, lack of targeted survey data should not prevent environmental agencies from the important task of protecting imperiled species and their habitats.

In the case of the eastern musk turtle (*Sternotherus odoratus*), designated as Special Concern under the Ontario Endangered Species Act (2007), and as Threatened under the federal Species at Risk Act (2009), its populations were once widespread throughout Ontario, Canada, but recently, their numbers have declined substantially in southern Ontario. Currently, only a few areas within the Laurentian Great Lakes still support viable populations (Edmonds and Brooks 1996; Edmonds 1998; COSEWIC 2012), one of them being Georgian Bay, which is the large eastern arm of Lake Huron. The coastal wetlands of eastern Georgian Bay tend to be small and widely distributed (< 2 ha; Midwood et al. 2012; Fracz and Chow-Fraser 2013), many of which are not accessible by road (DeCatanzaro et al. 2009). Difficult terrain and limited access has resulted in lower levels of anthropogenic disturbance compared to other Great Lakes (Campbell 2005), and this is a major factor contributing to high quality habitat that

supports many species of birds, fish, amphibians and reptiles (Chow-Fraser 2006; Cvetkovic 2008). While the remote location (Maynard and Wilcox 1996) and relatively unique geomorphology (Maynard and Wilcox 1996; Rokitnicki-Wojcik et al. 2011) keep these wetlands in pristine condition, they also impede field campaigns, and limit the ability of managers to conduct targeted surveys at the landscape scale. Consequently, populations of eastern musk turtles are assumed to be stable, even though their abundances and distribution are unknown (COSEWIC 2012), and no data are available to assess the status of their populations or critical habitat.

The primary goal of our study was to investigate landscape and site characteristics that influence musk turtle occupancy of coastal wetlands. Given the documented sensitivity of this species to anthropogenic disturbance (including shoreline modification) and changes in land cover (DeCatanzaro and Chow-Fraser 2010), we predict that wetlands with higher density of docks, cottages and roads will have a lower probability of musk turtle occupancy compared with those with little or no anthropogenic disturbance. Additionally, we predict that coastal wetlands with a higher proportion of surrounding natural habitats, such as forest cover or additional wetlands, will have a higher probability of musk turtle occupancy. Our second goal was to incorporate detection probability to determine the applicability of occupancy modelling for musk turtles. Lastly, by producing occupancy maps, we will also provide insight into the current status of musk turtles and their habitats to guide conservation decisions.

# <u>Methods</u>

# Study site selection

A long-term synoptic survey of 89 coastal wetland units of Georgian Bay (Lake Huron) occurred between 2002 and 2015. Although the primary target of these surveys were Great Lakes fish, freshwater turtles were caught incidentally, among which were many eastern musk turtles. Since they were not the target species of the sampling effort, we developed a set of criteria to identify comparable sites that would be suitable for development of musk turtle occupancy models. First, we excluded wetlands that occurred within 500 m of another to eliminate the chance of an individual musk turtle being recaptured in multiple wetlands; thus allowing us to treat each site as independent sampling units. We only counted a turtle once for every group of wetlands occurring within 500 m of each other and therefore none of the occupied units were greater than 80 ha in size, a threshold that falls between the published home range size between 6.2 ha (Carrière et al. 2009) and 205 ha (Laverty et al. 2016), with majority being <50 ha in size (Belleau 2008; Picard et al. 2011; COSEWIC 2012).

We also restricted our study area to the Parry Sound Ecodistrict to maintain consistency among landscape, habitat, and geological parameters (Crins et al. 2009). Lastly, we excluded years with fewer than 10 sampled wetlands to minimize no-data records. The application of these three criteria led to the exclusion of 31 sites, leaving 58 coastal wetland units spanning the eastern shore of Georgian Bay from the French River to Severn Sound, that had been sampled during 7 years across a 13-year period (i.e., 2003–2006, 2009, 2014, 2015).

# Sampling methods

Following the protocol established by Seilheimer and Chow-Fraser (2006), all wetlands had been surveyed with modified fyke nets between late May and early September. As part of this survey protocol, fyke nets were deployed overnight according to a modified technique designed to reduce stress on non-target species such as turtles. Nets were secured in place with metal poles at the 1-m depth contour, with the top of the net extending above the surface of the water, allowing turtles that had been captured to access air. Additionally, floats were placed in the nets to ensure there were air pockets in case unexpected weather events dislodged the nets during the 24-hour soak time. In this fashion, a total of three sets of paired, unbaited fyke nets were deployed at each site (Seilheimer and Chow-Fraser 2006). These included two pairs of large nets (13 and 4 mm bar mesh, 4.25 m length, 1 m x 1.25 m front opening) and one pair of small nets (4 mm bar mesh, 2.1 m length, 0.5 m x 1 m front opening), which were set parallel to shore in locations where there were a good mix of floating, emergent, and submergent vegetation types. Fyke nets were set with pairs facing each other connected by a lead, with 2.5 m wings attached at a 45° angle to the net opening. All turtle species captured were identified and immediately released. Although we originally targeted coastal wetland sites for fish community surveys, previous research has also found that modified fyke nets are an effective trapping method for freshwater turtles (Vogt 1980; Smith et al. 2006; DeCatanzaro and Chow-Fraser 2010).

We want to clearly point out differences between our protocol and that used in commercial fishing, in which fyke-nets have been shown to negatively impact freshwater

turtle populations (Larocque et al. 2012a; Larocque et al. 2012b; Stoot et al. 2013; Midwood et al. 2015). The protocol we used did not pose the same threats to freshwater turtles as do commercial protocols, which require nets to be completely submerged underwater and left to soak for several days, often resulting in high turtle mortality (Midwood et al. 2015).

#### Model development and variables

We used PRESENCE 6.9 (Proteus Wildlife Research Consultants, Dunedin, New Zealand; Hines 2006) to estimate occupancy ( $\psi$ ; probability a site is occupied), conditional occupancy ( $\psi_c$ ; probability a site is occupied, given observed detection history), and detectability (p; probability of detecting a species using fyke nets, given it is present) of musk turtles in Georgian Bay coastal wetlands. Conditional occupancy refers to the probability that a wetland supports musk turtles even though we had not detected them during our sampling effort. Including this in our model allows us to more accurately identify truly unoccupied sites and allocate conservation resources accordingly. **PRESENCE** uses detection and non-detection data (i.e., binary data [0,1]) to establish occurrence within a sampling unit, and models are fit with maximum likelihood techniques (MacKenzie 2006). In this study, we defined a sampling unit as a wetland site. The single-season occupancy model accounts for species detection resulting in improved estimates of occupancy. Improved estimates are achieved by including multiple surveys of the same site to more accurately estimate true and false absences, thus providing a detection history. In our case, wetlands were surveyed across multiple years, which were treated as multiple surveys. If a wetland had not been surveyed every year, we included

these years as no-data records. For example, a wetland with a detection history of "00.11" indicates that no musk turtles were caught the first two years, the wetland was not sampled in the third year, and that musk turtles had been caught in the final two sampling years.

We used available geospatial data to develop a set of predictor variables hypothesized to influence musk turtle occupancy of coastal wetlands (Table 5.1), and quantified all variables in ArcGIS 10.2.2 (ESRI, Redlands, CA, USA). We obtained wetland boundaries from the McMaster Coastal Wetland Inventory (Midwood et al. 2012), and calculated surface area (ha) of each wetland unit. Road density, building density, and dock density were used as proxies for anthropogenic disturbance (e.g., shoreline modification, human population density, traffic volume). In addition, we included the percentage of wetland (includes surrounding coastal and upland wetlands) and forest to investigate the relative influence of availability of natural land cover on musk turtle occupancy. To elucidate the effect of spatial scale on turtle occupancy, we calculated density and percent land cover at two buffer sizes (250 m and 1 km) to account for the range of daily movements recorded for the eastern musk turtle (0.1 m to 1 km observed; Belleau 2008, Laverty et al. 2016). Both buffer sizes were generated as circular buffers (radius of 250 m or 1 km) centered on the fyke net location.

We calculated road density as road length (km) per buffer area (km<sup>2</sup>) using the 2014 road network file from the National Topographic Database. The number of cottages and docks were digitized and enumerated from a combination of IKONOS satellite photos (2002 – 2008; Geoeye, Dulles, VA, USA), spring orthophotos (South Central

Ontario Orthophotography Project, 2013) and Google Earth image data (Digital Globe, 2015). We calculated all density variables as the number of docks or buildings per buffer area (km<sup>2</sup>). Publicly available bathymetric data were obtained from NOAA (National Oceanic and Atmospheric Administration) with a spatial resolution of 5 m (contour spacing) and 1–2 m in some nearshore areas. We used these merged data (provided by D. Weller, McMaster U., unpub data) to calculate maximum wetland slope and range in slopes to assess the effect of wetland morphology on occupancy.

To remove multicollinearity and reduce redundancy among model variables, we performed a principal component analysis (PCA) using all 13 variables in JMP 12 statistical software (SAS Institute Inc., NC, USA). Prior to running the PCA, all data were z-transformed to standardize variables to a mean of zero. PCA is an ordination technique that extracts eigenvalues and eigenvectors from the original set of variables. It produces as many PCs as there are variables, which are weighted linear combinations of the original set of variables (Singh et al. 2004). Therefore, by using the first several PC axes as variables, we reduced model redundancy while accounting for a high amount of variability without losing important information. The resulting principal component (PC) scores were used as variables in our models.

### Model selection

We ran all combinations of candidate models using the single-season model in PRESENCE 6.9 and assumed that detection was constant for all wetland sites. Constant detection means that there is equal likelihood that a turtle would be detected if it were present in a wetland and a fyke net had been used as the sampling technique. Additionally, for the single-season model, we set our biologically relevant time period to the average life span of a musk turtle (14 to 20 years; COSEWIC 2012) to target one generation. This sampling scope is appropriate given the long generation time for this species. We ranked models using the corrected Akaike's information criterion (AIC<sub>c</sub>) and considered models with a  $\Delta AIC_c \leq 2$  as parsimonious with no single model outperforming another (Burnham and Anderson 2002). In situations where multiple models were considered equivalent, we calculated average occupancy for each wetland site using the following equation:

Average 
$$\psi$$
 (site<sub>i</sub>) =  $\psi$  (model<sub>1</sub>)\*AIC $\omega_1 + \psi$  (model<sub>2</sub>)\*AIC $\omega_2 + \dots$  (1)

where average  $\psi$  (site<sub>i</sub>) is the average occupancy for a wetland site when considering all parsimonious models. We calculated average occupancy using the occupancy estimate from the first parsimonious model (model<sub>1</sub>), multiplied by the corresponding AIC weight (AIC $\omega_1$ ), which was subsequently added to the product of the remaining parsimonious models.

#### Model validation

To validate our occupancy model, we randomly selected 25% of our wetland sites to be held back for model validation (14 sites) and used the remainder to develop the model (Figure S5.1). To ensure our resulting model was robust and not dependent upon sites used for model development, we randomly selected 3 different sets of development and validation datasets (herein referred to as selection 1, 2, and 3). This allowed us to conduct a sensitivity analysis to compare the model outputs resulting from the 3 different datasets. If our models were robust, the results would remain consistent across the 3 trials and give us confidence regarding model results.

We used R 3.2.1 (R Core Team 2015) to assess model performance, by plotting the receiver operating characteristic curve (ROC) to illustrate the performance of our model as the threshold is varied (Pearce and Ferrier 2000). The ROC plot examines the tradeoff between the true positive rate (sensitivity) and true negative rate (specificity), where a 45° diagonal line provides a visual representation of model accuracy. The closer the ROC curve to the diagonal line, the closer the model is to being random; the further the ROC curve from the diagonal line, the better the model is at describing the phenomenon. We calculated the area under the curve (AUC) as a measure of model accuracy; an AUC value of 0.5 indicates a model that makes predictions randomly (correct 50% of the time), whereas a value of 1.0 indicates that the model makes predictions that agree completely with observations (correct 100% of the time; Fielding and Bell 1997). From the ROC plot, we estimated the optimal cutoff value by selecting the threshold value that balanced the true positive rate with the true negative rate.

To provide an additional measure of model performance, we estimated overall raw accuracy by dividing the total number of correct classifications by the total number of sites. We used the derived threshold value to determine the cutoff value for estimating presence or absence. In other words, if the occupancy rate was greater than the threshold value, the site would be classified as occupied. Alternatively, if the occupancy rate was lower than the threshold value, the site would be classified as unoccupied. These predictions were then compared to the observed survey data to determine total number of

correct classifications.

#### <u>Results</u>

Our 58 wetland sites were sampled up to 4 times during the 13-year sampling period (2003-2015). Overall, raw occupancy (also known as naïve occupancy) ranged between 59–64% among the 3 model selections. Of the 29 wetlands with positive survey results, there were only 6 wetlands where sites were sampled multiple years and musk turtles were always captured. Majority of the wetlands sampled had mixed survey results (combination of detections and no detections). In general, we had a 64–71% chance of detecting a musk turtle in a coastal wetland using the modified fyke net protocol.

# Model variables

The first 3 axes of the PCA explained 67% of the total variation in the dataset (Table 5.2). PC1 reflected the degree of anthropogenic disturbance within 1 km of the wetland, at the landscape-level (27.3% variation), whereas PC2 was most associated with characteristics within 250 m of the wetland, or site-level characteristics (23.3% variation), and PC3 corresponded to differences in wetland morphology (16.3% variation). Accordingly, sites with positive PC1 scores corresponded to those associated with higher densities of roads, buildings and docks within 1 km of a wetland (0.85, 0.74, 0.58, respectively; Table 5.2). In addition to the size of the wetland (0.58), building density and percent of wetland within 250 m of the wetland were also correlated with PC1 (0.54 and 0.65, respectively). Sites with positive PC2 scores corresponded to sites impacted by site-level variables, and were associated with higher densities of docks and buildings within 250 m of the wetland (0.71, 0.68, respectively), in addition to higher

forest cover (0.54). Sites with negative PC2 scores were associated with larger wetlands and higher amounts of wetland in 250 m and 1 km buffers (-0.60, -0.60, -0.66, respectively). Finally, sites with positive PC3 scores reflected wetland morphology, and were associated with coastal wetlands that have steeper and more variable slope gradients (0.94, 0.94, respectively).

# Model selection and sensitivity analysis

We ran all combinations of reduced variables (e.g., PC1, PC2, PC3) to produce 7 occupancy models per selection round. Models with a  $\Delta$ AIC  $\leq 2$  were considered parsimonious and therefore were not eliminated (Table S5.1). In total, four models were considered to be equivalent; models A, B, C, and D (Table S5.1). Our sensitivity analysis revealed that occupancy estimates from each of the 3 development datasets were comparable (ANOVA, F(2,129) = 0.07, p = 0.93), where probabilities only varied by an average of 7% (±0.8 SE) for an individual coastal wetland. Similarly, estimates for conditional occupancy were comparable among the 3 models (Wilcoxon,  $\chi^2 = 1.13$ , df = 2, p = 0.57) and only varied by 3% (±1.4 SE) for an individual coastal wetland. Since estimates were consistent across the 3 models we calculated means of the 3 datasets to derive an averaged model of turtle occupancy.

In the final averaged model (Table 5.3), site-level characteristics were an important predictor of occupancy (PC2); as proportion of forest cover within 250 m of the coastal wetland increased, so did occupancy. Wetland size and percent of wetland in the landscape did not increase the probability of occupancy. Although human

modifications such as docks and buildings within 250 m of a wetland appeared to increase occupancy, modifications within 1 km of a wetland decreased musk turtle occupancy in coastal wetlands (PC1). Lastly, more variable bathymetric slopes and wetlands with a greater maximum slope were associated with musk turtle occupancy (PC3).

#### Model validation and predictive mapping

Area under the receiver operating characteristic curve (AUC) was 0.83 (lower 95% CI = 0.66, upper 95% CI = 0.92), which indicates that the averaged model was a better predictor of occupancy than was the null model (AUC = 0.5; Figure 5.1). The detection-non detection cutoff or threshold value of 0.52 resulted in a raw accuracy (total number of sites correctly predicted/total sites) of 74%. Specifically, the model's ability to correctly predict when a wetland was occupied (sensitivity) was 80% and its ability to correctly predict when a wetland was unoccupied (specificity) was 71%.

We mapped average occupancy and conditional occupancy estimates of predicted musk turtle occurrences within our study area (Figure 5.2). Musk turtles were more likely to occupy coastal wetlands associated with higher surrounding forest cover (at the sitelevel), lower densities of docks, cottages, and roads (at the landscape-level), and more variable bathymetric slopes (Figure 5.2a). When detection history was accounted for, predicted occupancy generally increased across the study area (conditional occupancy; Figure 5.2b). A few clusters of coastal wetlands were predicted to have lower conditional occupancies (Figure 5.2b); these sites tended to be associated with lower forest cover, higher levels of anthropogenic disturbances and very shallow or very steep slopes (reduced slope range). Therefore, our model predicted majority of the coastal wetlands in the Parry Sound ecodistrict to be currently occupied by musk turtles.

## Discussion

Consistent with our expectations, we identified land cover as an important predictor of coastal wetland occupancy. Specifically, coastal wetlands with the lowest densities of buildings, docks, and roads (our proxy for anthropogenic disturbance) within 1 km and highest proportions of forest cover within 250 m, had the highest probability of occupancy. We also found musk turtles were associated with more variable bathymetric slopes. Coastal wetlands with more variable bathymetric slopes typically support a more diverse plant community (Duarte et al. 1986; Randall et al. 1996; Lougheed et al. 2001; Wei and Chow-Fraser 2007; Cvetkovic 2008). Being a highly aquatic species, musk turtles rely heavily on wetland vegetation, particularly submergent aquatic vegetation (SAV), for shelter, foraging, and aquatic basking (Ernst 1986; Ford and Moll 2004). Typically, anthropogenically-disturbed coastal wetlands are more likely to be characterized by high nutrient concentrations and suspended solids, which decreases light penetration, and therefore are expected to have lower diversity of SAV (Lougheed et al. 2001). On the other hand, coastal wetlands surrounded by undisturbed land (i.e., forest) have been shown to have reduced nutrient and sediment runoff and therefore a higher diversity and areal cover of SAV (Dillon and Kirchner 1975; Reckhow et al. 1980; Beaulac and Reckhow 1982; Mohammad and Adam 2010).

Contrary to our predictions, we found an inverse relationship between occupancy and proportion of surrounding wetland habitat. We had expected turtles to use other

wetlands in the surrounding landscape, but this may not be applicable to wetlands in Georgian Bay. Highly variable topography (Kor et al. 1991; Campbell 2005) may prohibit upland movements among distinct wetlands, especially since musk turtles do not tend to move great distances on land (Ernst 1986; Buhlmann and Gibbons 2001). Longer distance movements tend to occur within water (e.g., Laverty et al 2016). Buildings and docks within 250 m of a coastal wetland was also not a significant predictor of occupancy (PC2). However, we do not want to over-interpret this because it may be an artifact of the low number of docks in our dataset (i.e., <1 dock/km<sup>2</sup> within the 250 m buffer). To test the effect of dock density at this scale would require a dataset with a range of dock densities and such conditions are not realistic in our study area nor desirable. It wasn't until the 1 km buffer size that densities of anthropogenic disturbance varied among wetlands. The more important site characteristics driving PC2 is likely the amount of forest cover within 250 m of the coastal wetland, which did have a significant effect on turtle occupancy. On average, wetlands that supported musk turtles had 70% forest cover within a 250 m buffer, whereas wetlands without musk turtles had a lower cover of 53%.

Musk turtles rely on surrounding upland habitat for oviposition, usually nesting within 50 m of a waterbody (Steen et al. 2012). A similar association between musk turtles and forest cover was found in the Thousand Islands ecosystem in southeastern Ontario (Quesnelle et al. 2013). Presumably, high dock densities are associated with increased motorboat traffic, which has been shown to contribute to musk turtle mortality (Bancroft et al. 1983; Bennett and Litzgus 2014); this could be related to motorboat injuries sustained by turtles when they bask just below floating vegetation (Edmonds
1998). These results are consistent with turtles' requirement for undisturbed wetlands with low nutrients and suspended solids (DeCatanzaro and Chow-Fraser 2010; Wieten et al. 2012), and confirm the negative impacts of land conversion and shoreline modification. Although Laverty et al. (2016) found that low-impact activities such as campsites do not severely impact musk turtles, our results indicate the wetland occupancy by musk turtles appear to be negatively affected by human activity. Therefore, increased density of docks, buildings, and roads within 1 km of an occupied wetland should be regulated.

Including detection history or how often a turtle is located can improve occupancy estimates, determine the number of times a wetland should be sampled before declaring absence, and evaluate effectiveness of a survey method. Furthermore, conditional occupancy results are important for small-bodied, secretive species like the musk turtle that can be extremely difficult to locate (COSEWIC 2012). If detection probabilities are not accounted for when estimating distribution of occupied habitat, severe underestimates may result. When detection was considered (conditional occupancy), coastal wetlands with a 61–100% probability of occupancy increased from 13 wetlands to 36 wetlands (Figure 5.2). The increase in number of wetlands that have suspected occupancy is desirable for conservation purposes; this designation is based on the likelihood that a wetland with low anthropogenic disturbance at the landscape-level, higher amounts of forest surrounding the wetland, and more variable bathymetric slopes would support musk turtles. Conversely, we had 8 coastal wetlands with low occupancy estimates (21–40%) that decreased even further when detection was considered (0–20%; Figure 5.2).

Despite the wetlands having land cover covariates that indicate suitable habitat, the wetland had been sampled on multiple occasions without a musk turtle being detected, and therefore has a high probability of being truly unoccupied.

We had a 64–71% probability of detecting a musk turtle using the modified fyke net protocol, if they were actually present in the coastal wetland. This means if a wetland is surveyed 5 times using our protocol, musk turtles should be captured during 3 of these surveys, if they were present in the wetland. By creating the model at the landscape level, we have not accounted for other factors that may influence wetland occupancy and may account for 30% of the time we did not detect turtles even though they were present. For example, competition for resources (Lindeman 2000; Luiselli 2008), risk of predation (Harding 1997; Marchand et al. 2002; Ernst and Lovich 2009), and use of specific microhabitats (Edmonds 1998; Picard et al. 2011), may influence where turtles are found within the wetland or affect their willingness to enter the fyke net. False absences can also be attributed to the fyke net protocol and time of year that nets are set. Since nets used in our study are restricted to the 1 m depth contour, musk turtles will only be detected if they are near these areas.

# **Conclusions and Management Implications**

Although our model is limited to the unique landscape of Georgian Bay (featuring granitic bedrock and thin soils), our framework can be adapted and applied to other geographic regions and for other species. Estimating occupancy at the landscape level allows for a regional approach to conservation decision-making, and provides an assessment of habitat quality and insight into the status of a population. Recently,

Environment Canada proposed a recovery strategy for the musk turtle, emphasizing land conversion and shoreline alteration as major concerns for the recovery of the species (Environment Canada 2016). If eastern Georgian Bay continues to be developed, water quality and wetland habitat will continue to degrade, which may have detrimental effects on the musk turtle population. Based on our results, we recommend that increases in the number of building, docks, and roads within 1 km of coastal wetlands be monitored and regulated and declines in forest cover within 250 m be limited to ensure long-term occupancy and persistence of musk turtles in Georgian Bay coastal wetlands. Occupancy modeling is most applicable for species that are long-lived and habitat specialists, and can be used to scientifically assess factors that govern occupancy for sensitive species across large spatial scales. Given that musk turtles are a small-bodied, secretive species, nontarget species data can improve our knowledge of their distribution while limiting resources spent on large-scale targeted surveying efforts, and can provide critical information where data gaps exist. Our occupancy maps can be used to guide future musk turtle surveys and identify coastal wetlands with high probability of occupancy to ensure site-level protection and population persistence.

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Variable	Description	Buffer Radius
Dock density (number/km <sup>2</sup> )	Density of docks within the specified buffer area	250 m, 1 km
Road density (km/km <sup>2</sup> )	Density of roads within the specified buffer area	250 m, 1 km
Building density (number/km <sup>2</sup> )	Density of buildings within the specified buffer area	250 m, 1 km
Forest (%)	Percent of buffer area classified as deciduous, coniferous, or mixed forest	250 m, 1 km
Wetland (%)	Percent of buffer area classified as wetland	250 m, 1 km
Maximum slope	Maximum slope within the wetland unit determined from bathymetry	n/a
Slope range	Range of slopes within the wetland unit determined from bathymetry	n/a
Wetland area	The total surface area of the wetland unit (ha)	n/a

**Table 5.1:** Description of predictor variables considered during model development.

PC axis	Variance	Variable	Loading
	Explained		
	(%)		
PC1	27.3	Road density (1 km)	0.85
Landscape-		Building density (1 km)	0.74
level		Wetland % (250 m)	0.65
		Wetland area	0.58
		Dock density (1 km)	0.58
		Building density (250 m)	0.54
PC2	23.3	Forest % (250 m)	0.54
Site-level		Building density (250 m)	0.68
		Dock density (250 m)	0.71
		Wetland % (1 km)	-0.66
		Wetland % (250 m)	-0.60
		Wetland area	-0.60
PC3	16.3	Maximum slope	0.94
Morphology		Slope range	0.94

**Table 5.2:** The first three principal components (PC) explained 67% of the variation in the dataset. Variables with a loading value higher than 0.54 are included in the table. All variables included in the PCA were standardized.

**Table 5.3:** Untransformed estimates of regression coefficients for predictor variables. Model A, B, C, and D are the models selected as parsimonious based on their AIC values. Regression coefficient estimates  $(\hat{\beta}_1, \hat{\beta}_2, \hat{\beta}_3)$  are provided for each of the 3 randomly selected model development groups.  $\overline{\hat{\beta}}$  denotes the average of the three regression coefficient estimates.

		•		•		•		^	
Model	Variable	$\widehat{f eta}_1$	$SE_1$	$\widehat{\beta}_2$	$SE_2$	$\hat{\beta}_3$	SE <sub>3</sub>	$\hat{\overline{\beta}}$	SE
А	PC2	0.47	0.23	0.39	0.22	0.38	0.24	0.41	0.22
	PC3	0.48	0.29	0.10	0.24	0.02	0.23	0.20	0.23
В	PC2	0.46	0.23	0.40	0.22	0.38	0.24	0.41	0.23
С	PC1	-0.03	0.21	-0.04	0.20	-0.18	0.21	-0.08	0.21
	PC2	0.45	0.23	0.40	0.22	0.36	0.24	0.40	0.23
D	PC1	-0.05	0.19	0.05	0.18	-0.21	0.21	-0.07	0.19



**Figure 5.1:** The receiver operating characteristic (ROC) curve generated using data from validation sites across the 3 model selections. The dashed lines indicate the 95% confidence intervals. The area under the curve for our occupancy model is 0.82. The ROC curve of a null model has an AUC of 0.5; models with greater AUC values perform better than the null. A model that perfectly predicts occupancy has an AUC value of 1.0.



**Figure 5.2:** Mean probability of (a) coastal wetland occupancy ( $\psi$ ) and (b) conditional occupancy ( $\psi_c$  occupancy given detection) for musk turtles. **Table S5.1:** Results of AICc-based model selection. Change in AICc ( $\Delta$ AICc) and AIC weights (AIC $\omega$ ) are shown for each of the 3 randomly selected development datasets. The weights resulting from the 3 development datasets were averaged to produce AIC $\overline{\omega}$ .

Model	Predictor Variables	ΔAICc	AICω
		$\Delta AICc_1$	$AIC\omega_1$
А	PC2, PC3	0.00	0.38
В	PC2	0.92	0.24
		$\Delta AICc_2$	AIC <sub>w2</sub>
В	PC2	0.00	0.47
		ΔAICc <sub>3</sub>	AIC <sub>\$\phi_3\$</sub>
В	PC2	0.00	0.37
С	PC1, PC2	1.63	0.16
D	PC1	1.78	0.15
			$AIC\overline{\omega}$
А	PC2, PC3		0.38
В	PC2		0.36
С	PC1, PC2		0.16
D	PC1		0.15



**Figure S5.1:** Framework used to complete occupancy modeling. Solid boxes represent a main step in the framework and dashed boxes represent the process that is occurring.

# Chapter 6: Effects of *Phragmites australis* on spatial ecology of Blanding's turtles

(Emydoidea blandingii) in wetlands

By

Chantel Markle and Patricia Chow-Fraser

Presented in manuscript format and under review.

### <u>Abstract</u>

Phragmites australis australis (European common reed) is an aggressive invader of North American wetlands that forms dense monocultures and replaces native flora. Dense patches of *Phragmites* generally provide poor habitat for many species, although specific impacts on at-risk turtles are largely unknown. Using radio tracking data for 46 adult Blanding's turtles within two wetland complexes in southern Ontario, we created three predictive scenarios to relate the amount of *Phragmites* in the landscape to amount of effective habitat for Blanding's turtles. At the home-range scale (2<sup>nd</sup> order), turtle home ranges were distributed irrespective of location of *Phragmites* patches within the population range, resulting in a positive association between turtle home range and *Phragmites*. At the individual scale (3<sup>rd</sup> order), however, turtle radio locations were significantly farther from *Phragmites* patches than from random points, consistent with the hypothesis that they avoided *Phragmites* patches locally. When we analyzed habitat selection by sex, results were similar to population-level results except for nesting females. During nesting migrations, females did not avoid *Phragmites* patches at the 3<sup>rd</sup> order scale, but instead, interacted with *Phragmites*, potentially placing themselves at risk of being stranded within dense patches of common reed. Our results are consistent with the hypothesis that invasion of *Phragmites* reduces the amount of effective habitat for atrisk turtles in wetlands, since Blanding's turtles significantly avoided *Phragmites* patches at the third order scale This is the first quantitative evidence of the negative consequences of *Phragmites* invasion on availability of effective habitat for the Blanding's turtles and on their spatial ecology.

# Introduction

European common reed, *Phragmites australis australis* (herein referred to as *Phragmites*), is a perennial grass that has aggressively invaded freshwater wetlands, watercourses, and beaches in North America (Chambers et al. 1999, Saltonstall 2002). *Phragmites* first establishes in high marsh or recently disturbed habitats and grows clonally from underground rhizomes to invade low marsh habitats (Amsberry et al. 2000). These patches become tall and dense (Haslam 1972), crowding out native flora by reducing solar radiation to the ground (Meyerson et al. 2000, Rice et al. 2000). Once established, *Phragmites* forms dense patches that result in altered habitat structure (Rice et al. 2000) and reduced plant diversity (Silliman and Bertness 2004).

The expansion of *Phragmites* and subsequent alteration to native habitat structure has affected many wetland-dependent fauna species including birds, amphibians, and reptiles (Meyerson et al. 2000). The effect of *Phragmites* on marsh birds appears mixed (Gagnon Lupien et al. 2015); while some ecologically sensitive species (e.g., ducks, heron, egrets, and sandpipers) have been unable to use monotypic patches (Benoit and Askins 1999), more robust species (e.g., marsh wren [*Cistothorus palustris*] and red-winged blackbird [*Agelaius phoeniceus*]) have been able to do so. Where herpetofauna are concerned, however, there is consensus in the literature. *Phragmites*-dominated sites supported significantly fewer species of reptiles and amphibians compared to sites with little to no *Phragmites* (Mifsud 2014). Loss of shallow aquatic habitat due to aggressive colonization of *Phragmites* has been linked to long-term declines of the Fowler's toad (*Anaxyrus fowleri*; Greenberg and Green 2013). Shading from tall stands of *Phragmites* 

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on beaches have lowered the temperature of the surrounding micro-environment and reduced hatching success of freshwater turtles (Bolton and Brooks 2010). Therefore, presence of invasive *Phragmites* is particularly detrimental to sensitive species in wetland habitats (Catling 2005, Gilbert et al. 2014).

The Blanding's turtle (*Emydoidea blandingii*), a medium-sized, semi-aquatic turtle relies on wetlands for feeding, mating, and overwintering, and often makes extensive over-land movements (Edge et al. 2010, Innes et al. 2008, Markle and Chow-Fraser 2016, Newton and Herman 2009). In Ontario, the Blanding's turtle is listed as Threatened (Ontario Government 2007) and the Canadian Great Lakes-St. Lawrence population was recently upgraded to Endangered (Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2016). While studies have shown the negative effects of *Phragmites* stands on turtle nests (Bolton and Brooks 2010, Cook 2016) and others have focused on mapping and understanding the distribution and spread of *Phragmites* in the landscape (e.g., Catling and Mitrow 2011, Bourgeau-Chavez et al. 2013, Samiappan et al. 2016), no study has been completed to understand how *Phragmites* effects habitat selection by at-risk turtles.

The purpose of our study was to determine if *Phragmites* affects the spatial ecology of Blanding's turtles. We hypothesized that Blanding's turtles can have 3 different responses to *Phragmites* which we will call the fixed home range (FHR) hypothesis (scenario A; Fig. 6.1A), the dynamic home range (DHR) hypothesis (scenario B; Fig. 6.1B), and the dynamic home range plus saturation (DHR+S) hypothesis (scenario C; Fig. 6.1C). In scenario A, we hypothesized that interannual distribution of

turtle home ranges will have a high degree of consistency (i.e., fixed) regardless of *Phragmites* invasion. This hypothesis predicted that at the home-range scale, turtles will not avoid *Phragmites* and may even appear to select for it when the amount of *Phragmites* increases in the landscape. At the individual scale, however, turtles will avoid patches of *Phragmites* because they are difficult to move through and do not provide optimal basking or foraging opportunities. Overall, as the amount of *Phragmites* in the landscape increases, the amount of effective or useable habitat will decrease since *Phragmites* will take over potentially suitable habitat that would subsequently be avoided by turtles (Fig. 6.2A).

In scenario B, we hypothesized that home ranges can, to some degree, vary spatially each year (i.e., dynamic). Therefore, the dynamic home range hypothesis predicted that turtles would alter habitat selection at the home-range scale to avoid *Phragmites*. In this scenario, turtles would use areas of the population range that do not contain *Phragmites* or contain less *Phragmites*. With comparatively less *Phragmites* in a turtle's home range relative to the population range, avoidance of *Phragmites* at the individual selection scale would not be detected. Therefore, as the amount of *Phragmites* in the landscape increases, the amount of effective or useable habitat would remain constant (Fig. 6.2B).

In scenario C, we hypothesized that home ranges can vary spatially each year (analogous to scenario B); however, *Phragmites* invasion will proceed until such a time that patches within the population range could no longer be avoided. The DHR+S hypothesis includes a saturation component that predicts turtles altering selection at the

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home-range scale to avoid *Phragmites* until *Phragmites* becomes so ubiquitous within the landscape that turtles can no longer avoid patches and may even appear to select for them. Nevertheless, at the individual scale, we predicted that turtles would avoid *Phragmites* because dense patches do not provide optimal basking or foraging opportunities and are difficult to move through. Therefore, availability of effective habitat would be constant at the beginning of the *Phragmites* invasion and eventually become reduced when a threshold of expansion is reached (Fig. 6.2C).

Relatively little is known about the effects of *Phragmites* on habitat selection by Blanding's turtles and their spatial ecology. To date, no published study has quantified the threat of invasive *Phragmites* on the effective habitat of at-risk species. Our study aims to provide insights on how habitat selection by the Blanding's turtle is affected by the distribution pattern of *Phragmites*, and inform managers on the importance of controlling this invasive species when designing recovery strategies for remaining populations of this sensitive freshwater species.

# Study Area

We conducted our study in two separate wetland complexes in southern Ontario located 125 km apart (herein referred to as study sites A and B). These wetlands were cattail marshes and located in agriculturally-dominated watersheds. Both wetlands support a variety of species at risk, including the Blanding's turtle and were first colonized by invasive *Phragmites australis* 45 to 70 years ago (Wilcox et al. 2003, Catling and Mitrow 2011).

In each wetland complex we delineated and classified habitat using ArcGIS 10.3 (ESRI, Redlands, California, USA). We classified habitat into 6 classes, including: aquatic marsh, emergent marsh, open water, *Phragmites*, treated *Phragmites*, and upland (Table 6.1). All remaining landscape features were grouped into broad habitat classes since we were mostly interested in the distribution of *Phragmites* within the wetlandupland matrix. Portions in both study sites had been treated to control invasive *Phragmites*; in site A, *Phragmites* were treated by rolling during the fall of 2009, and in site B, patches were sprayed with herbicide and then cut down during the fall of 2014. We selected image data that aligned temporally with turtle radio tracking data to minimize temporal discrepancies and used field-collected data to ground-truth the habitat delineations. For study site A, we classified 2010 orthophotos (Southwestern Ontario Orthophotography Project) to complement 2011/2013 radio tracking data. For study site B, we classified 2015 orthophotos (Southwestern Ontario Orthophotography Project) and 2015 image data collected by an unmanned aerial vehicle to complement 2014/2015 radio tracking data.

#### <u>Methods</u>

#### *Turtle movements*

We captured adult Blanding's turtles opportunistically by hand, dip net, or in baited hoop nets. We identified the sex of each turtle using secondary sex characteristics. We attached AI-2F radio transmitters (Holohil Systems Ltd., Carp, Ontario, Canada, 19 g) with quick dry and plumber's epoxy. The transmitter did not exceed 5% of the turtle's body mass. We released turtles at their original capture site immediately after tagging. In study site A, we radio tracked 8 females and 9 males. During the active season of 2011 (12 April 2011–23 August 2011) we tracked 4 males and 2 females, and in 2013 (27 April 2013–22 September 2013) we tracked 5 males and 6 females. In study site B, we radio tracked 15 females and 14 males during the active seasons of 2014 (21 April 2014–27 August 2014) and 2015 (12 April 2015–20 August 2015). Overall, we collected data for 46 adult Blanding's turtles (23 males and 23 females). We used a 3-element Yagi antenna (Wildlife Materials International, Murphysboro, Illinois, USA) and a Lotek Biotracker Receiver (Lotek Wireless, Newmarket, Ontario, Canada) to track each turtle, and we recorded GPS location and habitat data at each tracked location. All work was carried out under approved animal use protocols from McMaster University (#11-02-05, #14-09-35) and site-specific permits (WSCA 1076122/1073523, SARA-0R-2014-0260, ESA M-102-6326447130, AY-B-005-13).

### *Home range size estimation*

We estimated individual turtle home ranges and the population range in Geospatial Modelling Environment 0.7.2.1 (Spatial Ecology LLC,

www.spatialecology.com, 9 Aug 2016) and ArcGIS 10.3. To calculate individual turtle home ranges for the purposes of determining habitat selection, we selected a kernel density bandwidth (or smoothing factor, h) that resulted in the 95% contour area equaling the area of the 100% minimum convex polygon (Row and Blouin-Demers 2006). This modified approach has been used to study habitat selection in turtles (e.g., Edge et al. 2010, Paterson et al. 2012) and delineates home range based on the distribution of radio locations while allowing for a more standardized application when matched to the area of

the corresponding minimum convex polygon. For individual turtles that were tracked in multiple years, we pooled data for home range estimation. We estimated population range as the 100% minimum convex polygon surrounding all turtle radio locations and subsequently buffered this estimate (452 m for site A; 315 m for site B) to encompass home ranges of individual turtles (Edge et al. 2010, Paterson et al. 2012).

### Habitat selection

We used Euclidean distance analysis (EDA) to analyze habitat selection (nonrandom habitat use) at the 2<sup>nd</sup> order and 3<sup>rd</sup> order scale (Johnson 1980). We chose to use EDA, a distance-based analysis, over compositional analysis, a classification-based analysis (Aebischer et al. 1993), because it is more tolerant of radio tracking and GPS locational errors, can be applied at multiple spatial scales, and avoids the unit sum constraint (Conner and Plowman 2001). Euclidean distance analysis provides a method to compare observed habitat use to random habitat use by calculating distance ratios ( $d_{ii}$  =  $u_{ii}/r_{ii}$ ). To determine if habitats are being used non-randomly at the 2<sup>nd</sup> order scale, we compared habitat use in individual turtle's home ranges to the availability of habitat in the population range (Johnson 1980). Therefore,  $u_{ij}$  is the mean distance from random locations within an individual's (i) home range to the nearest edge of each habitat type (j; represents use) and  $r_{ii}$  is the mean distance from random locations within the population range to the nearest edge of each habitat type (represents availability). We also tested for non-random habitat use at the 3<sup>rd</sup> order scale by comparing habitats used by turtles to what was generally available within their home range (Johnson 1980). At this selection scale,  $u_{ij}$  was the mean distance from individual radio locations to the nearest edge of

each habitat type and  $r_{ij}$  was the mean distance from random locations within an individual's home range to each habitat type. If habitat use is random at either scale, we would expect the mean ratio of used distances ( $u_{ij}$ ) to random distances ( $r_{ij}$ ) to equal 1.0. If the turtle is closer to a particular habitat type than is expected by random, the distance ratio ( $d_{ij}$ ) would be less than 1.0 (selection). Conversely, if the turtle is farther from a particular habitat type than is expected by random, the distance ratio ( $d_{ij}$ ) would be greater than 1.0 (avoidance).

We tested for evidence of non-random habitat use using a MANOVA to identify any distance ratios which significantly differed from 1.0. We then used Wilcoxon signed rank tests to determine which habitats the turtles selected (d < 1.0) or avoided (d > 1.0). We used Holm's sequential correction when making multiple comparisons (Holm 1979). All spatial analyses were completed in ArcGIS 10.3 and statistical tests in JMP 13 (SAS Institute Inc.).

# Home range shift and overlap

We estimated the change in home range location between years for individual turtles in study site B that had a complete tracking dataset in two consecutive years. In particular, we excluded females if we did not capture the nesting migration in both years as this could artificially alter the home range location. For each individual turtle, annual home range was estimated using a 100% minimum convex polygon. We used ArcGIS 10.3 to determine the centroid of the home range and measured the distance between the 2014 home range centroid and the 2015 home range centroid. The calculated distance provides an estimate of far a turtle home range can shift between years. For the same

subset of turtles, we also calculated the percent of the 2015 home range that overlapped with the 2014 home range. While the centroid shift provides context to the overall location of the home range within the landscape, the percent overlap determines the amount of area that was used in both years.

# **Results**

We radio tracked 46 adult Blanding's turtles (26 males and 26 females) and collected 1,105 radio locations across four years. We collected an average of 20 ([SE]  $\pm$  4) radio locations per turtle (17 turtles) in study site A, and an average of 25 ( $\pm$  2) locations in study site B (29 turtles). When data were pooled for both populations, mean home range area was 32.8 ha ( $\pm$  6.4), ranging from 0.62 ha to 260 ha. Although the average area of female home range (46 ha  $\pm$  11.9) compared to male (20 ha  $\pm$  3) was not significantly different (Z = -1.8, P = 0.07), female home range size was double the area of males. In addition, females' home ranges were 0.67 km longer than males (1.6 km  $\pm$  0.2 vs. 0.93 km  $\pm$  0.1, respectively; Z = -2.5, P = 0.01).

Blanding's turtles in study site A had a population range that was 110 ha larger than those in site B (1293 ha vs. 1183 ha, respectively), but the amount of habitat invaded by *Phragmites* was comparable. Within both population ranges, 13–14% of area susceptible to invasion (i.e., wetland habitats and beach) was infiltrated by *Phragmites*. Although the amount of *Phragmites* within the population range was comparable, the distribution of *Phragmites* differed. Study site B had 5 times more individual *Phragmites* patches than did site A (581 vs. 153, respectively), resulting in patches occurring 12 m closer together in B than in A (20 m apart vs. 32 m apart, respectively; Figure 6.3).

# Habitat selection

Blanding's turtles were using habitats non-randomly at the 2<sup>nd</sup> order or homerange scale (Wilks'  $\Lambda = 0.77$ ,  $F_{10,1148} = 15.7$ ,  $P \le 0.001$ ). The most preferred habitat classes were emergent and aquatic marshes (lowest mean distance ratio; Fig. 6.4A). Turtles selected all habitat classes (Fig. 6.4A), including *Phragmites*. Patches of *Phragmites* were located closer to random locations within home ranges than to random locations within the population range (Z = -540.5,  $P \le 0.001$ ; Fig. 6.4A), indicating that turtles selected *Phragmites* at the home-range scale.

At the 3<sup>rd</sup> order or individual scale, there was also evidence of non-random habitat use (Wilks'  $\Lambda = 0.91$ ,  $F_{10,1148} = 5.44$ ,  $P \le 0.001$ ), but in this case, we identified avoidance of *Phragmites* within the home range (Z = 212.5, P = 0.01; Fig. 6.4B). Similar to the 2<sup>nd</sup> order scale, turtles selected wetland habitats within their home ranges. We also found turtles strongly selecting patches of treated *Phragmites* at the individual scale, whereas upland habitat and open water were used randomly with respect to availability within the home range (Fig. 6.4B).

We conducted the analyses separately for males and females. At the home-range scale, we confirmed non-random habitat use by males (Wilks'  $\Lambda = 0.67$ ,  $F_{10,568} = 12.3$ ,  $P \le 0.001$ ), all females (Wilks'  $\Lambda = 0.82$ ,  $F_{10,566} = 5.95$ ,  $P \le 0.001$ ), and for females when nesting migrations were excluded (Wilks'  $\Lambda = 0.86$ ,  $F_{10,568} = 2.07$ ,  $P \le 0.001$ ). For all 3 groups, patches of *Phragmites* were closer to locations within the home range compared to the mean distance at the population range (Male, Z = -138,  $P \le 0.001$ ; Female, Z = -138,  $P \le 0.001$ ; Female excluding nesting, Z = -138,  $P \le 0.001$ ; 2<sup>nd</sup> order; Fig. 6.5).

Habitats were also used non-randomly at the individual or 3<sup>rd</sup> order scale (Males, Wilks'  $\Lambda = 0.92$ ,  $F_{10,568} = 2.28$ , P = 0.01; Females, Wilks'  $\Lambda = 0.88$ ,  $F_{10,566} = 3.70$ ,  $P \le 0.001$ ; Females excluding nesting, Wilks'  $\Lambda = 0.79$ ,  $F_{10,568} = 6.95$ ,  $P \le 0.001$ ). Selection of *Phragmites* by males was similar at the population and home-range scale (Z = 77, P = 0.01; Fig. 6.5). For all females, however, they no longer appeared to avoid *Phragmites* at the 3<sup>rd</sup> order (Z = 31, P = 0.36; Fig. 6.5), except when the nesting migrations were excluded (Z = 99, P = 0.001; Fig. 6.5).

### Home range shift and overlap

We had comparable tracking datasets in 2014 and 2015 for 20 Blanding's turtles in study site B (11 males, 9 females). On average, the home range centroid shifted by 209 m ( $\pm$  38m [SE]) and home ranges overlapped by 53% ( $\pm$  6% [SE]). We also had two turtles where 94–100% of their home ranges overlapped and the centroid moved between 100–110 m.

### Discussion

We found evidence that turtles avoided *Phragmites* patches at the local scale. This is consistent with both the fixed home range (FHR) hypothesis (scenario A) and the dynamic home range plus saturation (DHR+S) hypothesis (scenario C). We eliminated the dynamic home range hypothesis (scenario B) from further consideration because Blanding's turtles appeared to select *Phragmites* at the home-range scale (2<sup>nd</sup> order). Since patterns of habitat selection can only be differentiated between scenarios A and C at the start of *Phragmites* colonization (See Fig. 6.2), and *Phragmites* has already been

established for 45 to 75 years in our study sites, we cannot eliminate either hypothesis. To be able to reject the DHR+S hypothesis, we would need time-series data of a population just beginning to experience *Phragmites* invasion. With such information, we would then be able to determine if turtles can change the location and orientation of their home range to avoid *Phragmites*, at least during the early stages of invasion. However, some studies have shown that individual Blanding's turtles' home ranges can vary on an annual basis (Grgurovic and Sievert 2005, Schuler and Thiel 2008), lending support towards the DHR+S hypothesis. In our study, we determined that home range centroids shifted by approximately 210m between 2014 and 2015 for 20 Blanding's turtles and home range area overlapped by 53%. Although this is only between 2 years and for a limited sample size, it does indicate that the location of Blanding's turtles' home ranges is dynamic to some degree. At the same time, the tendency for Blanding's turtles in our study to exhibit fidelity to resident wetlands may limit the extent of annual home-range variability. Therefore, a high degree of overlap in home ranges (over 50%) from year to year may be adaptive for turtles that overwinter in the same resident wetlands, given that there is limited connectivity to other suitable wetlands in a fragmented landscape.

Regardless of which hypothesis applies (FHR or DHR+S), avoidance of *Phragmites* at the individual scale has serious implications for the Blanding's turtles in an invaded wetland. Avoiding *Phragmites* at the individual but not at the home-range scale means a reduced amount of effective or usable habitat in the turtle's home range. In our study, *Phragmites* occupied approximately 13–14 % of wetland habitat, and this means that over 170 ha of habitat is no longer available for the Blanding's turtles. Without pre-

invasion data, we do not know if turtles used habitat patches that are now occupied by *Phragmites*. We do know, however, that turtles selected both aquatic and emergent marsh at the individual scale and that *Phragmites* was the only habitat avoided. Based on these data, the invasion of *Phragmites* and the subsequent replacement of wetland habitat reduced the amount of available effective habitat for Blanding's turtles. It is well documented that Blanding's turtles use aquatic and emergent marsh for feeding, mating, and overwintering (e.g., Bury and Germano 2003, Ernst and Lovich 2009, Edge et al. 2010, Markle and Chow-Fraser 2014). Therefore, loss of these critical habitats will likely have detrimental effects on the population in the long term. Even if Blanding's turtles varied their home range from year to year, reduction in total amount of effective habitat would be a certainty, as is a change in the spatial ecology of Blanding's turtles.

When males and females were analyzed separately, we found that results at the home-range scale were consistent with the pooled, population-level results. At the individual scale, however, females did not avoid *Phragmites* when nesting migrations were included. We attribute the female's lack of avoidance of *Phragmites* during nesting migrations to the need of gravid females to access nesting habitat on beaches as quickly and directly as possible, even if this means crossing through continuous barriers of *Phragmites*. Because of long-distance movements during the nesting season, the home ranges of females were 0.67 km longer and twice as large compared to those of males, leading to increased interactions with *Phragmites* patches. The tendency for females to exhibit nest-site fidelity (e.g., Markle and Chow-Fraser 2014; Standing et al. 1999) and thus use the same travel corridor and nesting area every year, means that their chance of

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encountering *Phragmites* patches would increase as these stands continue to expand. Therefore, gravid females may be especially susceptible to *Phragmites* invasions and may be at risk of dessication if they become stranded in dense stands during nesting migrations. This does not mean that males are safe from *Phragmites*; at study site A, a male Blanding's turtle was stuck in a dense *Phragmites* stand and desiccated, and other researchers have observed similar fatal situations (Gilbert et al. 2014).

We recommend that treatment and removal of invasive *Phragmites* be used to remediate at-risk turtle habitat. Treated patches of *Phragmites* were strongly selected at the individual scale (Fig. 6.4A). In study site A, we located tagged Blanding's turtles submerged in water under the stems of rolled *Phragmites*. In study site B, following spraying and cutting of *Phragmites*, we observed many radio tagged and untagged Blanding's turtles and other at-risk turtles basking in these newly open areas. Although this evidence is only anecdotal, it confirms that turtles can respond almost immediately and use newly treated patches.

In our study, managers concentrated treatment efforts on patches located in areas that had been frequently used by turtles. This may explain why we found a high positive association between radio locations and treated patches and why the treated patches were used so quickly. An appropriately designed study should be conducted to determine how turtles resume use of treated patches at various stages of revegetation. The scenarios presented in this study may assist interpretation of results from studies to determine how much effective habitat can be reclaimed after large-scale eradication of *Phragmites* is achieved.

# Management Implications

Our study is the first to provide quantitative evidence of the negative effects of Phragmites invasion on turtle habitat selection and spatial ecology. We found that invasive *Phragmites australis* influenced the movement of Blanding's turtles and led to avoidance of *Phragmites* patches at the 3<sup>rd</sup> order scale, thus reducing the amount of effective habitat within the landscape. In this study, we quantified a loss of 170 ha of marsh habitat, critical for Blanding's turtles for feeding, breeding, and overwintering. Gravid females were most at risk because of their nesting migrations and nest-site fidelity that resulted in increased interactions with *Phragmites* patches. This is concerning because population persistence is sensitive to small increases in the mortality of adult female turtles (Congdon et al. 1993). Desiccation of one male in this study means that both sexes are vulnerable to stranding in dense *Phragmites* patches. Given how rapidly *Phragmites* has spread across southern Ontario (Catling and Mitrow 2011), there is an urgent need to understand the long-term consequences that *Phragmites* avoidance, reduction of effective habitat, and alteration of thermal properties of habitats may have on the long-term viability of at-risk turtle populations in the province.

In light of our findings, we stress the importance of *Phragmites* control in wetlands, specifically wetlands that support species at risk. Although only relatively small patches of *Phragmites* were treated in our study sites, turtles selected treated patches at the 3<sup>rd</sup> order scale and we observed turtles using newly sprayed and cut patches for basking, and rolled patches for shelter. Based on our results, we recommend the continued treatment of *Phragmites* to restore marsh habitat used by Blanding's turtles for
feeding, mating, and overwintering. Given the current herbicide limitations in Ontario when treating *Phragmites* in the presence of standing water (Ontario Ministry of Natural Resources and Forestry [OMNRF] 2011), it is even more critical to continue developing and testing effective treatment methods for *Phragmites* in wetlands. While a vast number of treatment methods exist such as cutting, rolling, burning, or tarping (OMNRF 2011), a number of challenges to successful control of *Phragmites* remain. These include the timeconsuming nature of field implementation, short window of opportunity for assessment, and the variable response of wetland plants, including *Phragmites*, to control strategies (Hazelton et al. 2014).

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**Table 6.1:** Habitat types classified within delineated Blanding's turtle population rangesin two study areas in southwestern Ontario. Habitats were classified in2011/2013 for site A and in 2014/2015 for site B.

Habitat type	Description	Site A (ha)	Site B (ha)
Aquatic marsh	Greater than 25% cover of floating species (e.g., <i>Nymphaea odorata, Nuphar lutea, Brasenia schreberi</i> ), greater than 25% submerged species, and less than 25% emergent species (e.g., <i>Schoenoplectus</i> ).	23.0	123.0
Emergent marsh	Greater than 75% cover of cattails ( <i>Typha</i> ) with homogenous appearance. Seasonally-flooded meadow marsh dominated by grass and sedge hummocks.	312.0	504.0
Open water	Water deeper than 2 m with less than 25% vegetation cover.	310.0	238.0
Phragmites australis	Dense invasive <i>Phragmites australis</i> typically growing in circular shapes throughout the marsh.	73.0	103.0
Treated Phragmites australis	Invasive <i>Phragmites australis</i> treated through rolling (site A) or spraying and cutting (site B).	11.0	2.0
Upland	Any terrestrial area dominated by coniferous and/or deciduous trees or shrubs, including open or shrubby sand beach. Also includes anthropogenic land uses such as agricultural fields, campgrounds, open fields, roads, road shoulders, or trails.	420.0	214.0
Other	Habitats unique to either population and excluded from analyses (e.g., treed/thicket swamp).	144.0	0.0



**Figure 6.1:** Conceptual landscape schematic representing the (A) fixed home range hypothesis (FHR), (B) dynamic home range hypothesis (DRH), and (C) dynamic home range plus saturation hypothesis (DRH+S). Each schematic depicts the locations of a Blanding's turtle, the individual's home range, and the population range in relation to the distribution of *Phragmites australis*.



**Figure 6.2:** Simplified prediction curves corresponding to the (A) fixed home range hypothesis (FHR), (B) dynamic home range hypothesis (DHR), and (C) dynamic home range plus saturation hypothesis (DHR+S). Each curve relates the amount of effective turtle habitat to the amount of *Phragmites* in the landscape. The asterisk shown in (C) denotes that the inflection point is only an approximation and may occur elsewhere along the x-axis.



**Figure 6.3:** Comparison of *Phragmites australis* distribution in a portion of study site A (A) versus a portion of study site B (B).



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- **Figure 6.4:** The  $2^{nd}$  order (A) and  $3^{rd}$  order (B) mean (± SE) distance ratios for habitat types found in our two study areas in southwestern Ontario. Distance ratios were calculated using data collected from 46 adult Blanding's turtles in 2011, 2013, 2014, 2015. The asterisk denotes that the distance ratio is significantly different from one (dashed line). Mean distance ratios < 1 indicate that the habitat type was used more often than expected (selected), whereas mean distance ratios > 1 indicate that the habitat type was used less often than expected (avoided). Lack of significant difference between distance ratios and 1 indicates the habitat was neither selected or avoided more than expected.





**Figure 6.5:** Mean distance ratio for *Phragmites australis* at the 2<sup>nd</sup> order and 3<sup>rd</sup> order scale calculated using data from 46 adult Blanding's turtles in two study areas (southwestern Ontario) in 2011, 2013, 2014, 2015. The asterisk denotes that the mean distance ratio is significantly different from one (dashed line). Mean distance ratio < 1 indicate that the habitat type was used more often than expected (selected), whereas mean distance ratio > 1 indicate that the habitat type was used less often than expected (avoided). Lack of a significant difference distance ratio and indicates that *Phragmites australis* was neither selected for or avoided more than expected. Movements made and locations acquired during the nesting migration were removed when we calculated the distance ratio for the 'Female without nesting' category.

# Chapter 7: The true cost of partial fencing: Evaluating strategies to reduce reptile road mortality

By

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# Abstract

One of the deadliest roads in North America for species at risk fragments a marshlake ecosystem. To reduce road mortality, stakeholders installed >5 km of exclusion fencing along a southwestern Ontario, Canada, causeway in 2008–2009. Between 2012 and 2014, 7 culverts were installed to provide safe crossings. We evaluated the success of these mitigation strategies by 1) comparing results of road surveys conducted 5 years before and 5 years after fencing installation and 2) monitoring use of culverts by turtles using motion-activated cameras at culvert openings and stationary antennas placed to detect movements of passive integrated transponder (PIT)-tagged turtles (68 Blanding's turtles [*Emydoidea blandingii*] and 30 spotted turtles [*Clemmys guttata*]). We also radiotracked 30 Blanding's turtles to measure culvert use in relation to home ranges. Turtle and snake abundance was 89% and 53% lower, respectively, in completely fenced road sections than in unfenced sections; abundance was 6% and 10% higher, respectively, between partially fenced and unfenced sections. After mitigation, locations where we found reptiles on the road were associated with fence ends, underscoring the importance of fence integrity and ineffectiveness of partial fencing as a mitigation strategy. We confirmed use of culverts by Blanding's turtles, northern map turtles (Graptemys geographica), snapping turtles (*Chelydra serpentina*), and midland painted turtles (*Chrysemys picta*). Through radio-tracking, we determined that male and female Blanding's turtles home ranges overlapped with different segments of the causeway. We recommend that stakeholders emphasize ensuring fence integrity and continuity, limiting impact of edge effects, and conducting a comprehensive monitoring program.

## Introduction

Roads are a significant threat to biodiversity, often resulting in declines in sensitive species (e.g., Fahrig et al. 1995, Ashley and Robinson 1996, Steen and Gibbs 2004, Aresco 2005*a*, Fahrig and Rytwinski 2009). In addition to causing mortality, roads can have direct negative effects through habitat loss and fragmentation, and indirect effects through reduced landscape connectivity and gene flow (Jackson and Griffin 2000). Roads can also influence thermal and hydrological regimes, noise and light levels, and invasive species colonization beyond the road edge, generally known as the roadeffect zone (Andrews et al. 2008, Beckmann et al. 2010). In southwestern Ontario, Canada, roads are of particular concern because of the high incidences of species-road interactions, given that the great majority of all land in this region is within 1.5 km of a road (Gunson 2010). For reptiles, these interactions may be more frequent because they can be attracted to roads for thermoregulation (Sullivan 1981) and nesting (Andrews et al. 2008), or need to cross roads to access habitat for foraging, brumation, and mating. Road mortality can be detrimental for many species but especially for turtles, which have lifehistory traits (low juvenile recruitment and delayed sexual maturity) that increase their susceptibility to population declines following even small increases in adult mortality (Marchand and Litvaitis 2004, Steen and Gibbs 2004, Aresco 2005a), and snakes, which already face a number of threats in this region, including heavy persecution by humans (Ashley et al. 2007).

The conservation or establishment of corridors is often recommended to mitigate negative effects of habitat fragmentation by roads (Beier and Noss 1998). To properly

implement this mitigation strategy, a 2-stage approach is required. The first step is to construct exclusion fencing to prevent animals from accessing the road and guide them to crossing structures. Exclusion fencing can be installed in a variety of ways such as along one side of the road, both sides of the road, or certain segments of the road (Gleeson and Gleeson 2012); however, installation of any exclusion fence could restrict movement of target species across the landscape (Jaeger and Fahrig 2004). Therefore, the second step is to build culverts to permit safe passage under the road that fragments the landscape, or build wildlife bridges to permit passage over the roadway. Implemented crossing structures (i.e., corridors or eco-passages) can include drainage pipes (Mata et al. 2008), concrete box culverts, open-grate underpasses (Jackson 2003), large aquatic underpasses (Kaye et al. 2006, Baxter-Gilbert et al. 2015), or large overpasses (Clevenger and Waltho 2000, Healy and Gunson 2014).

Installation of fencing and culverts is a costly mitigation strategy that often results in limited resources being allocated towards a rigorous monitoring program. Consequently, little is known about overall strategy effectiveness (Lesbarrères and Fahrig 2012). We 1) analyzed the effectiveness of fencing to reduce reptile abundance on the road, 2) determined if target species used culverts and compared culvert monitoring techniques, and 3) established how Blanding's turtle (*Emydoidea blandingii*) ranges overlapped with a causeway. The causeway we investigated is one of the deadliest roads in North America for imperiled species (Aresco 2005*b*), especially semi-aquatic turtles such as the Blanding's turtle and spotted turtle (*Clemmys guttata*) that live in the area. First, we used data 5 years pre- and post-mitigation to analyze the effectiveness of

fencing, making this one of the longest evaluation periods documented in available literature, considering that the average post-monitoring period is 1.7 years (van der Ree et al. 2007). We hypothesized that exclusion fencing would mitigate the negative effects of roads, and predicted that turtle and snake abundance would significantly decrease along sections of the road with exclusion fencing in the post-mitigation time period compared to the pre-mitigation time period. Furthermore, if our mitigation strategy eliminated negative effects of roads, then we would expect reptile locations to be randomly distributed in relation to fence ends and culverts. Secondly, we wanted to confirm use of recently installed culverts by our target species to travel between habitat fragments and compare strengths and weaknesses of available culvert monitoring techniques. Specifically, we used passive integrated transponder (PIT) tags and stationary antennas to monitor culvert use by Blanding's turtles and spotted turtles, and motion-activated cameras to monitor use by all species. Thirdly, we established if male and female Blanding's turtles require road mitigation efforts at different locations because of differences in home ranges and movements. Blanding's turtles are of particular interest in road mitigation studies because they are designated a Threatened species at the provincial level and Endangered species at the federal level (Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2016, Ontario Government 2007), and known to make long-distance movements throughout the active season (COSEWIC 2005).

# Study Area

Our study took place along a 2-lane paved causeway (3.6 km) in southwestern Ontario. On average, 2,780 vehicles per day use the causeway between April and October, quadrupling on summer weekends (Wilson and Craig 2009). Following its initial construction in the 1920s, the causeway was raised by 1.5 m in the 1950s to reduce flooding across the road, which severed 3 of the natural marsh-lake connections. In 2012, one of these natural marsh-lake connections was restored, and efforts are underway to reconnect the remaining 2. To the east of the causeway was an open bay (referred to as bay or lake habitat herein) and to the west was a 1,200-ha wetland complex (Fig. 7.1). This wetland complex provided critical habitat for waterfowl, fish, and many Threatened and Endangered species (Environment Canada 2015). High levels of road mortality were documented in road-kill surveys conducted in 1979, 1980, 1992, and 1993 (Ashley and Robinson 1996). In response to growing concern over the high level of mortality, a project steering committee (consisting of government and non-government participants) was formed in 2006 to develop an action plan to mitigate against road mortality. This resulted in >5 km of exclusion fencing being installed along various portions of the causeway in 2008 and 2009 (Table S7.1). Silt fencing (1 m in height) was initially installed along the causeway, but later replaced with a woven geotextile (Hinspergers Poly Industries Ltd., Mississauga, Ontario, Canada). The 122-cm (48-inch) geotextile material was mounted on  $5 \times 10$ -cm ( $2 \times 4$ -inch) pressure-treated wooden posts using non-corrosive, large washers and deck screws. The bottom of the fencing was buried in a 20–25-cm (8–10-inch) excavated trench that was later backfilled. Following

complications due to substrate and environmental conditions, some sections of the geotextile were replaced by small gauge (0.32 cm or 1/8 inch) polyvinyl chloride (PVC) mesh netting (obtained from a fishing company in Nova Scotia, Canada), with small gauge (0.32 cm or 1/8 inch) galvanized hardware cloth used in drier, upland areas. Because segments of the road bordered privately owned property that could not be fully fenced off, there were segments of the causeway with only partial fencing (incompletely fenced on one or both sides of the road; sections A and D). Sections B and C had complete fencing (both sides fully fenced) and section E had no fencing throughout the 10-year study. Between 2012 and 2014, one hydraulic concrete box culvert, 3 terrestrial open-grate culverts, and 3 concrete box culverts were installed to provide safe passage under the causeway (Table S7.2). An additional culvert was installed in 2014 but remained blocked off and inaccessible to species for the duration of our study.

#### Methods

#### Field methods

Road mortality surveys began 5 years before (2003–2007) exclusion fencing installation in 2008–2009 and continued 5 years after (2010–2014) beginning in April and concluding in October. Between 2003 and 2007, we surveyed both sides of the 2-lane causeway on foot, or by vehicle. In the 5 years surveyed in the pre-mitigation period, we conducted an average of 22 surveys each month and a total of 154 surveys. From 2010 to 2014, we conducted road surveys on foot every Monday, Wednesday, and Friday. In the 5 years surveyed in the post-fencing period, we conducted an average of 40 surveys each month and a total of 284 surveys. On each survey occasion, we identified and counted all

reptiles that were alive or deceased in each of the 5 road sections (A to E in Fig. 7.1, Table S7.1). When we encountered a live individual, we moved it off the road in the direction it was headed. We considered both live and deceased individuals in our analyses because presence of either on the road represents a failure in exclusion fencing.

Culvert monitoring took place during 2014 and 2015 and involved the use of PIT tags with stationary antennas and motion-activated cameras. In spring of both years, we used baited hoop nets and dip nets to capture 68 adult Blanding's turtles (38 M, 30 F) and 30 adult spotted turtles (17 M, 13 F). In 2014, we affixed PIT tags (Biomark, Boise, ID, USA) to the carapace of 30 Blanding's turtles (15 M, 15 F); in 2015, we inserted PIT tags under the skin of 38 Blanding's turtles (23 M, 15 F) and 30 spotted turtles (17 M, 13 F). To detect the PIT tags as turtles traveled through the culverts, we mounted a stationary cord antenna attached to a custom-built PVC frame at the west entrance of 3 culverts each spring (Table S7.3). Both ends of the antenna were attached to an IS1001 portable enclosure (Biomark) powered by 2 deep-cycle batteries (12-volt, 75-amp hour, sealed lead acid; DCM0075, Interstate Batteries, Dallas, TX, USA). We housed the IS1001 portable enclosure and batteries in a plastic container covered by tarp and placed it on a raised wooden platform to prevent water damage. Any time a PIT-tagged turtle passed through the antenna, PIT-tag readers logged the date, time, and identification of the turtle. Additionally, we monitored culverts using motion-activated, time lapse cameras (PC900 HyperFire Professional, Reconyx, Holmen, WI, USA; Table S7.3). Cameras were set to medium-high sensitivity and on a 30-minute timer. Each time the motion-activation was

set off, 3 consecutive photos were taken. Each week, we changed antenna and camera batteries, downloaded data, and tested all equipment for proper functioning.

In 2014, we fitted 30 Blanding's turtles (15 F, 15 M) with radio-transmitters and 5 individuals also carried global positioning system (GPS) devices (Lotek Wireless, Newmarket, ON, Canada, 10 g; Telemetry Solutions, Walnut Creek, CA, USA, 30 g). We weighed all turtles to ensure attachments did not exceed 5% of the turtles total mass. We cleaned rear marginal scutes to allow for the best attachment of the AI-2F transmitter (Holohil Systems Ltd., Carp, ON, Canada, 19 g). We used a combination of speed set epoxy and putty epoxy to attach the transmitter. Transmitter and epoxy were colored black for camouflage. Once the epoxy was hard to the touch, we checked the transmitter for proper operation and immediately released the turtle at its capture location. Throughout the 2014 and 2015 active seasons, we radio-tracked each turtle at a minimum once a week with a 3-element Yagi antenna (Wildlife Materials International, Murphysboro, IL, USA) and a Lotek Biotracker Receiver (Lotek Wireless, Newmarket, ON, Canada). Whenever a turtle was located, we recorded the date, time of day, GPS location (accuracy <3 m), and activity. We carried out all work out under approved animal use protocols from McMaster University (no. 11-02-05, no. 14-09-35) and sitespecific permits (NWA-2014-02, WSCA 1076122, SARA-0R-2014-0260, ESA M-102-6326447130).

## Statistical analysis

We used a Poisson generalized linear mixed model in R 3.3.1 (R Core Team 2015) to determine if number of adults on the road differed before and after exclusion

fencing in road sections with complete fencing, partial fencing, or no fencing at all. We used a Poisson distribution to model our nonparametric count data and included amount of fencing (complete, partial, none) and period (before mitigation, after mitigation) as fixed effects. Because our data were repeated measures, we included year (2003–2014) and road section (A–E) as random effects to control for lack of independence. We conducted a larger survey effort post-mitigation; thus, to facilitate comparisons, we explicitly accounted for varying survey effort by including number of surveys as an offset term.

We used the SANET v4.1 (www.sanet.csis.u-tokyo.ac.jp, accessed 1 Sep 2015) extension toolbar in ArcGIS 10.2 (ESRI, Redlands, CA, USA) to test if locations where we found reptiles on the road (after mitigation) were independently and identically distributed along the causeway (i.e., unaffected by culvert locations or fence ends). Because we considered reptile road locations to be on-network events, we used the cross K function method in SANET v4.1 to analyze reptile road locations under the complete spatial randomness (CSR) hypothesis. We used locations of culverts or fence ends as our structural point inputs and reptile road locations as our temporal point inputs. We ran 1,000 iterations for Monte Carlo simulations and accepted significance at  $\alpha = 0.05$ .

We estimated Blanding's turtle population range, population core range, and male and female home ranges and core ranges using kernel density estimation (KDE) and the least-squares cross validation (LSCV) estimator. Kernel density estimation yields utilization distributions based on distribution of locations (Kernohan et al. 2001, Franklin 2010), providing an estimation of space use. We calculated all utilization distributions

using at least 50 locations as suggested by Seaman et al. (1999). We used the resulting utilization distributions or kernel density surfaces to calculate 95% and 50% isopleths to estimate range boundaries (Worton 1989, Seaman et al. 1999, Powell 2000). We used the 95% isopleth to estimate home range and excluded 5% of the outermost locations, which we considered to be casual forays and thus not part of the home range (Burt 1943). We used the 50% isopleth to estimate the core range, excluding 50% of the outermost locations. In ArcGIS 10.2, we calculated overlap of home ranges and core ranges with habitat types beyond the management boundaries of the marsh our study was conducted in. These habitat types included privately managed land, additional roads, agricultural land, and local conservation authority land. We calculated overlap to identify areas of potential conservation concern and direct future management actions. We calculated home range to estimate average Blanding's turtle dispersal distance (square root of home range area; Bissonette and Adair 2008). The average dispersal distance provides a measure of how far a turtle may move to access a culvert or bypass the fence through a compromised area or move around a fence end (Baxter-Gilbert et al. 2015).

In the event that an individual Blanding's turtle used a culvert, we estimated the individual's range and used ArcGIS 10.2 to determine the percent of overlap with the causeway. To estimate individual ranges, we used the minimum convex polygon method (MCP) because we did not have more than 50 locations per turtle to use the KDE method (Seaman et al. 1999). The MCP method is commonly used because of its simplicity and calculated by creating the smallest polygon around a designated set of locations (Mohr 1947, Burgman and Fox 2003). We calculated all kernel density estimations and isopleths

in GME 0.7.2.1 (Spatial Ecology LLC, Brisbane, Australia) and minimum convex polygons in ArcGIS 10.2.

#### <u>Results</u>

Between 2003–2007 and 2010–2014, we recorded 1,153 deceased reptiles (498 were hatchlings or juveniles). Mortality was greatest in April, with >90% of deaths corresponding to turtles and snakes. Reptile mortality continued to be high in May and June, consisting of almost half of all recorded species. Of the 13 reptile species recorded during the 10-year survey period, 6 had sufficient abundance data to be used for in-depth analyses of the effectiveness of exclusion fencing as a mitigation strategy. These species were the Blanding's turtle, spotted turtle, snapping turtle (*Chelydra serpentina*), eastern foxsnake (*Pantherophis vulpinus*), eastern ribbonsnake (*Thamnophis sauritus*), and eastern garter snake (*Thamnophis sirtalis sirtalis*).

For species at risk such as the Blanding's turtle (Endangered; COSEWIC 2005), spotted turtle (Endangered; COSEWIC 2004), and ribbonsnake (Special Concern; COSEWIC 2002), the number of individuals found per survey, post-mitigation, declined by 79%, 88%, and 96%, respectively. After the installation of fencing, we observed an 18% increase in the number of snapping turtles (Special Concern; COSEWIC 2008*a*) found per survey and an 8% increase for foxsnakes (Endangered; COSEWIC 2008*b*).

The average abundance of turtles (i.e., Blanding's, spotted, and snapping turtles) found on the road declined by 16% after installation of exclusion fencing. Of the total number of turtles found, 91% were deceased. There was a significant interaction between mitigation period (pre-fencing vs. post-fencing) and amount of fencing (complete, partial,

none) on turtle abundance (Fig. 7.2;  $\chi_2^2 = 36$ , P < 0.001). When the road was completely fenced, average turtle abundance declined by 89% between the pre-mitigation and post-mitigation periods, relative to the no fencing condition (Z = -3.91, P < 0.001). Although not significant, mean turtle abundance was 6% greater in sections with partial fencing compared to those with no fencing (Z = 0.47, P = 0.64)

The average abundance of snakes (i.e., eastern foxsnake, ribbonsnake, and eastern garter snake) found on the road declined by 13% after installation of exclusion fencing. Of the total number of snakes found, 93% were deceased. There was a significant interaction between mitigation (pre-fencing vs. post-fencing) and amount of fencing (complete, partial, none) on snake abundance (Fig. 7.3;  $\chi_2^2 = 15.9$ , P < 0.001). When the road was completely fenced, average snake abundance declined by 53% between the pre-mitigation and post-mitigation periods, relative to the no fencing condition (Z = -1.7, P = 0.08). Although not significant, mean snake abundance was 10% greater in sections after installation of partial fencing compared to those with no fencing (Z = 0.69, P = 0.49).

## Culvert use

Of the 30 PIT-tagged spotted turtles and 68 PIT-tagged Blanding's turtles, we confirmed that 2 male Blanding's turtles used the large aquatic culvert to safely cross under the road, one individual using the culvert in consecutive years (Table S7.4). Of the 15 male Blanding's turtles we radio-tagged, these were the only 2 to use the inner bay. Both male Blanding's turtles spent up to a month in the bay, traveled no farther than 65 m from the road and maintained a small active range of 20–25 ha (MCP) of which less than 15% overlapped with the causeway. In one instance, we tracked a turtle to the bay but

had no corresponding record of culvert use. After examining the fencing, we ascertained that the turtle crossed over the road through a compromised area. We immediately repaired the fencing and the turtle used the culvert later in the summer to cross back to the marsh.

Based on motion-activated, time-lapse cameras, we confirmed that the terrestrial open-grate culverts were used by northern map turtles, midland painted turtles, and snapping turtles, and terrestrial concrete box culverts (flooded throughout season) were used by Blanding's turtles (Table S7.4). Throughout the summer, our cameras also captured photos of snakes, but it was nearly impossible to identify these to species because of their small body size and most of them crossed during the night. We had a similar issue with species identification when culverts were flooded and most of the individual was submerged. In some instances, we could not determine culvert use because the camera did not capture a photo of the turtle on both sides of the culvert or inside the culvert. Instead, we interpreted a single photo of a turtle as an investigation of the culvert rather than as a confirmed usage.

#### Cross K function

In our cluster analysis, we found that post-mitigation reptile road encounters were distributed randomly and independent of the location of culverts, indicated by the observed curve falling within 95% confidence intervals. The observed curve of post-mitigation reptile road encounters indicated that events significantly clustered between 35 m to 75 m from the fence ends. We also found that reptile road encounters clustered

significantly between 90 m and 800 m from the fence ends. At the remaining distances from fence ends, road abundance was distributed randomly.

#### Home range analysis

We estimated population range, core range, and home range using kernel density estimation (LSCV estimator) with locational data from the 30 Blanding's turtles (15 F, 15 M) surveyed in 2014 and 2015 (Fig. 7.4a, b). We collected 349 locations for females and 433 locations for males over 2 years. As expected, female home range (828 ha) was larger than male home range (217 ha), given the longer distances females migrate to nest. But this was also true with respect to core range; female core range (159 ha) was larger than that for males (39 ha). Based on home range areas, average female dispersal distance was 2.9 km and average male dispersal distance was 1.5 km. In addition, home ranges indicated different areas of conservation concern. Female home range extended over unfenced section E and partially fenced section A, both of which lacked culverts. In contrast, male home range was highly concentrated over areas of the causeway that had culverts and complete fencing. Although male and female home ranges overlapped about 11–12% with the causeway, male core range overlapped 8% and female core range overlapped by <1%. Approximately 20% of the female home range stretched beyond the habitat managed by the federal government, and included a beach that was managed by the local conservation authority (2%), agricultural fields (5%), a public road (6%), and a privately managed marsh (6%).

With 790 Blanding's turtle locations (radio-tracked data and opportunistic finds), the population range spanned 3.1 km with a length of 4 km. Area of the 95% population

range was 526 ha (dispersal distance of 2.3 km) and the area of the 50% core population range was 83 ha. Even though the population range (13%) and core range (8%) overlapped with majority of the causeway (Fig. 7.4c), there were gaps in the existing mitigation strategy, notably in sections A and E, where there was limited fencing and no culverts. An estimated 11% of the population range extended over another road (south of the marsh; 3%), a private marsh (5%), section of protected beach (1%), and agricultural land towards the north (2%).

#### **Discussion**

Similar to other studies, we identified seasonal patterns in road mortality (e.g., Ashley and Robinson 1996, MacKinnon et al. 2005). Spring is a particularly vulnerable time for reptiles after they emerge from their overwintering ground; therefore, fences damaged from the previous winter should be repaired as early in the season as possible. A challenge when conducting a decade-long study on road mortality is maintaining consistent funding for the many aspects of the project (e.g., materials, installation, personnel, monitoring). As a result, the number of surveys conducted post-mitigation was greater than pre-mitigation (40 surveys/per month vs. 22 surveys/per month, respectively). When more surveys are conducted, the expected bias would be towards a greater number of individuals recorded on the road; however, this was not the case in our data. When accounting for survey effort in our analysis, we found reduced reptile road abundance in areas with complete fencing. In all likelihood, we underestimated reptile road abundance pre-mitigation, and the reduction in number of reptiles on the road was even greater with complete fencing than what we reported.

For Blanding's turtles, spotted turtles, and ribbonsnakes, there was a decrease in number of individuals found per survey following mitigation by 79%, 88%, and 96%, respectively. There was a 16% reduction in abundance of turtles and 13% reduction in abundance of snakes on the road post-mitigation; however, abundance varied significantly across the different road sections. We found 89% fewer turtles and 53% fewer snakes in sections with complete fencing. For turtles, sections with partial fencing did not reduce road abundance and even resulted in 6% greater road abundances. Implications of partial fencing were even more severe for snakes, resulting in 10% greater abundances on the road compared to pre-mitigation conditions. In the case of foxsnakes, we found 8% more snakes per survey after mitigation, but where they were found along the causeway changed, with more occurrences in areas with partial fencing. This finding is not surprising for the eastern foxsnake because fencing currently in use has limited effectiveness for larger climbing snakes. For adult snapping turtles, abundance increased by 18% after mitigation efforts, occurring primarily in sections of roads that had only partial fencing. Increased mortality had also been observed in central Ontario, where turtles were forced to make multiple crossings and increased their time on the road because of partial or compromised fencing (Baxter-Gilbert et al. 2015). In the central Ontario study, turtles accessed the road through an unfenced area, only to meet a fence on the other side of the road, and were then forced to cross back over the road again. Turtles are also known to retreat into their shell when cars pass by individuals on the road, a response that may increase time on the road (Andrews et al. 2015). Because the population of snapping turtles in our study area also appear to use the bay more

frequently than do Blanding's and spotted turtles (S. D. Gillingwater, Upper Thames River Conservation Authority, unpublished data), we advocate for complete fencing on both sides of the road and the development and installation of escape hatches to exit the fenced portion of the road where complete fencing is not possible.

Fences are the key to a successful road mitigation strategy but require a well thought out design to be effective (van der Ree et al. 2015), and finding effective fencing is a common challenge (e.g., Langen 2011). Initially, we installed silt fencing (commonly used to control erosion at construction sites) along the causeway. Although inexpensive, the material suffered from ultraviolet and wind damage, and the metal staples that attached the fencing to the wooden stakes often rusted away. A year later, we replaced the silt fencing with a woven geotextile; however, after 2 years, the geotextile fencing began to fail in areas exposed to high winds. The high winds would eventually cause the geotextile to rip off fence posts or sag between posts. We replaced some of these sections with mounted galvanized hardware cloth. Once again, these failed in some areas because the hardware cloth rusted away and ripped in damp marsh conditions. In marsh areas, we replaced the hardware cloth with a PVC mesh netting capable of withstanding both windy and wetter conditions, with a small enough mesh size to not entrap snakes. Since 2012, the main fencing system has consisted of geotextile material with smaller sections of PVC mesh netting in upland, windy areas. Even with this system, regular maintenance of fencing must be carried out to ensure tears, gaps, uprooted fence bottoms, flooded ditches, and vegetation do not compromise the effectiveness of the fencing. Going forward, Animex fencing (Animex International, Fareham, United Kingdom) will be

installed and tested as a more durable, long-term solution. Based on our findings, it is important that the new fencing design consider features to prevent species from climbing over the fencing (e.g., foxsnakes). More permanent concrete or metal sheet piling barriers have been beyond the financial limitations of the project.

Based on our cluster analysis, we found that majority of reptiles that are still getting onto the road do so by traveling around fences. We detected significant clusters within 35-75 m and >90 m of fence ends. Because the causeway has 6 fence ends that are at least 400 m apart, clusters identified at large distances (90–800 m) are likely identifying neighboring fence end clusters. Based on the average dispersal distance of a female Blanding's turtle, an individual would travel up to 2.9 km (straight line distance) during a movement event. Given that the causeway is 3.6 km in length, a Blanding's turtle is likely to encounter one of the 7 culverts under the roadway. This makes it imperative to have intact fencing to direct turtles to the culvert openings, because they will gain access to the road if they encounter a compromised area in the fencing. Instead of fences ending abruptly, we suggest that fencing be angled away from the road to lead animals back to safety, as demonstrated in Florida (Aresco 2005b) and recommended in Ontario (Ontario Ministry of Natural Resources and Forestry 2013). Length of the angled fencing will likely depend on the habitat features, but based on our results, should reach 75 m if possible. If the entire 3.6 km roadway is fenced, featuring curved ends, even species that make large distance movements will be less likely to circumvent the fence. Furthermore, with the addition of new culverts at the end of 2016, the causeway will

provide a total of 12 safe crossing opportunities, increasing the chance an individual will encounter a culvert before a fence end or compromised area.

We identified use of the large aquatic culvert (culvert 3) by 2 male Blanding's turtles using PIT tags and radio-tracking. Photos obtained with motion-activated cameras were unable to confirm Blanding's turtles traveling through a culvert; however, we did confirm a Blanding's turtle investigating a large terrestrial culvert (culvert 5). Neither Blanding's turtles nor spotted turtles were found to use or investigate the remaining culverts (culverts 1, 2, 4, 6, 7) during the period surveyed (May–Aug), although it is possible some movements to or from overwintering sites had not been captured. Lack of confirmation for the spotted turtle may mean that this species needs more time to discover and use culverts because radio-tracking data from 2004 to 2007 indicate that spotted turtles do cross the road in this marsh complex (S. D. Gillingwater, unpublished data). We were able to confirm that northern map turtles, painted turtles, and snapping turtles used terrestrial open-grate culverts (1, 2, and 7), all of which were only recently installed in the fall of 2014 (and operational for the first time in 2015). This suggests that with correct placement within the landscape, culverts can reconnect habitat in the next active season following installation.

The 3 approaches we used to monitor culvert usage had different advantages and disadvantages (Table S7.5). Motion-activated cameras were the least costly (\$700 per camera) and required minimal field work (10 hr/week), and have been widely used (e.g., Dodd et al. 2004, Crosby 2014, Taylor et al. 2014, Baxter-Gilbert et al. 2015); however, in this study, they were difficult to use to confirm usage of culverts by animals, nor allow

us to positively identify small-bodied species. Furthermore, they were ineffective for aquatic culverts because cameras could not capture images of animals below the water surface. The PIT tag and stationary antenna cost about \$4,000 per set up plus \$10 per PIT tag. This approach allowed us to confirm usage of culverts regardless of the animal being submerged. We were also able to easily identify species and sex of the animal within the culvert because they had been tagged at the beginning of the study. This method required about 30 minutes of processing per week to download data and change batteries, but was limited to the number of animals fitted with a PIT tag. The third approach was to combine the PIT tag and stationary antenna with radio-tracking. This option was the most costly (~\$13,000 for 30 turtles) and also required up to 40 hours per week of tracking, but it allowed us to determine how the target species made use of their fragmented habitats. Given these available options, we recommend that terrestrial culverts (ones which remain dry throughout the season) be monitored with cameras, whereas aquatic culverts (or those that flood for part or all of the season) be monitored with PIT tags and antennas. Most importantly, a radio-tracking program should be implemented before and after the mitigation strategy to determine whether or not culverts are actually reconnecting habitat fragments (Clevenger and McGuire 2001, Dodd et al. 2004, Lesbarrères and Fahrig 2012).

Population and home range estimates for Blanding's turtles were useful for identifying vulnerable sites within the landscape and to direct next steps. For example, despite the overlap with privately managed marsh and agricultural land, <2% of the population range occurred in agricultural fields; hence, conservation efforts on farm land

should not receive top priority in this instance. Instead, mitigation should be focused on specific areas within the population home range where there are currently no culverts and that have limited or partial fencing. This study also revealed that 3% of the population and 6% of the female range included the unpaved road at the southern end of the study area, where Blanding's turtles crossed to access nesting habitat. Although this represents a proportionately small amount of the adult range, the unpaved road likely results in significant mortality of hatchlings as they make their way from the beach to the marsh. Safe access to nesting habitat is key to long-term sustainability of the population. This beach habitat should be protected from further anthropogenic alterations. Our movement and habitat-use data were also consistent with our Blanding's turtle culvert crossing data, showing that only a relatively small proportion of the tracked turtles and associated home ranges included the causeway in 2014–2015. We expect the percentage of turtles using the culvert to fluctuate on a long-term basis depending on environmental conditions from year to year. Through mark-recapture studies carried out on Blanding's turtles and spotted turtles from 2003 to 2016 (S. D. Gillingwater, unpublished data), we have observed shifts in habitat use and behavior of turtles that reflect shifts in vegetation and water levels within the marsh.

## Management Implications

One of the most important findings in our decade-long study was the failure of partial fencing in mitigating against road mortality. We found that partial fencing had no significant effect on reducing road abundance and was no better than road sections with no fencing. The second important lesson is the need to curve fence ends (up to 75 m) to

redirect species towards interior habitat. Although culverts can help connect fragmented habitats, fencing is what keeps animals off the road and directs them to the culverts. Therefore, fence integrity is key to success and a vigilant inspection and maintenance program is essential. When selecting fence materials, it is important to consider your target species but also your site (van der Ree et al. 2015). A combination of upland, windy areas and wet, marsh conditions required 2 different types of fencing in our study area (PVC mesh and woven geotextile). Although this fencing system withstood site conditions, regular maintenance was still required to repair damages, and it did not prevent larger climbing snakes from accessing the road. We recognize that in some cases complete fencing cannot be installed; in our study area, gaps in the fencing were necessary because of private driveways and marinas. In these situations, improved designs that prevent access to the road must be considered (van der Ree et al. 2015). Research to determine effectiveness of culvert placement and type, length of lag time before use, and species-specific preferences will require a carefully designed long-term before-after-control-impact monitoring program. In developing mitigation strategies, future projects should consider incorporating movement and habitat-use data, in addition to road mortality data, to identify the most vulnerable road segments.

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Figure 7.1: The 2-lane paved causeway in our study area is 3.6 km in length and separates the inner bay (east) from a wetland complex (west) in southwestern Ontario, Canada. We surveyed the causeway 5 years before (2003–2007) exclusion fencing installation in 2008–2009 and continued to survey 5 years after (2010–2014) beginning in April and concluding in October. On each survey occasion, we identified and counted all species that were alive or deceased in each of the 5 road sections. Sections A and D were partially fenced, sections B and C were completely fenced on both sides of the road, and section E had no fencing. Seven culverts (solid circles) were installed under the causeway during the post-mitigation period (2010–2014). Culverts 3–5 were constructed in the fall of 2012 and culverts 1, 2, 6, and 7 were constructed in the fall of 2014. Another road runs east-west along the southern end of the marsh. Built refers to any residential or commercial areas.



**Figure 7.2:** Results of a Poisson generalized linear mixed model displaying the mean number of turtles (± SE) found on a 3.6-km paved causeway in southwestern Ontario, Canada, during the pre-mitigation period (2003–2007, dashed line) and the post-mitigation period (2010–2014, solid line) in road sections with no fencing, partial fencing, or complete fencing.



**Figure 7.3:** Results of a Poisson generalized linear mixed model displaying the mean number of snakes (± SE) found on a 3.6-km paved causeway in southwestern Ontario, Canada, during the pre-mitigation period (2003–2007, dashed line) and the post-mitigation period (2010–2014, solid line) in road sections with no fencing, partial fencing, or complete fencing.



**Figure 7.4:** The 95% isopleth calculated from the kernel density estimation used to estimate home range (solid line) and the 50% isopleth used to estimate core range (dashed line) for 15 adult male Blanding's turtles radio-tracked in 2014 and 2015 (a) and 15 adult female Blanding's turtles radio-tracked in 2014 and 2015 (b) in a marsh complex in southwestern Ontario, Canada. We estimated the population range from the 95% isopleth and core range from the 50% isopleth using data from all adult Blanding's turtles radio-tracked in 2014 and 2015 (c). The population range and core range overlapped with marsh habitat, agricultural land, and surrounding roads (causeway and southern road).

**Table S7.1:** Ashley and Robinson (1996) divided the causeway in our study area in southwestern Ontario, Canada into 5 sections (A–E). We described habitat from 2008 to 2015.

Section	Habitat to west	Habitat to east	Exclusion fencing
A	Dominated by cattails and <i>Phragmites</i> , large open pool about 40 m from road.	Marina and trailer park development; no natural habitat.	Partial fencing
В	Dominated by cattails and <i>Phragmites</i> .	Dominated by cattails and <i>Phragmites</i> .	Complete fencing
С	Dominated by cattails and <i>Phragmites</i> . A ditch and dyke system are located about 40 m from road.	Dominated by cattails and <i>Phragmites</i> .	Complete fencing
D	Dominated by cattails and <i>Phragmites</i> . A ditch and dyke system are located about 40 m from road and are parallel to southern portion of this section.	Cottage, marina, trailer park development; some areas dominated by cattails and <i>Phragmites</i> .	Partial fencing
E	Dominated by cattails and <i>Phragmites</i> .	Boat houses; area dominated by cattails and <i>Phragmites</i> .	No fencing

Culvert	Dimensions (m)	Model	Provider	Installation date
1 and 2 (Terrestrial open-grate)	$12.00 \times 0.50 \times 0.48$	AT500	ACO Systems	Fall 2014
3 (Hydraulic concrete box)	18.30 × 3.00 × 2.10	Reinforced non- standard concrete box culvert	M-CON Pipe and Products	Fall 2012
4 (Terrestrial concrete box)	16.30 × 1.80 × 0.90	Reinforced non- standard concrete box culvert	M-CON Pipe and Products	Fall 2012
5 (Terrestrial concrete box)	$16.20 \times 0.50 \times 0.48$	AT500	ACO Systems	Fall 2012
6 (Terrestrial concrete box)	17.00 × 1.80 × 0.90	Reinforced non- standard concrete box culvert	M-CON Pipe and Products	Fall 2014
7 (Terrestrial open-grate)	$\begin{array}{c} 13.00 \times 0.50 \times \\ 0.48 \end{array}$	AT500	ACO Systems	Fall 2014

**Table S7.2:** The dimensions (length  $\times$  span  $\times$  rise), model, and provider for the 7 culverts<br/>along the causeway in our study area in southwestern Ontario, Canada.

Culvert	Monitoring start	Monitoring end	Monitored with camera	Monitored with antenna
1	11 May 2015	27 Aug 2015	$\checkmark$	
2	11 May 2015	27 Aug 2015	$\checkmark$	
3	1 May 2014	29 Aug 2014	$\checkmark$	$\checkmark$
	11 May 2015	28 Jun 2015		$\checkmark$
4	22 Jun 2014	29 Aug 2014	$\checkmark$	$\checkmark$
	11 May 2015	27 Aug 2015	$\checkmark$	
5	22 Jun 2014	29 Aug 2014	$\checkmark$	$\checkmark$
	11 May 2015	27 Aug 2015		$\checkmark$
6	11 May 2015	27 Aug 2015		$\checkmark$
7	11 May 2015	27 Aug 2015	$\checkmark$	

**Table S7.3:** Time period and method(s) used to monitor each culvert in our study area in southwestern Ontario, Canada.

ccies Sex Culvert Method Confirm use?	Method	Used culvert to move east	Used culvert to move west	Total in b

Species	Sex	Culvert	Method	Confirm use?	Used culvert to move east	Used culvert to move west	Total days in bay
Graptemys geographica	N/A	1	Camera	Yes	N/A	N/A	N/A
Chelydra serpentina	N/A	1	Camera	Yes	N/A	N/A	N/A
Clnysemys picta marginata	N/A	I	Camera	Yes	N/A	N/A	N/A
Chelydra serpentina	N/A	2	Camera	Yes	N/A	N/A	N/A
Emydoidea blandingii	Male	3	PIT tag–antenna	Yes	2 Jun 2014	5 Jul 2014	34 days
Emydoidea blandingii	Male	3	PIT tag–antenna	Yes	23 Jul 2014	30 Jul 2014	7 days
Emydoidea blandingii	Male	3	PIT tag–antenna	Yes	N/A	23 May 2015	5 days
Emydoidea blandingii	N/A	S	Camera	No, only investigation on 25 Jun 2014	N/A	N/A	N/A
Chelydra serpentina	N/A	7	Camera	Yes	N/A	N/A	N/A
Chrysemys picta mareinata	N/A	7	Camera	Yes	N/A	N/A	N/A

	Camera	PIT tag	PIT tag and radio tracking
Resulting data	Identifies investigation of culvert	Identifies usage of culvert regardless of above or under	Identify habitat use and movement patterns before and after culvert
	Minor chance of identifying usage	water Identify species and	use Determine home range
	Animal must be above water	sex	to guide future conservation efforts
Cost	\$700/camera (including security enclosure)	\$4,000/setup plus batteries and installation supplies	\$4,000/setup plus batteries and installation supplies
	\$150/camera for	\$10/PIT tag	\$10/PIT tag
	rechargeable batteries, charger,		\$250/radio tag
	and memory cards to switch out		\$600 tracking equipment
Time processing	10 hr/week	30 min/culvert/week	30 min/culvert/week; up to 40 hr/week
Potential to inform management	Low	Medium	High

Table S7.5: Comparison of methods to monitor culvert use (in CAD dollars) by reptiles.

# Chapter 8: Long-term habitat changes in a protected area: Implications for herpetofauna habitat management and restoration

By

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Presented in manuscript format and under review.

### <u>Abstract</u>

Point Pelee National Park, located at the southern-most tip of Canada's mainland, historically supported a large number of herpetofauna species; however, despite nearly a century of protection, six snake and five amphibian species have disappeared, and remaining species-at-risk populations are thought to be in decline. We hypothesized that long-term changes in availability and distribution of critical habitat types may have contributed to the disappearance of herpetofauna. To track habitat changes we used aerial image data spanning 85 years (1931–2015) and manually digitized and classified image data using a standardized framework. Change-detection analyses were used to evaluate the relative importance of proportionate loss and fragmentation of 17 habitat types. Marsh habitat diversity and aquatic connectivity has declined since 1931. The marsh matrix transitioned from a graminoid and forb shallow marsh interspersed with water to a cattail dominated marsh, altering critical breeding, foraging, and overwintering habitat. Reduced diversity of marsh habitats appears to be linked to the expansion of invasive *Phragmites australis*, which invaded prior to 2000. Loss of open habitats such as savanna and meadow has reduced availability of high quality thermoregulation habitat for reptiles. Restoration of the northwestern region and tip of Point Pelee National Park to a mixed landscape of shallow wetlands (cattail, graminoid, forb, open water) and eradication of dense *Phragmites* stands should improve habitat diversity. Our results suggest that long-term landscape changes resulting from habitat succession and invasive species can negatively affect habitat suitability for herpetofauna and protection of land alone does not necessarily equate to protection of sensitive herpetofauna.

# Introduction

Reptiles and amphibians, collectively referred to as herpetofauna, are two of the most endangered taxonomic groups facing global population declines [1-3]. Species population declines and disappearances are frequently linked to habitat loss, fragmentation, and degradation [1,2,4,5]. The direct loss of habitat prohibits or significantly impairs species' ability to carry out critical life activities such as foraging, overwintering, and breeding. Furthermore, when habitat areas are reduced in size, local species extinctions will occur until a new equilibrium of fewer species is reached that can be sustained in the smaller area [6,7]. Herpetofauna depend on diverse wetland habitat for breeding, foraging, and winter refugia. In addition to wetlands, many reptiles and amphibians rely on adjacent terrestrial habitat [8]. For instance, turtles require terrestrial habitat for nesting and estivation [9,10], snakes overwinter on land [11,12], and after breeding, some anurans will move upland to forage and overwinter [13]. A tight spatial coupling between wetland and terrestrial habitat is critical for a diverse range of herpetofauna to successfully carry out life activities.

Distinct from habitat loss is habitat fragmentation, the process of a continuous habitat being broken down into distinct patches that ultimately changes the original habitat configuration [14]. Fragmentation can isolate critical habitats or entire populations [e.g., 15], reduce genetic diversity [e.g., 16], and decrease the potential for rescue effects [e.g., 17]. For example, a road can not only lead to direct mortality from vehicular collisions, but it can also fragment habitat and prevent access to essential patches [see 18,19]. Habitat destruction or degradation may be the result of fragmentation

or other stressors that threaten the health of herpetofauna, such as nutrient or sediment enrichment, contamination by organic and inorganic pollutants, presence of invasive species, or changes in habitat composition [1,20]. Beyond habitat loss, fragmentation and degradation, species population declines and even local extirpations have been linked to mass mortality events because of disease [21], illegal harvesting for food or the pet trade [20], or some unknown factor(s) [22].

The protection of natural lands is vital for conservation of biodiversity [23]; however, protection of lands alone is not always sufficient for maintaining historic species diversity. This seems to be the case for PPNP located at the southern-most tip of Canada's mainland, which has lost several herpetofauna species despite nearly a century of protection from human disturbance [24]. Over time, the park has seen the extirpation of five amphibian (i.e., Northern cricket frog [Acris crepitans], Eastern tiger salamander [Ambystoma tigrinum], Fowler's toad [Anaxyrus fowleri], gray treefrog [Hyla versicolor], American bullfrog [*Lithobates catesbeianus*]) and six snake species (i.e., blue racer [Coluber constrictor foxii], timber rattlesnake [Crotalus horridus], gray ratsnake [Pantherophis spiloides], Eastern hog-nosed snake [Heterodon platirhinos], milksnake [Lampropeltis triangulum], massasauga [Sistrurus catenatus]), with a suspected extirpation of the Endangered spotted turtle (*Clemmys guttata*) that was last seen in the park in the early 1990s [25]. Currently, the park supports several at-risk species including the Blanding's turtle (*Emydoidea blandingii*) and snapping turtle (*Chelydra serpentina*); though there are concerns over the status of these species because evidence suggests that recruitment is limited [Chelydra serpentina; 26]. Although agricultural practice and

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human infrastructure has been limited within the protected park, there have been suspected changes in appropriate habitat for herpetofauna. Continued loss of herpetofauna is of conservation concern and identification and quantification of habitat changes in the park can inform management and habitat restoration efforts.

Given the successional properties of ecosystems, the proportion of habitat classes are expected to change over time. While habitat succession is a natural process, it can affect landscape suitability either positively or negatively depending on the target species. Therefore, our primary objective was to quantify the amount of habitat change that has occurred within PPNP over the last 85 years. Given that PPNP has been protected from anthropogenic stressors such as land-use alteration, we hypothesized that long-term changes in availability and distribution of habitats types may have contributed to the disappearance of herpetofauna. Specifically, we tested for changes in shallow marsh habitats suitable for breeding, feeding and overwintering for turtles [10] and anurans [13], upland and sandy habitats that provide nesting habitat for turtles [10], and thermoregulation habitat and refugia for snakes [27,28]. Our secondary objective was to identify any areas of habitat loss and fragmentation within the park that could be targeted for possible habitat restoration efforts or a feasibility study of habitat restoration strategies.

# Methods

#### Study site

Point Pelee is a 16 km<sup>2</sup> National Park located on the north shore of Lake Erie at the southern-most tip of Canada's mainland. The park was established in 1918 and is a

popular tourist destination hosting 300,000–500,000 visitors annually [24]. Point Pelee National Park is located within the Carolinian zone, one of the most diverse Canadian regions for herpetofauna. Point Pelee is also recognized as an Important Bird Area and a Wetland of International Significance by UNESCO [29].

#### Habitat delineation and classification

We acquired image data for the years 1931, 1959, 1973, 1977, 1985, 1990, 2000, 2004, 2010 and 2015 to complete a multi-date data classification (Table 8.1). We digitized image data and classified habitats in ArcGIS 10.3 (ESRI, Redlands, California, USA) at a map scale of 1:1500. When examining habitat change derived from image data, our detection analyses are only as accurate as each individual classified product [30]. These errors are inherent with any historical image classification since derived data cannot be ground-truthed. We minimized compound errors by creating and adhering to a formal delineation and classification framework for each image (Table 8.2). To maintain nomenclature consistency, we adapted habitat ecosite and vegetation names from Dougan and Associates [31] who previously completed an Ecological Land Classification for Point Pelee National Park. Although we classified the delineated habitats to ecosite, not all ecosite classes were used to facilitate comparisons among years. Therefore, agriculture, constructed, forest, meadow, woodland, savanna, thicket and swamp were retained at their community class level in our final analyses (Table 8.2).

### Habitat loss and fragmentation

We measured habitat areas in ArcGIS 10.3 and calculated change in total area on a temporal scale. To account for changes in total park area through time (due to variations

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in water level of Lake Erie), we expressed all habitat types as a percentage of total delineated park area in order to examine relative changes in habitat losses and gains through time. To determine if serial autocorrelation was present in our dataset, we used the Durbin-Watson test. We also used a Spearman's correlation to determine if change in habitat area was correlated with Lake Erie water levels (Data obtained from Great Lakes Water Level Dashboard, www.glerl.noaa.gov).

We calculated a variety of metrics in FragStats 4.2 [32] to quantify habitat loss and fragmentation within the park at both the landscape and habitat class scale. For the purposes of our study, we defined the landscape as Point Pelee National Park, comprising a mosaic of habitat patches. At the landscape scale, we investigated changes to the area and distribution of patches, as well as overall habitat diversity using Shannon's and Simpson's diversity indices and Simpson's evenness. At the class scale, we analyzed changes to the area and distribution of specific habitat classes. We used linear regression to determine the relationship between fragmentation metrics and time. Prior to calculating metrics, we converted habitat data to rasters with a cell size of 2 m to ensure patch boundaries were accurately represented and that visually connected features remained connected in the raster surface. To characterize fragmentation, we quantified change in landscape division and patch distribution using effective mesh size and splitting index [32]. The splitting index is the number of patches after dividing the landscape into patches of equal size that would result in the same degree of landscape division, and is irrespective of patch size, shape and relative location. Effective mesh size is the size of

the patches when the landscape is divided in S areas of the same size (where S = splitting index).

We quantified habitat change using patch and core area metrics standardized by the proportional abundance of each habitat type [area-weighted metrics; 32]. We calculated mean patch area and the mean radius of gyration to measure patch extent and to determine the average distance a species can move within a specific patch before reaching the patch boundary. Lastly, we calculated core area metrics to estimate the amount of interior habitat after accounting for edge habitat [32]. We quantified the change in core area, number of disjunct core areas and the core area index (i.e., the percentage of core area). Examining core area is important because the measure integrates both the size and shape of the patch. The amount of core area will differ between two patches of the same total size if one patch is a perfect circle and the second is elongated and narrow. Core area metrics provide an indication of how far a species would have to travel before accessing another habitat type, as well as the ratio of edge to interior habitat, a variable that is relevant to species adversely affected by edges [33] or that select for edge habitat [34,35]. When determining core area, we treated 15 m as edge habitat based on the average daily distance moved by spotted turtles [30 m; 36,37] and previous research on habitat use by snakes [34,35].

# Change-detection

To identify change hotspots that could be used to direct restoration, we conducted a change-detection analysis in ArcGIS 10.3. We examined change-detection rasters to identify which habitat types replaced those that had been lost. We chose images that had been acquired many decades apart. We could not use the earliest image acquired in 1931 due to low resolution, but instead used the 1959 image, which had a suitably high resolution, and the 2015 image, which is the most recently available.

#### <u>Results</u>

# Landscape-level habitat changes

At the landscape scale, habitat in Point Pelee National Park has become less diverse and more homogenous. In all cases the Durbin-Watson test was not significant (DW < 2, p > 0.5); therefore, no serial autocorrelation was present in our dataset. Mean patch area and mean patch extent (radius of gyration) increased at a rate of 1.84 ha per year and 2.98 ha per year, respectively (Table 8.3). The amount of mean core habitat in PPNP increased by 200% from 1931 to 2015 (Table 8.3). The mosaic of unique natural habitats declined through time from 26 to 8 patches (splitting index) and, on average, each patch increased by 128 ha (effective mesh size; Table 8.3).

# Class-level habitat changes

Substantial changes in land cover proportions occurred in PPNP over the last 85 years (Fig 8.1), with the greatest occurring in the marsh (Fig 8.2a). Most notably, cattail organic marsh has doubled in total area from 309 ha to 625 ha (Fig 8.2a), while graminoid shallow marsh has decreased by 80% (Table S8.1). Following a peak in 1959, the areal cover of forb shallow marsh has generally declined (Fig 8.2a). Although *Phragmites* had invaded the park prior to the end of the last century, the 2000 image was the earliest in which we could positively identify its presence. Over the course of the following 15 years, the number of *Phragmites* patches expanded from 4 to 166 unique

patches, increasing to a density of 11 patches per 100 ha (0.27 patches in 1931). The amount of open water was relatively stable through time, comprising about 20% of the park's total area. Although not to the same extent as that of the marsh, there were also changes in upland habitat classes (e.g., forest, thicket, savanna, and meadow classes; Fig 8.1); there was an increase in forest and thicket habitat types, while the amount of swamp remained fairly consistent (Fig 8.2b). Total forest area increased as mean patch area increased to 50 ha and patch extent reached 560 m (Table 8.3). Noticeable declines were observed for savanna and meadow habitats. About half of the core meadow habitat was lost between 1931 and 2015 (core area index; Table 8.3). The amount of sand dune habitat significantly declined by 30 ha ( $R^2 = 0.90$ , p < 0.0001). Active management within the park boundaries led to the decommissioning of all agricultural lands and reduced the number of buildings and roads since the 1930s (Fig 8.2c).

In 1931, the matrix (i.e., dominant habitat type in which patches are distributed throughout PPNP; Forman and Godron 1986) was graminoid organic shallow marsh, with open water and forb organic shallow marsh interspersed. We detected a major change in the matrix that occurred between 1959 and 1973, when cattail organic shallow marsh became the dominant and most extensive feature and continued to take over the marsh until present-day conditions. Mean area for a cattail patch increased significantly from 64 ha to 336 ha, expanding at a rate of 4.12 ha per year (Table 8.3). The mean radius of gyration increased by 7.43 m per year, increasing from 377 m to almost a kilometer in extent. The core area of dense cattail habitat currently comprises 42% of the park

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landscape, with the largest patch totaling 28% of the cattail area. Furthermore, when we divided the landscape into patches of equal sizes, the amount of cattail increased from 13 ha to 142 ha (Table 8.3). In contrast to cattail habitat, percentage of available forb core area has been completely lost from the park (declined to 0% from 50%; Table 8.3), leaving the remaining 1.2 ha as edge habitat. Although the amount of open water remained consistent through time (Fig 8.2a), the percentage of available core area has increased to 83%, but the number of disjunct areas has declined from 79 to 33 patches, indicating an overall reduction in aquatic connectivity (Table 8.3).

Point Pelee is a sand spit and is constantly subjected to erosional and depositional forces. Throughout 1931 to 2015, erosional forces have been greater than depositional forces along the eastern shorelines. Although the western shoreline has seen some deposition, there was a net loss in shoreline area totaling 72 ha. Total length of the shoreline has also decreased from 19.2 km to 18.0 km. The area of mineral shoreline and sand barrens/dunes has been declining through time, with some observed fluctuations (Fig 8.2c; Table S8.1). We found a significant negative correlation between water level of Lake Erie and the amount of shoreline (-0.75, p = 0.01) and open sand barren/dune habitat (-0.76, p = 0.01; Fig 8.3) in PPNP; such a significant correlation with water levels was not identified for any other habitat class. There was also a decline in total park area over time, which appeared to be associated with Lake Erie water levels (Fig 8.4). Although total park area tended to decline as water levels rose (1931–1985), it did not increase when lower water levels returned between 1990 to 2015 (Fig 8.4). Consequently, PPNP has experienced an overall net loss of 58 ha since 1931.

# Change-detection

Our change-detection analysis revealed that over 650 ha (43–44%) within the park underwent a change in habitat type between 1959 and 2015. Most notably, loss of forb and graminoid marsh was succeeded by 139 ha and 188 ha of cattail marsh, respectively. Over 50 ha of open water was infilled by cattail marsh. As a result of management actions, approximately 89 ha of constructed and agricultural land was reclaimed as forest or thicket. The increase in amount of forest was also because of succession of woodland areas (72 ha).

We identified five hotspots within the park that experienced major changes (Fig 8.5). There are two hotspots in the marsh where habitat transitioned from a diverse mixture of broad- and narrow-leaved emergent interspersed with pools of water in 1959 to almost being completely infilled by dense cattails in 2015 (Hotspots A and B; Figs 8.5a and 8.5b). In 2015, over 90% of the *Phragmites* distribution had displaced marsh habitats (i.e., cattail, forb, graminoid OSM), while the remaining 10% occupied open water, swamps, and sand dunes. Concurrent with the spread of *Phragmites* throughout the park, large homogenous stands infiltrated marsh habitat towards the northern end (Hotspot C; Fig 8.5c); long stretches of *Phragmites* also became established along majority of the eastern beach, creating a barrier between the beach and marsh (Hotspots D and E; Figs 8.4d and 8.4e).

# Discussion

# Long-term habitat changes

Consistent with our hypothesis, we confirmed that long-term changes in habitat availability and distribution had occurred in Point Pelee National Park. First, both the diversity of marsh habitat and connectivity of aquatic habitat patches have been reduced over the past 85 years, similar to changes noted in other Ontario wetlands [38]. Between 1959 and 1973, the marsh matrix transitioned from a graminoid organic shallow marsh interspersed with patches of open water and forb organic shallow marsh, to a cattaildominated marsh with very few patches of open water (Fig 8.5). Such major changes in habitat distribution and isolation would certainly have affected movement patterns and limited herpetofauna's ability to access required habitat types during the active season. For instance, in 1931, there had been abundant shallow aquatic habitat that would have provided important foraging, mating, and overwintering habitat for herpetofauna [10,13]. By 1973, however, the dispersed shallow pools had coalesced into large deep pools and had become surrounded by large dense stands of cattails (Fig 8.2a). In such a habitat matrix, herpetofauna might have been forced to travel up to a kilometer through dense cattail stands to access critical habitat (Fig 8.2a). Since patches throughout the landscape continued to expand at a rate of 1.84 ha per year, unfavourable habitat patches (e.g., *Phragmites*) would have become increasingly difficult to traverse, while critical habitats would have become more isolated and disconnected.

High-quality thermoregulation habitat likely became a limiting factor for herpetofauna in PPNP. For turtles, thermoregulation opportunities may have become

severely reduced as dense, homogenous cattail beds formed, leaving almost no open water or open canopy basking sites [25]. For snakes, loss of open terrestrial habitats such as savannas and meadows may have reduced important thermoregulation habitats. Open terrestrial habitats have been found to provide a variety of environmental temperatures, and by basking or seeking shelter, snakes can control their body temperature [39]. Moreover, the decline in treed and shrubby sand dunes reduces availability of cover objects known to be important for certain snakes [28]. In a similar thermal environment to PPNP, snakes near Ottawa, Ontario, preferred open habitats over forest likely because it enabled behavioural thermoregulation [39]. The lower thermal quality of forests compared to open habitats resulted in snakes preferring open habitat at both the microhabitat and macrohabitat scale [40]. In PPNP, there had been a succession from woodland habitat with open canopy to forests with closed canopies, as well as a decline in savanna and meadow that resulted in a total loss of 150 ha of open habitat; such a change could have resulted in reduced thermoregulatory opportunities [41] and a subsequent loss of snake species [42].

The third major habitat change in PPNP was the invasion of *Phragmites australis* into marsh and beach habitats, posing a significant threat to the quality and amount of potential breeding and nesting habitat for herpetofauna. Growth of *Phragmites* along the eastern beach has created a potential barrier for species, particularly turtles, from moving between the marsh and beach during the nesting season. In addition, growth of *Phragmites* in known turtle nesting beaches can lower nest temperatures through shading [43]. In a similar sand dune and shallow marsh ecosystem, the Fowler's toad (*Anaxyrus*)

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*fowleri*) experienced population declines following loss of breeding habitat to *Phragmites*, even though there had been minimal loss of adult habitat [44]. Although *Phragmites* was only at image-detectable levels in 2000, it had invaded PPNP sometime between 1970 and 1990 [45], making it possible for this invasive species to be at least partially responsible for the extirpation of 3 of the 11 herpetofauna species after the 1970s [24]. *Phragmites* continues to threaten other amphibians and reptiles in the park, including the spotted turtle, which is on the brink of extirpation.

Legislation can protect a parcel of land from being altered by human activities but it cannot protect it from changes in hydrological variability nor natural successional processes that can affect marsh habitat and impact species that are reliant on a diverse, interconnected system [e.g., 46]. Moreover, habitat availability and suitability are expected to evolve with climate change in light of predicted increases in temperature and decreases in precipitation [47]. As both natural and anthropogenically accelerated forces alter sensitive marsh habitats, more active management will be required if the goal is for habitat to continue functioning as a refugium for species at risk, particularly herpetofauna. In the case of PPNP, although designated a protected area since 1918, it continued to be stressed by invasive species (e.g., *Phragmites australis*) and climate change, and had undergone natural ecosystem succession. If the intent is to promote overall biodiversity and to devise species-specific recovery strategies, then the Park must engage in active management, including removal and control of *Phragmites* and the enhancement of marsh habitat diversity and connectivity. Overall, the management of a marsh towards a more diverse vegetative and structural state will provide a range of

opportunities for feeding, breeding, overwintering, and thermoregulating and likely benefit the highest number of native and rare species.

#### Habitat management and restoration

We identified five hotspots for habitat restoration, or a feasibility study of habitat restoration strategies, based on areas that have undergone the greatest amount of habitat change (Fig 8.5). The first two hotspots (Figs 8.5a and 8.5b) were once characterized by a mix of shallow water wetlands (cattail, graminoid, forb, open water) but have been completely succeeded by cattails. Monotypic cattail stands have been controlled with a variety of techniques [48,49] and management towards a hemi-marsh condition [50] with a mix of open water and vegetation interspersed has been used to enhance waterfowl diversity [51,52]. Similarly, open channels have been cut into dense cattail stands to restore connectivity for fish in the St. Lawrence River [53]. Efforts to increase the number of open-water patches within cattail beds could provide movement corridors and additional basking opportunities for reptiles; however, management towards a hemimarsh has not been studied from a herpetological perspective. Moreover, evidence suggests that decreasing the size of cattail beds would allow for expansion of wet meadow, graminoid, and forb marsh habitat, and lead to restoration of habitat diversity [54].

We identified the next three hotspots as areas where *Phragmites* has either formed a barrier wall between the marsh and the beach (Figs 8.5d and 8.5e) or formed large, dense monospecific patches (Fig 8.5c). The removal and control of *Phragmites* is extremely important to recovering beach nesting habitat and marsh habitat, while

ensuring *Phragmites* do not colonize habitat where cattail removal has been suggested. In particular, the barrier of *Phragmites* between the beach and marsh is concerning for turtles in search of nesting habitat. Resident wetlands and nesting sites must be closely linked and the connecting corridor must provide passable habitat [55], which is likely not the case along the eastern beach. If *Phragmites* continues to expand into sandy habitats, high water level years can compress the amount of available nesting habitat since sand barren and dune habitats are correlated with changes in Lake Erie water levels. Since 1931, we have identified a net loss of 58 ha of park area because of erosion and the beach is vulnerable to further losses. Removal of *Phragmites* along the beach is key in restoring connectivity to the marsh and maintaining high quality nesting habitat.

## Additional stressors

In addition to habitat restoration and increasing habitat suitability within the park, there are other factors that could have played a role in observed herpetofauna declines such as (1) visitor disturbances, (2) mesopredators, (3) historic use of chemicals, (4) reduction in park size and (5) park isolation. Prior to the 1800s, the marshes of Point Pelee used to extend 2000 ha beyond the current park boundary; however, Hecnar and Hecnar [24] determined that this loss of area only accounted for the disappearance of two to four reptile species and one or two amphibian species. Point Pelee has lost six reptile and five amphibian species, not including the spotted turtle. These results suggest that additional stressors may have led to the extirpation of the remaining species, such as the geographic isolation of the park. Point Pelee is spatially isolated from other wetland complexes; therefore, species must move further to reach other wetlands and this may

result in decreased immigration and rescue effects [17]. The isolation of marsh habitats in the southern Ontario landscape has resulted in decreased gene flow among foxsnake populations [56]. For extirpated species, the distance from Point Pelee to the nearest population ranged from 14 to 500 km. These distances were significantly farther than for species currently in PPNP, and support the hypothesis that isolation impacts species richness [24].

In addition to the unanticipated negative effects of habitat change, we must also consider the unintended negative effects of human disturbance through park visitation in PPNP [57]. Literature shows that even small levels of turtle adult mortality, for instance through vehicular collisions or illegal poaching, could have impacts on population stability [e.g., 58,59]. Increased human contact can also elevate densities of mesopredators within the park and lead to increased egg depredation of at-risk turtles [60,61]. For example, recreational activities in protected areas, such as use of trails and roads, have been shown to indirectly restructure predator-prey communities despite protection from major anthropogenic landscape change [62]. Changes in predator distribution may potentially have a greater negative impact on herpetofauna given that some turtles do not nest every year [63] and the high probability of nest predation could have seriously lowered population recruitment for turtle populations in PPNP [26]. Since amphibians are highly sensitive to pesticides (Howe et al. 2004), the historical use of these chemicals may have caused population declines or extirpations; evidence shows that the last observations of Fowler's toads in PPNP aligns with the use of DDT [64].

# **Conclusion**

Without actions to recover lost and degraded habitat, the spotted turtle will likely become extirpated from the park, and other herpetofauna species will continue to lose critical habitat. Long-term persistence of herpetofauna is dependent upon access to critical habitats for mating, foraging, nesting, and overwintering. We have provided several lines of evidence to show how critical habitat has become lost and/or fragmented in Point Pelee over the course of 85 years, even though this social-ecological-system had been "protected" as a national park. We acknowledge that many factors contribute to the extirpation of a species, and without data on annual population sizes, we cannot identify the exact causes that led to the disappearance of 11 herpetofauna species and the suspected extirpation of spotted turtles. Nevertheless, loss, fragmentation and degradation of habitat within the park boundary has undoubtedly played a pivotal role in the decline of herpetofauna in PPNP, given that these are the primary causes of species declines worldwide. Therefore, future management plans should include restoring some of the original habitat features and functions that we have identified in this study (Fig 8.5) in order to maintain and then eventually increase overall biodiversity. The most timesensitive step is removal and control of invasive *Phragmites australis* to prevent further encroachment on critical reproductive and thermoregulatory habitat. Habitat restoration alone will not be enough to recover species at-risk but evaluation of historical habitat and long-term changes is the cornerstone to assessing restoration needs and designing an effective management approach. The creation of suitable habitat and assessment of longterm changes can contribute to a comprehensive feasibility study for re-establishing the
spotted turtle or other lost herpetofauna, and eventually return some of the incredible floral and faunal diversity of Point Pelee National Park [29].

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Year	Source	Date of Acquisition	Season	Spectral Range	Scale
1931	Air photo; obtained from PPNP	4/18/1931	Leaf off	Black and White	1:10,000
1959	Air photo; obtained from PPNP	4/29/1959	Leaf off	Black and White	1:4,000
1973	Air photo; obtained from PPNP	1/13/1973	Leaf off	Black and White	1:10,000
1977	Air photo; obtained from PPNP	11/2/1977	Leaf off	Black and White	1:4,000
1985	Air photo; obtained from PPNP	Summer 1985	Leaf on	Black and White	1:30,300
1990	Air photo; obtained from PPNP	4/8/1990	Leaf off	Black and White	1:9,000
2000	Air photo; obtained from PPNP	3/22/2000	Leaf off	Black and White	1:6,250
2004	Air photo; obtained from PPNP	4/9/2004	Leaf off	Black and White	1:10,000
2010	Land Information Ontario; Southwestern Ontario Orthophotography Project (SWOOP)	Spring 2010	Leaf off	True Colour	1:10,000
2015	Land Information Ontario; Southwestern Ontario Orthophotography Project (SWOOP)	Spring 2015	Leaf off	True Colour	1:10,000

**Table 8.1:** Image data processed for change-detection analyses in Point Pelee National Park (PPNP).

Habitat	Description	Ecological Land Classification
Agriculture	Active or inactive agricultural fields	OAGM (Agriculture, coniferous plantation, orchard)
Constructed	Buildings, roads and trails	CV (Residential, transportation, utilities)
Common Reed	Dense invasive Phragmites australis australis typically growing in circular	MAMMI-12
Graminoid MMM	shapes throughout the marsh	
Cattail OSM	Very low edge-to-interior ratio (high density) of cattails ( <i>T. latifolia, T. angustifolia, T. x glauca</i> ) with homogenous appearance, almost no visible pools of open water	MASOI-1
Graminoid OSM	Medium edge-to-interior ratio of mixed broad-leaved or narrow-leaved emergents interspersed with water (medium density)	MASOI
Forb OSM	High edge-to-interior ratio of broad-leaved or narrow-leaved emergents with many small pools (low density)	MASO2
Graminoid MMM	Little to no standing water, dense monospecific grass stands	MAMMI-3
Mixed MMM	Small, wet meadow with diverse floral communities, often found between dune and extensive marsh communities	MAMM3
Meadow	Open terrestrial area with homogenous spread of grasses, < 25% tall shrub cover	ME (DF graminoid, mixed meadow)
Forest	> 75% cover of deciduous or coniferous, or > 25% both coniferous and	FO (DF cedar, poplar, deciduous and white hime forest EM nonlar forest)
Woodland	25 600 correr of devidious or coniference treas	W/O (DE cadar coniference decidions
W OODLAHD	- 00% cover of decidinois of contractors these	WO (DF cedar connerous, deciduous and mixed woodland)
Savanna	10 - 35% cover of deciduous or coniferous trees	SV (DF cedar coniferous, mixed savanna)
Thicket	Open area with $> 25\%$ tall shrub cover and little tree cover	TH (DF and FM deciduous thicket, deciduous shrub thicket)
Swamp	Wetland characterized by presence of deciduous or coniferous trees	SW (Maple mineral deciduous swamp, mineral deciduous thicket swamp)
Open Water	Open water, devoid of vegetation	OA
Sand Barren/ Dune	Low hill or ridge of sand either inland or lining the land, vegetation may include shrubs and trees	SB (Open, shrub, treed sand barren and dune)
Shoreline	Community that lies adjacent to Lake Erie, rarely any vegetation	SH (Mineral open and shrub shoreline)

parentheses: DF = drv-fresh: FM = fresh-moist: MMM = mineral meadow marsh: OSM = organic shallow. Table 8.2: Habitat types classified in image data for Point Pelee National Park. Ecosites presented in

scale. Each metric was regressed against year to determine rate of change during our study period (1931–2015). Table 8.3: Metrics used to describe habitat changes in Point Pelee National Park at the landscape and class

Scale	Scale Habitat	Metric	R <sup>2</sup>	Rate of change	p-value	1931	2015	
		AW Mean Patch Area	0.81	1.84 ha/yr	0.0009	67.7 ha	186.0 ha	
		AW Mean Radius of Gyration	0.67	2.98 m/yr	0.0066	532.1 m	714.1 m	
		AW Mean Core Area	0.82	1.64 ha/yr	0.0008	50.8 ha	155.0 ha	
T andecane	nla	Effective Mesh Size	0.84	1.92 ha/yr	0.0005	58.4 ha	186 ha	
adaramat	đ	Splitting Index	0.91	-0.23 patches/yr	< 0.0001	26.4 patches	8.0 patches	
		Shannon's Diversity Index	0.92	TA 800.0-	< 0.0001	2.4	1.8	
		Simpson's Diversity Index	0.90	-0.002 /yr	0.0001	0.88	0.75	
		Simpson's Evenness	0.89	-0.002 /yr	0.0001	0.93	0.80	
		AW Mean Patch Area	0.84	4.12 ha/yr	0.0005	63.9 ha	336.1 ha	
	Cattail OSM	AW Mean Radius of Gyration	0.82	7.43 m/vr	0.0007	377.4 m	903.2 m	
		Effective Mesh Size	0.83	2.12 ha/yr	0.0006	12.8 ha	141.8 ha	
112	Once Worker	AW Mean Core Area Index	0.57	0.11 %/yr	0.01	75.1%	83.4 %	
Class	Open water	Number of Disjunct Core Areas	0.55	-0.73 areas/yr	0.01	79	33	
	Forb OSM	AW Mean Core Area Index	0.57	-0.53 %/vr	0.02	49.6%	00.00 %	
8	Lorent	AW Mean Patch Area	0.82	0.47 ha/yr	0.0004	4.2 ha	49.8 ha	
	LOICEI	AW Mean Radius of Gyration	0.75	4.68 m/yr	0.0012	128.3 m	558.8 m	
	Meadow	AW Mean Core Area Index	0.46	-0.46	0.03	64.1%	33.7%	

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**Figure 8.1:** Habitat composition throughout the sampling period (1931 to 2015) in Point Pelee National Park. The quality of the 1990 image did not permit classification of marsh ecosites/vegetation types (i.e., common reed, cattail organic shallow marsh, graminoid organic shallow marsh, forb organic shallow marsh) and is only presented at the community level (marsh). Habitat types comprising < 0.5% of the park were excluded.



**Figure 8.2:** Land-cover trends over 85 years in Point Pelee National Park. (a) Marsh habitats, excluding 1990. (b) Upland habitats. (c) Shoreline, beach and anthropogenic classes. Habitat types comprising < 0.5% of the park were excluded.



**Figure 8.3:** Plot of areal extent of Point Pelee National Park and open sand barren/dune as a function of annual mean water level in Lake Erie (meters above sea level). Both areal extent of Point Pelee National Park and open sand barren/dune were significantly correlated with water level (p = 0.01); Spearman's rho correlation coefficient is shown separately for each correlation.



**Figure 8.4:** Total area of Point Pelee National Park from 1931 to 2015. A net loss of 58 ha was experienced over this time period. Years with a larger park area tend to be associated with higher water levels and vice versa.



**Figure 8.5:** Change in marsh habitat in Point Pelee National Park between 1959 and 2015. (a) and (b) indicate hotspots of major habitat change and infilling within the marsh (solid-lined boxes). (c), (d), and (e) indicate hotspots of invasive common reed (dashed-lined boxes).

<b>Table S8.1:</b> Landscape composition in hectares in Point Pelee National Park from 1931 to 2015. Habitat types are presented according to the greatest amount of change between 1931 and 2015. The quality of the 1990 image	prevented classification beyond marsh community class and therefore we have no data (nd) for ecosites/vegetation	types.	
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Habitat	1931	1959	1973	1977	1985	1990	2000	2004	2010	2015	Change in landcover 1931-2015
	2010/01/02	1000			0.00000	0.0000		1000 C			(na)
Cattail Organic Shallow Marsh	309.0	289.5	484.6	461.9	519.4	pu	663.6	656.9	673.0	624.9	+315.9
Forest	36.5	46.4	124.1	174.6	161.1	140.7	181.2	157.4	186.5	197.9	+161.4
Open Water	247.8	349.3	321.3	345.4	392.9	322.1	324.2	313.7	306.2	312.0	+64.2
Thicket	2.7	9.8	22.7	53.6	58.5	60.9	80.0	88.3	66.3	64.2	+61.5
Common Reed	0.0	0.0	0.0	0.0	0.0	pu	1.1	0.0	31.4	47.3	+47.3
Swamp	58.0	54.1	68.8	57.7	42.4	49.5	45.0	50.0	58.3	69.4	+11.4
Graminoid Mineral Meadow Marsh	0.0	2.7	3.8	5.7	0.0	pu	6.8	6.9	3.3	4.3	+4.3
Mixed Mineral Meadow Marsh	0.0	0.0	1.2	0.8	0.0	pu	0.7	0.3	0.2	0.2	+0.2
Agriculture	95.7	92.3	8.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-95.7
Graminoid Organic Shallow Marsh	110.0	210.8	217.8	200.4	109.4	pu	25.0	31.8	1.0	21.8	-88.2
Sand Barren/Dune	65.1	56.8	38.2	30.2	65.6	41.9	27.2	20.6	22.3	24.7	-40.4
Savanna	36.2	13.3	10.7	14.0	0.0	0.0	0.0	0.0	0.0	0.0	-36.2
Mineral Shoreline	70.4	34.4	21.5	32.7	30.0	50.2	50.3	47.0	41.5	39.6	-30.8
Woodland	80.2	98.8	74.2	49.6	90.1	119.0	51.1	84.1	64.8	51.9	-28.3
Meadow	31.5	13.2	60.7	33.4	9.3	4.9	10.9	10.0	8.5	10.1	-21.4
Forb Organic Shallow Marsh	10.7	194.5	10.6	14.7	18.7	pu	2.0	2.0	1.7	1.2	-9.5
Constructed	18.4	58.2	37	26.1	17.2	28.7	19.1	14.7	16.8	15.7	-2.7

## **Chapter 9: General Conclusion**

## <u>Summary</u>

A key step in generating effective recovery strategies for species at risk is to identify habitat used under a variety of geographic settings. Due in part to habitat loss and degradation, the Blanding's turtle (*Emydoidea blandingii*) is considered at-risk across most of its range. Because little information for this species exists for the many islands of Georgian Bay, the world's largest freshwater archipelago, in chapter 2, we conducted an intensive study on the habitat use of Blanding's turtles on a protected island. We found that both sexes utilized vernal pools and wet forest to move between habitat patches. Females used inland wetlands early in the year and coastal wetlands during the nesting season, whereas males maintained extensive use of inland wetlands during the entire active season. We also identified year-to-year differences in habitat selection by females during the nesting season that we attribute to differences in weather (amount of precipitation in the spring) and its effect on availability of vernal pools, an important temporary habitat that provides food, hydration, and shelter. We confirmed that Blanding's turtles used nesting habitat unique to the Georgian Bay landscape, lichenfilled cracks in bedrock, and observed fidelity to this habitat type and the nesting location. Overall, critical habitat types for both males and females included: 1) upland and coastal wetlands for annual use, 2) vernal pools, beaver ponds, and wet forest to access and travel between wetlands, and 3) rocky outcrops for nesting sites. To the best of our knowledge, this was the first study to determine habitat selection by Blanding's turtles in the Georgian Bay archipelago.

Relatively little is known about thermal tolerances and requirements of the Blanding's turtle during the overwintering period. In chapter 3, we used water temperature data from confirmed and unconfirmed overwintering habitats to characterize thermal suitability of overwintering habitat. We found that water temperatures of all occupied overwintering habitats ranged from 0.44° C to 3.68° C, with a mean of 1.77° C  $(\pm 0.03^{\circ} \text{ C})$ , and showed slow steady declines throughout the overwintering period. Regardless of location, average water temperatures at all confirmed overwintering habitats remained above the freezing point of turtle body fluids (- $0.6^{\circ}$  C). Average water temperature rarely dropped below 0° C, but dropped to -0.33° C for eight days at one location. Turtles in our Georgian Bay site were under continuous ice cover for 99 days, strongly supporting the hypothesis that Blanding's turtles are anoxia tolerant and supports water temperature as the primary selection criterion. Determining thermal parameters suitable for overwintering is one step towards more thoroughly understanding where turtles overwinter and why, and can provide knowledge useful for habitat creation and ensuring current habitats continue to provide suitable overwintering conditions.

With increasing anthropogenic pressures, maps indicating suitable habitat can aid management decisions and prioritize areas for protection. In chapter 4, we revealed that islands in Georgian Bay with higher proportions of wetlands and vernal pools were generally considered to be suitable for Blanding's turtles compared to those with lower proportions. Our findings highlight the importance of both permanent and temporary wet habitats for Blanding's turtles. Based on our final model, approximately 64% of

evaluated islands or 89% of the total mapped area in the archipelago is suitable for Blanding's turtles.

The assessment and evaluation of a species' status requires data on habitat occupancy, as well as identification of threats to their critical habitat, both of which are difficult to obtain when the species occurs across large spatial scales. In chapter 5, we found coastal wetlands which supported musk turtles were associated with higher proportions of forest cover, lower densities of roads, buildings, and docks within 1 km of the wetland, and more variable bathymetric slopes. We also had a 64-71% probability of detecting a musk turtle whenever present in the wetland using our modified fyke net protocol. As a whole, high coastal wetland occupancy across majority of our study area indicates that at present, habitat quality in eastern Georgian Bay is in good condition; however, land-use alterations and development should be limited to ensure continued musk turtle presence.

Dense patches of *Phragmites australis* generally provide poor habitat for many species, although specific impacts on at-risk turtles are largely unknown. At the home-range scale (2<sup>nd</sup> order), we found that turtle home ranges were distributed irrespective of location of *Phragmites* patches within the population range, resulting in a positive association between turtle home range and *Phragmites* (chapter 6). At the individual scale (3<sup>rd</sup> order), however, turtle radio locations were significantly farther from *Phragmites* patches than from random points. During nesting migrations, females did not avoid *Phragmites* patches at the 3<sup>rd</sup> order scale, but instead, increased their interactions with *Phragmites*, placing themselves at increased risk of being stranded within dense

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patches. Our results indicate that invasion of *Phragmites* reduces the amount of effective habitat for at-risk turtles in wetlands, since Blanding's turtles significantly avoided *Phragmites* patches wherever they proliferated within the home range. This was the first quantitative evidence of the negative consequences of *Phragmites* invasion on availability of effective habitat for the Blanding's turtles and on their spatial ecology.

In chapter 7, we evaluated the success of road mortality mitigation strategies by 1) comparing results of road surveys conducted 5 years before and 5 years after fencing installation and 2) monitoring use of culverts by turtles using motion-activated cameras at culvert openings and stationary antennas placed to detect movements of passive integrated transponder (PIT)-tagged turtles. In completely fenced road sections, turtle and snake abundance was 89% and 53% lower compared to unfenced sections. In partially fenced sections, however, turtle and snake abundance was 6% and 10% higher compared to unfenced sections. After installation of exclusion fencing, locations where we found reptiles on the road were associated with fence ends, underscoring the importance of fence integrity and ineffectiveness of partial fencing as a mitigation strategy. We confirmed use of culverts by 4 species of turtles, some using recently installed culverts to safely cross under the road. Lastly, we used radio tracking to determine that male and female Blanding's turtles home ranges overlapped with different segments of the road, directing future mitigation efforts.

In chapter 8 we studied a protected landscape to identify any long-term changes in habitat availability and distribution. Since 1931, marsh habitat diversity and aquatic connectivity declined. The marsh matrix transitioned from a graminoid and forb shallow

marsh interspersed with water to a cattail dominated marsh, altering critical breeding, foraging, and overwintering habitat for reptiles and amphibians. Furthermore, reduced diversity of marsh habitats appears to be linked to the expansion of invasive *Phragmites australis*, which invaded prior to 2000. Continued loss of open habitats such as savanna and meadow has limited high quality thermoregulation habitat for reptiles.

## Recommendations

Based on the results from this thesis, I make the following recommendations that will advance the conservation of freshwater turtles.

- An effective conservation strategy for Blanding's turtles in Georgian Bay must include protection of inland and coastal wetlands, in addition to the surrounding upland matrix and connecting corridors.
- 2) We found a few male Blanding's turtles that dispersed long distances to use an area that was unique compared to our other tagged turtles. This behavior may aid in gene dispersal if a male mates with females from different resident wetlands. It is important to be aware of the turtles that travel long distances as they could be important for sustaining the population and an effort should be made to identify and protect the habitat used as travel corridors.
- Determining overwintering sites is important for conservation planning and research on microhabitat may determine key site features. In particular, thermal parameters of overwintering sites can provide knowledge useful for habitat

restoration and creation to ensure habitats provide suitable overwintering conditions in the face of global climate change. The impacts of climate change on turtles are largely unknown, but we can improve population resilience with targeted wetland conservation and restoration. For example, managers and planners can record wetland water temperatures during the winter months as a cost-effective framework to determine and monitor suitable overwintering sites.

- 4) I recommend an integrative approach be applied to create habitat suitability maps for other species at risk in Georgian Bay. Since our habitat suitability models are intended for use in conservation, false absences are more problematic than false presences, especially for a species at risk. I therefore recommend using a model that tends to predict suitable habitat even though the species cannot be detected. While the extent of suitable habitat will always be larger than a species' realized distribution and its overestimation may be preferred, the model should have reasonably good performance so that money and resources are not wasted.
- 5) Given that musk turtle occupancy of coastal wetlands is negatively affected by human activity, we recommend that anthropogenic alterations (i.e., construction of docks, buildings, and roads) within 1 km of an occupied wetland should be regulated and declines in forest cover within 250 m be limited to ensure long-term persistence of musk turtles in Georgian Bay.

- 6) I recommend occupancy maps be used to guide future musk turtle surveys and identify coastal wetlands with high probability of occupancy to ensure site-level protection and population persistence. In particular, I recommend that occupancy maps for any species at risk explicitly incorporate detection probabilities to avoid underestimating occupied habitat.
- 7) We recommend the continued treatment of *Phragmites australis* to restore marsh habitat used by Blanding's turtles for feeding, mating, and overwintering.
- 8) We recommend that stakeholders emphasize ensuring fence integrity and continuity, limiting impact of edge effects by curving fence ends up to 75 m to redirect species, and conducting a comprehensive monitoring program. Most importantly, resources should be allocated towards complete fencing on both sides of the road to ensure road mortality declines; partial fencing is not an effective strategy to reduce road mortality for reptiles, in fact, it is no better than having no fencing. Since fence integrity is key to success, a vigilant inspection and maintenance program is essential. Selection of fence material is also vital. In our study, 2 different fencing types were required to withstand upland, windy conditions and wet, marsh conditions.
- 9) Our results suggest that long-term landscape changes resulting from habitat succession and invasive species can negatively affect habitat suitability for

herpetofauna and protection of land alone does not necessarily equate to protection of sensitive herpetofauna. To enhance marsh habitat diversity and achieve a more diverse vegetative and structural state (e.g., hemi-marsh condition), active management should include removal and control of *Phragmites* and monotypic cattail patches.

 We recommend use of unmanned aerial vehicles to collect high spatial and temporal resolution image data to revolutionize the way we address and answer ecological questions.

#### Future Work

During the research completed for this thesis, many new questions developed that would continue our work and provide valuable data towards improving conservation of freshwater turtles.

1) Inter-annual differences in weather patterns may influence usage of wet and dry habitats and should be investigated further, especially in light of predicted changes associated with global climate change. Changes to timing and duration of inundated habitats may have consequences for the long-term viability of turtle populations. Therefore, future research should focus on differences in precipitation from year to year and how they may affect the timing of migration and the use of temporary habitats.

- 2) Aquatic overwintering poses many risks to turtle survival, and increasing air temperatures and decreasing precipitation associated with global climate change may have negative ramifications on the suitability of overwintering sites. Future research should investigate changes in phenology attributed to climate change because there is the potential for dissociation between times when turtles emerge and when resources become available. With warmer winter temperatures and reduced ice cover, turtles may emerge multiple times throughout the winter season. Additional energy reserves would be required to allow turtles to move into and out of overwintering sites, but these may not be forthcoming if food resources are unavailable during the winter months.
- 3) Future research should begin to determine the structural requirements of an overwintering site to provide suitable water temperatures. Parameters outlining suitable temperatures and additional research on the physical structure of suitable overwintering habitats can then help improve wetland restoration and creation projects to ensure they provide necessary overwintering refugia. As additional field data become available, temperature thresholds determined from *in-situ* research will have the greatest applicability for habitat conservation purposes, especially in the face of a changing climate.
- 4) Since our Blanding's turtle habitat suitability models were developed during the summers of 2011 and 2012, all habitats were mapped and suitability predicted

following a sustained period of low water levels in Georgian Bay beginning in 1999. Given that the structure of vegetation communities in coastal wetlands are significantly affected by inter-annual variation of water-levels (or lack thereof), our model may be used in a comparison to investigate how changes in coastal wetland habitat affects habitat use by Blanding's turtles under different waterlevel regimes.

- 5) To my knowledge, no one has reported individual turtles using multiple islands in Georgian Bay; although there is no reason to believe that multiple island use may not occur. Future research could determine the extent at which Blanding's turtles can swim to access habitats across multiple islands.
- 6) Majority of the satellite imagery used for habitat suitability mapping was collected during mid-summer (July and August), meaning that I was not able to use these images to map vernal pools. Instead, I used orthophotos acquired during spring, when pools are usually fully inundated and canopy cover is minimal. But, even with the combination of spring imagery and some ground truthing, it is likely that presence of vernal pools was underestimated. Future research should focus on more accurate and large-scale mapping applications.
- 7) An appropriately designed study should be conducted to determine how turtles resume use of treated *Phragmites australis* patches at various stages of

revegetation. Future research should determine how much effective habitat can be reclaimed after large-scale eradication of *Phragmites* is achieved. Furthermore, the collection of time-series data of a population just beginning to experience *Phragmites* invasion would determine if turtles can change the location and orientation of their home range to avoid *Phragmites*, at least during the early stages of invasion. On a related note, it is critical to continue developing and testing effective treatment methods for *Phragmites australis* in the presence of standing water given the current herbicide limitations in Ontario.

- More attention should be paid to developing and installing an exclusion fencing system that can effectively prevent larger climbing snakes from accessing the road.
- 9) I recognize that in some cases partial fencing is the only option because of private property. For these cases, improved fencing designs that prevent animal access to the road must be developed and tested.
- Research to determine effectiveness of culvert placement and type, length of lag time before use, and species-specific preferences will require a carefully designed long-term before-after-control-impact monitoring program.

- 11) Evidence suggests that controlling a marsh towards a hemi-marsh condition would provide both vegetative and structural diversity; however, this has not been explicitly studied from a herpetological perspective and would provide direct evidence to support future marsh restoration efforts.
- 12) Protection of land alone is not always sufficient to ensure long-term persistence of herpetofauna; thus, there is an urgent need to assess the resilience of protected areas and implement evidence-based restoration strategies. First, future research should assess the resilience of protected areas to a changing climate, surrounding development, and Canada's worst invasive plant, *Phragmites australis*. Second, it will be important to investigate and evaluate the efficacy of habitat restoration as an adaptation strategy to improve resilience. Third, it will be vital to mobilize new knowledge to aid on-the-ground implementation and application of resulting strategies. Results from this research should provide significant data towards assessing and restoring the current and future resilience of protected areas and allow us to prioritize the implementation of habitat restoration strategies.
- 13) Under the proposed Wetland Conservation Strategy for Ontario 2017-2030 (Ontario Ministry of Natural Resources and Forestry 2017), 'lands that are seasonally flooded by shallow water' are included under the definition of a wetland. Despite this, vernal or ephemeral pools are not classified as one of the major types of wetlands. The explicit consideration of vernal pools as a unique

wetland category is the first critical step towards acknowledging their importance as habitat for at-risk reptiles and ensuring vernal pools receive proper protection. The importance of vernal pools for species such as the Blanding's turtle (see chapters 2 and 4) cannot be overlooked if we intend to protect critical habitat required for Blanding's turtles to successfully carry out life cycle activities. Furthermore, the proposal to monitor wetland conservation success through the use of no net loss targets will likely prove to be challenging. Many freshwater turtles, including the Blanding's turtle, use the same wetlands year after year for mating, feeding, and overwintering (see chapters 2-4). From a turtle's perspective, the application of a general no net loss policy may not be sufficient to ensure long term population persistence. This is especially true if the new wetland is created in an area that is disjunct from the original location and turtle population. Even if the new wetland is created with the intention to provide habitat for at-risk reptiles, this field of research is young. There are many decades of research ahead of us before we can confidently say that the function of an existing wetland as at-risk reptile habitat can be replicated. As the human population continues to grow and development needs evolve, the protection of wetlands and at-risk turtle habitat will become even more important and likely make this field of research even more critical.

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# Chapter 10 / Appendix A: Georgian Bay, Lake Huron: freshwater turtles and their wetland habitat in a changing landscape

By

Chantel Markle and Patricia Chow-Fraser

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# Introduction

Georgian Bay, the eastern arm of Lake Huron, is home of the world's largest freshwater archipelago, a World Biosphere Reserve (UNESCO, 2014) that contains more than 30,000 islands (Figure 1). Considered the 6<sup>th</sup> Great Lake by some (Barry, 1978), Georgian Bay spans 15,000 km<sup>2</sup>/5,800 square miles and is almost as large as Lake Ontario (18,960 km<sup>2</sup>/7,320 square miles). Unlike Lake Ontario, Lake Erie, and Lake Michigan, which are underlain by sedimentary rock (sandstone and limestone), bedrock in Georgian Bay is Canadian Shield, which is granitic with only a very thin layer of soil (Georgian Bay Ecoregion; Figure 10.1). This bedrock geology, together with exposure to prevailing winds, has inspired the iconic image of windswept pines on top of rocky shorelines popularized by Canada's Group of Seven artists. Georgian Bay is also unique because its convoluted shoreline has led to the development of many small coastal wetlands. In fact, more than 12,600 distinct wetlands were identified within the bay's coastal zone (Midwood, Rokitnicki-Wojcik, and Chow-Fraser 2012). This is more than twenty-four times as many coastal wetlands than Lake Michigan (524), Lake Huron (493), Lake Ontario (488), Lake Superior (307), and Lake Erie (149; Ingram et al., 2004). Although most of the wetlands in Georgian Bay are small (< 2 ha/0.008 square miles in size), they tend to form complexes that function like larger wetlands, providing reproductive and foraging habitat for many fish species and aquatic wildlife (Midwood and Chow-Fraser, 2014). In addition to having the highest density of coastal wetlands in the Great Lakes basin, it has a disproportionate number of contiguous pristine wetlands (Cvetkovic and Chow-Fraser, 2011).

Recently, only two of the 175 coastal wetlands surveyed in eastern Georgian Bay showed signs of degradation attributable to human activities per the Water Quality Index (Chow-Fraser, 2006). Although the remaining 99% of surveyed coastal wetlands are in good to excellent condition, there are increasing threats to wetlands from road expansion, as well as cottage and residential development along the shoreline (Niemi et al., 2007; Walton and Villeneuve, 1999). This is particularly true in southern Georgian Bay, where coastal wetlands have been developed at the expense of wildlife habitat. Since southern Georgian Bay is only a two-hour drive north of Toronto, it is easily accessible to weekend users, making it a popular vacation destination. Georgian Bay also contains the busiest recreational waterway in Canada (Walton and Villeneuve, 1999). These factors put species and their habitats at risk (Bywater, 2013), although not to the same extent that urbanization and agricultural development impacts habitat south of the Canadian Shield. Another factor affecting coastal wetlands in Georgian Bay has been fifteen years of sustained low water levels (1999 to 2014) (Canadian Hydrographic Service, 2012); only recently have water levels rebounded above the long-term average. The loss of interannual variation of water levels significantly affects the structure of vegetation communities in coastal wetlands (Midwood and Chow-Fraser, 2012). The loss of vegetation structure and diversity can have cascading effects on species reliant on coastal wetlands. Given the unprecedented nature of this disturbance, we do not know how long it will take before coastal wetlands will recover, or indeed, if they will transition to an altogether different state, especially with a changing climate. All these threats could
fundamentally change ecosystem processes in coastal marshes of Georgian Bay, and we have the rare opportunity to study them as they adapt to new conditions.

These coastal and upland wetlands provide critical breeding, foraging, and overwintering habitat for many rare avian, fish, and reptile species, and it is well documented that degradation in wetland quality affects their abundance (DeCatanzaro and Chow-Fraser, 2010; Seilheimer et al., 2007; Smith and Chow-Fraser, 2010). A good example is the thirty-three species of reptiles and amphibians that live in the Georgian Bay archipelago, a higher herpetofauna diversity than anywhere else in Canada (Parks Canada, 2013). Of these species, the coastal zone of Georgian Bay supports six species of freshwater turtles: spotted turtle (*Clemmys guttata*), snapping turtle (*Chelydra serpentina*), painted turtle (*Chrysemys picta marginata*), northern map turtle (*Graptemys geographica*), eastern musk turtle (*Sternotherus odoratus*), and the Blanding's turtle (*Emydoidea blandingii*). All except the painted turtle are listed as species at risk in this region (Figure 10.2).

Although turtles are one of the oldest species in the world and have survived ice ages and mass extinctions, habitat loss and degradation following European settlement over the past 200 years have led to their current decline (Ernst and Lovich, 2009). In particular, the Blanding's turtle is listed as a threatened species provincially and endangered federally in its Ontario Great Lakes range due to habitat alteration and destruction (Government of Canada, 2017; Marchand and Litvaitis, 2004; Ontario Government, 2007; Steen and Gibbs, 2004). The Blanding's turtle is an important focal species because it is a semi-aquatic species known to make extensive use of both aquatic

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and terrestrial habitats. Across the species' range, Blanding's turtles are known to use aquatic habitats such as vernal pools, bogs, marshes, and fens (Edge et al., 2010; Hartwig and Kiviat, 2007; Markle and Chow-Fraser, 2014), and terrestrial habitats (e.g., forest) throughout the active season (Ernst and Lovich, 2009). Their use of habitat mosaics makes them the perfect candidate for landscape-level conservation approaches (Figure 10.3). Furthermore, they can be thought of as an umbrella species. This means that if we can identify and protect habitat required for the Blanding's turtle, we will likely protect many other species in the process. This is especially beneficial in Georgian Bay because it is difficult to carry out extensive field sampling for many species in remote wetlands. By designing a detailed landscape conservation plan for an umbrella species, we can better direct resources while having the greatest positive impact.

#### Statement of the issue

In response to increased pressure from human development, which threatens freshwater turtles and their habitats in this region, we conducted research to enhance conservation strategies for Blanding's turtles in the Georgian Bay archipelago. The geologically distinct landscape that dominates the coastal islands here make it difficult for us to directly apply information to our study sites from populations living elsewhere in Canada and the United States. (e.g., Beaudry, deMaynadier, and Hunter, 2009; Edge et al. 2009, 2010; Hartwig and Kiviat, 2007; Millar and Blouin-Demers, 2011). When designing landscape approaches, it's important to apply them in the appropriate setting. Therefore, we collected data to develop a habitat suitability model specific to the Georgian Bay landscape. We developed the model using information collected from a

population of Blanding's turtles on a protected island and then applied our results to the entire archipelago to produce regional maps for conservation and management purposes. To track types of habitat used by turtles during various activities (e.g., mating, feeding, nesting and overwintering, etc.), we used radio telemetry techniques to follow turtles for more than two years. This interdisciplinary approach combined field data, remote sensing, and statistical modeling to produce spatially explicit statistical models that should advance efforts to develop effective management plans for Blanding's turtles throughout the biosphere reserve. The overall goal is to identify all suitable islands in the archipelago to mark them for protection in conservation plans before they become degraded or developed.

#### <u>Methods</u>

#### *Radio tracking*

Radio tracking allows us to locate a tagged turtle throughout the season to study movement patterns and habitat use. At the beginning of the study, we attached a radio transmitter to the back of each turtle's shell with a quick dry epoxy (Figure 10.4). We ensured the transmitter had a streamlined finish to allow the turtle to move easily through water and vegetation. We then used a receiver and antenna to scan for the unique radio frequency emitted by each transmitter (Figure 10.5). We radio tracked turtles at least once per week during the active season and approximately once a month during the winter. Whenever a turtle was located, we recorded the date, time of day, GPS location, turtle's activity, weather conditions, and details about the habitat. We used the series of

radio locations for each turtle to determine where the turtle moved throughout the year and the key habitats it used.

### GPS logger

In some cases, we also attached a GPS logger to a turtle to collect additional locational data on a pre-determined schedule (Figure 10.6). By changing the timing of when the GPS attempts to record a location, we could increase (e.g., every hour to capture small scale movement) or decrease the number of fix attempts (e.g., every few days to capture large scale movement). This is useful for collecting data on female nesting movements that tend to occur at night when researchers are generally not in the field to radio track a turtle. GPS loggers are an important tool to enable researchers to accurately map the extent of turtle movements (Christensen and Chow-Fraser, 2014).

# *Geographic information systems*

We used a geographic information system (GIS) to display and analyze our spatial data. In a GIS, we created maps of our study area, displayed turtle locations and movements, and mapped suitable habitats. In addition, we used aerial imagery of our study site. Aerial images allowed us to see the entire study area and map out the location of important habitats such as wetlands and forests. Depending on the time of year the aerial image was acquired, we could also determine the amount of vegetation that has grown in an area of interest. By using unmanned aerial vehicles (i.e., drones), we can capture high-resolution aerial images at the most desired time, even multiple times a season, or annually for several years to quantify habitat change. Unmanned aerial vehicles are especially useful in dynamic systems such as coastal wetlands where the

percent of floating and emergent vegetation fluctuates throughout the summer and requires current aerial images to produce accurate results (Marcaccio, Markle, and Chow-Fraser, 2016).

#### Findings

### Habitat use

Blanding's turtles use a mosaic of aquatic and terrestrial habitats, exhibiting fidelity to their residence wetlands, returning year after year to the same wetland (Congdon, Kinney, and Nagle 2011). Generally, they emerge in April or May from overwintering habitats such as permanent pools (Joyal, McCollough, and Hunter, 2001; Ross and Anderson 1990), streams (Newton and Herman, 2009), and wetlands (Edge et al., 2009; Kofron and Schreiber, 1985). During the spring, turtles often bask on logs or mats of vegetation to increase their body temperature and metabolism. Turtles may also move among wetlands in search of food, while males additionally search for mates. In early summer, females begin their journey to nesting sites, and this can include sojourns across multiple wetland types such as vernal pools, bogs and marshes (e.g., Beaudry et al., 2009; Markle and Chow-Fraser, 2014; Standing, Herman, and Morrison, 1999). In addition to diverse habitat use, males and females may move long distances to mate with adjacent sub-populations to increase gene flow (McGuire, Scribner, and Congdon 2013). Females have been known to move more than 6 km (3.73 mi) in search of a nesting site (Edge et al, 2010) and we found males moving 900 m (0.56 mi) in early summer in our study site (Markle and Chow-Fraser, 2014).

Our study highlights the extent and variety of habitat types used by Blanding's turtles in the Georgian Bay archipelago to carry out their life processes (Figure 10.7; Markle and Chow-Fraser 2014). Initially, females used inland wetlands for feeding and basking. Two bogs were resident wetlands for all tagged turtles (i.e., bearing transmitters; n = 15) and were dominated by 30 to 38 percent cover of *Sphagnum* moss mats. In late May, females used patches of swamp and isolated vernal pools to move to coastal wetlands -- considered staging habitat -- where they spent one to two weeks feeding before migrating to nesting grounds. Females nested in soil-filled crevices in open rocky outcrops, a nesting site type unique to the Canadian Shield. In southwestern Ontario, majority of nesting occurs along sandy beaches or roadsides in urbanized areas. Upon completion of nesting, females travelled about 400 m (0.25 mi) to return to their resident wetlands where all tagged turtles successfully overwintered. The males' habitat use and movements were less extensive than those of females. Over the course of the spring and summer, males spent most of their time in two bogs, using a chain of small vernal pools to move among them. Our study identified vernal pools -- seasonally inundated wetlands -- as a critical habitat type for Blanding's turtles in Georgian Bay. Both males and females used them as corridors to access permanent wetlands and nesting sites. Since vernal pools are sourced by snowmelt and spring rainwater, they begin to dry up through the summer before refilling in fall. However, this cycle of filling and draining depends on weather patterns. In years with reduced snowmelt and warmer winter temperatures, there was reduced distribution of vernal pools throughout the landscape, and this apparently resulted in turtles using other connecting corridors. Climate change is expected to

influence the abundance and accessibility of vernal pools, but the extent to which this may alter habitat connectivity and movements of Blanding's turtles is unknown and should be investigated.

#### Distribution of suitable habitat

By developing a habitat suitability model, we identified the primary habitat requirements for the Blanding's turtle in the Georgian Bay islands. We also created a map of all the islands with suitable habitats for the turtles, and by extension, additional wetland-dependent species. Our resulting maps scored the suitability of an island for Blanding's turtles on a scale of zero to one, and it was statistically derived to reflect the similarity of an island to the habitat composition required by our previously studied subpopulation of turtles. We interpreted an island with a score of zero as having a low probability of containing suitable habitat for Blanding's turtles. On the other end of the scale, a score of one would indicate that the island has a high probability of providing suitable habitat for them.

Our habitat suitability map indicates that approximately 64 percent of the evaluated islands or 89 percent (14,027 ha/54 square miles) of the total mapped area in the archipelago is suitable for Blanding's turtles. Our results confirm the assumption that this coastal zone provides high-quality habitat for species at risk. In comparison to the remaining habitat fragments in southwestern Ontario, it is likely that habitat in the archipelago contains much of the undisturbed habitat within the provincial range of the Blanding's turtle. That said, the presence of suitable habitat does not equate to turtle occupancy because of competition for food and other resources, predator abundance, and

quality of nesting and overwintering sites. Therefore, the distribution of suitable habitat will always be larger than the actual distribution of the species. For conservation purposes, however, it is better to overestimate distribution of suitable habitat and include areas with suitable habitat but no turtle population than to risk overlooking a single subpopulation of this at-risk species.

#### **Discussion and Conclusions**

Within Georgian Bay, freshwater turtles face many threats including habitat loss and degradation through shoreline modification and cottage development, road mortality, and climate change. Although these same threats exist in other regions, the geological landscape in Georgian Bay is unique compared to other areas within the turtle's range, making it difficult to automatically apply habitat data collected from other populations. Therefore, we collected data to use in developing regional management strategies for Blanding's turtles in the coastal areas of Georgian Bay. One of the first, and perhaps most important, steps when developing a regional strategy is to map suitable habitat for the atrisk species. Next, identifying threats to the population and habitat allows for targeted management strategies. Roads are a concern for many turtle populations because they fragment habitats, and such fragmentation can have a negative impact on species that migrate long distances, often resulting in individuals crossing roads and leaving protected areas. By knowing where suitable habitats exist in relation to roads, land managers and conservation authorities can mitigate road mortality by constructing fences and ecopassages to reconnect fragmented habitat (e.g., Aresco 2005; Beier and Noss 1998; Lesbarreres and Fahrig, 2012). Reduction of adult mortality can play a major role in the

recovery of declining populations because freshwater turtles have long life spans, delayed sexual maturity, and low rates of juvenile recruitment (Congdon et al., 1993; Marchand and Litvaitis, 2004; Steen and Gibbs, 2004). Therefore, protection of pristine habitat should be the first priority. Subsequently, populations experiencing adult mortality should should be identified and threats should be mitigated. Once a source of adult mortality has been addressed, other conservation measures such as nest protection and head-starting (i.e., incubating eggs, raising turtles, and releasing them into the wild when they are larger, giving them a 'head-start' at life) can be implemented to improve juvenile recruitment.

As a starting point, Georgian Bay conservation plans should emphasize protection of unique open-rocky nesting habitats, upland and coastal wetlands used for critical activities such as feeding, mating, and overwintering, and the connecting habitats used to travel among these key areas. Many studies have confirmed the frequent use of wetlands and vernal pools by the Blanding's turtles throughout the active season (e.g., Joyal et al., 2001; Millar and Blouin-Demers, 2011), and the importance of wetland areas in relation to increasing turtle occupancy (Fortin, Blouin-Demers, and Dubois, 2012). Other modeling work indicated that the risk of turtle extinction increased as vernal pools were lost from the landscape (Gibbs, 1993). Similarly, we found that vernal pools are important features in the Georgian Bay landscape with high ecological value within this ecosystem. We also found that use of vernal pools as a connecting corridor varied among years, and we hypothesize that variation in annual precipitation may affect the timing of turtle movements and use of temporary habitats. In the face of climate change, the spatial

distribution of vernal pools is likely to change and is expected to impact the timing and movement patterns of turtles throughout the landscape. There is an urgent need for natural resource managers to be aware of this because vernal pools receive no formal protection under the law as an independent category by provincial agencies. Currently, the only way to protect vernal pools is to classify each one, on a case-by-case basis, as part of a wetland complex through the Ontario Wetland Evaluation system (Ontario Ministry of Natural Resources and Forestry, 2013b) or the Blanding's turtle habitat regulation (Ontario Ministry of Natural Resources and Forestry, 2013a). Future research should aim to understand how a changing climate will affect the distribution of seasonal wetlands across the landscape.

Although most of coastal Georgian Bay and the archipelago has limited human disturbance, some of islands are being threatened by increasing development pressures. Therefore, ensuring valuable habitats are protected is essential for long-term conservation efforts. For areas experiencing higher levels of development, such as in Severn Sound and Honey Harbour, creating local habitat suitability maps can help guide more detailed management plans. Now that we have begun identifying suitable habitats, it is critical to begin a strategic monitoring program to survey the actual distribution of Blanding's turtles in this area. A lot of work remains if we intend to protect the turtles and high-quality habitat from certain degradation and fragmentation. Ensuring that the landscape provides the habitats required to sustain a population of any species at risk should be the most important aspect of any conservation or mitgation strategy. Without the habitat that

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species require, no amount of population augmentation, nest protection, and road fencing will ensure long-term population persistence.

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*Sistrurus catenatus catenatus*: population and habitat management issues in urban, bog, prairie and forested ecosystems, Toronto Zoo, Toronto, Ontario.



**Figure 10.1:** Georgian Bay is the eastern arm of Lake Huron. (Inset) The Georgian Bay Ecoregion is characterized by exposed Precambrian bedrock and mixed forests.

What is a species at risk?

A species that is facing population declines or whose habitat is facing threats.

Typically, this is due to human-based impacts. The species may become extinct if

nothing is done to reverse these threats.

Figure 10.2: What is a species at risk?

Landscape ecology and conservation

Landscape ecology is the study of spatial patterns at a variety of spatial scales. Research may involve the mapping or analysis of patterns in different landscapes, their change through time, and the impact of human disturbance. Studies may also examine the relationships and interactions among landscape features and habitat composition and its species. Conservation strategies at the landscape scale are designed to consider a more holistic, large-scale approach to managing a particular area.

Figure 10.3: Landscape ecology and conservation.



Figure 10.4: A radio transmitter is attached to a turtle's shell with epoxy.



Figure 10.5: A researcher uses a receiver and antenna to detect the signal from a radio transmitter and locate a tagged turtle.



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**Figure 10.6:** A GPS logger is attached with epoxy. Pictured is a GPS logger that does not have a radio transmitter built in, so the turtle is also outfitted with a radio transmitter.





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Chapter 11 / Appendix B: Use of fixed-wing and multi-rotor unmanned aerial vehicles to map dynamic changes in a freshwater marsh

By

James Marcaccio, Chantel Markle, and Patricia Chow-Fraser

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# Abstract

We used a multi-rotor (Phantom 2 Vision+, DJI) and a fixed-wing (eBee, senseFly) unmanned aerial vehicle (UAV) to acquire high spatial-resolution composite photos of an impounded freshwater marsh during late summer in 2014 and 2015. Dominant type and percent cover of three vegetation classes (submerged aquatic, floating or emergent vegetation) were identified and compared against field data collected in 176 (2m x 2m) quadrats during summer 2014. We also compared these data against the most recently available digital aerial true colour, high-resolution photographs provided by the government of Ontario (Southwestern Ontario Orthophotography Project (SWOOP), May 2010), which are free to researchers but taken every five years in leaf-off spring conditions. The eBee system produced the most effective data for determining percent cover of floating and emergent vegetation (58% and 64% overall accuracy, respectively). Both the eBee and the Phantom were comparable in their ability to determine dominant habitat types (moderate Kappa agreement) and were superior to SWOOP in this respect (poor Kappa agreement). UAVs can provide a time-sensitive, flexible and affordable option to capture dynamic seasonal changes in wetlands that ecologists often require to study how species at risk use their habitat.

# Introduction

In ecological research, especially in the field of conservation, aerial images are a prerequisite to creating effective management plans for ecosystems and species-at-risk. Without accurate knowledge of what habitat is present and how it is changing, it is difficult to form a management or recovery strategy for endangered species and places. The conventional method of image acquisition, using sensors mounted on planes or satellites, can collect image data for large areas at a time, but can cost tens or hundreds of thousands of dollars depending on the region of interest (Anderson & Gaston 2013). Although these methods can acquire image data for large areas, it can be difficult to use these to obtain data for a specific time period of interest (e.g., year, season or day). For instance, satellites can only obtain photos on days when the image sensor is in line with the study area, and then these photos take time to come to market. Air photos require detailed planning and can be limited by weather and flight regulations. Desired image data may never be obtained for a study site, and consequently researchers and management agencies often have to settle for whatever image data are available. For example, timing of aerial image data collection can limit ability of investigators to study movement patterns and habitat use of migratory animals (Markle and Chow-Fraser 2014), carry out change-detection analyses (Singh 1989), or monitor the spread of invasive species (Wan et al. 2014).

Recent advancements in technology have opened up a new source for aerial image data: unmanned aerial vehicles (UAVs), commonly referred to as drones. These systems

fly without an onboard operator and are controlled remotely from the ground. The proliferation of the 'flying camera' market for recreational users has permitted lower prices with consistent improvement in quality of all small-scale UAVs. One of the most important additions to UAVs has been global positioning systems (GPS), with live-feeds of video (first person view; FPV) and base stations that can determine the UAV's location. Equipped with these, a UAV can know its own location in three-dimensional space and apply this to its image data to allow operators to view the landscape from the UAV's point of view during flight.

Many potential uses of this new technology in the field of ecology are being explored, although not all have yet been attempted or brought to their full realization, especially for time-sensitive research (Rose et al. 2014). Martin et al. (2012) have brought this to light, using an artificial study identifying randomly placed and randomly covered tennis balls in the hopes that it can provide a crucial positive application to conservation. Researchers have attempted to quantify the accuracy (e.g., Chabot & Bird 2013; Gómez-Candón et al. 2013) and savings (e.g., Brekenridge et al. 2012) of a UAVbased mapping approach. Breckenridge et al. (2012) found that using a helicopter-style UAV for determining vegetation cover was 45% faster compared to in-field identification. In addition to faster surveys, they found no difference in vegetation cover interpretation between these techniques (Breckenridge et al. 2012), which could be due to the higher degree of texture seen in UAV image data as compared to traditional image data sources like satellites (Laliberte & Rango 2009). An approach with fixed-wing, plane-style UAVs has also been used, which yielded highly accurate images (Koh & Wich 2012; Chabot & Bird 2013). Gómez-Candón et al. (2013) used a quad-copter to produce image data suitable for monitoring agricultural crops, and Wan et al. (2014) monitored growth of invasive species in salt marshes of China. Moreover, they determined that flight paths 30 metres above ground only required a few ground-control points to maintain spatial accuracy of these images.

The purpose of our study is to compare the ability of recently available multirotor and fixed-wing UAVs to produce image data that permits accurate mapping of wetland vegetation when compared to field-collected vegetation data. We will also compare UAV image data with the most recently available digital aerial photographs provided by a consortium of governments in Ontario (Southwestern Ontario Orthophotography Project (SWOOP), May 2010). These orthophotos are true colour and have been acquired during spring (vegetation in leaf-off conditions) at 4-year intervals since 2002. They are commonly used in Ontario research projects because they are provided at no cost to researchers and cover almost all of southwestern Ontario. While many studies have assessed the merits of these technologies with respect to object-based image classification (Laliberte et al. 2011; Laliberte et al. 2012; Knoth et al. 2013), we present a comparison directly between image data and field data.

### Study site

Our study took place in a 90-ha impounded wetland located within a larger wetland complex along the northern shore of Lake Erie, Ontario (Figure 11.1). The owner of the dyked wetland regulates water levels within the impounded area to discourage establishment of invasive emergent species like the non-native *Phragmites australis spp. australis* and consequently only a few of these are found within the impoundment. This is in striking contrast to the edge of the impoundment, which is covered with this invasive subtype. Overall, the most common emergent vegetation (EM) in this area is cattail (*Typha* spp.) and swamp loosestrife (*Decodon verticillatus*), along with a variety of floating aquatic vegetation (FL) (e.g., *Nymphaea odorata, Nymphoides peltata*) and submerged aquatic vegetation (SAV) (e.g., *Ultricularia* spp., *Potamogeton* spp.). This diverse and dynamic vegetation community provides habitat for many at-risk turtles, snakes, and birds (Environment Canada 2015).

#### Materials and Methods

#### Piloted aircraft image acquisition

Image data from piloted aircraft used in this study were obtained from the Southwestern Ontario Orthophotography Project, herein referred to as SWOOP (SWOOP, 2010). Various levels of governments provide funds to acquire images (leafoff conditions) every 4 years for a large portion of southwestern Ontario. We use these image data from piloted aircraft because they are commonly used in Ontario for research and planning purposes, and are similar to aerial image data from piloted aircraft utilized in many countries. We use the most recent image data available, which were captured in spring (April/May) 2010 using a Leica geosystems ADS80 SH82 sensor. These image data have 20 cm resolution with 50 cm horizontal accuracy (see Table 11.1).

### Multi-rotor image acquisition

The multi-rotor UAV used in this study was a DJI Phantom 2 Vision+ (DJI, Nanshan district, Shenzen, China), herein referred to as Phantom, is a low-cost unit that is extremely popular amongst recreational UAV pilots. This was operated with a Samsung Galaxy S3 (running Android 4.3 "Jelly Bean") and the DJI Vision application. The total weight of the system is 1242 g with a DJI 5200mAh LiPo battery. We kept the remote control at factory settings and flew the UAV with both S1 and S2 levers in the upright position. The S1 lever in this position indicates it is in GPS hold configuration. That is, if the UAV is not given a command it will hold its position regardless of external factors such as wind effects. The S2 lever in the upright position turns off intelligent orientation control. This means that the directional input is always relative to the UAV. For example, pushing the lever forwards will make the drone move forward from its current position, whereas with intelligent orientation control on, pushing the lever forward will move the drone forward with respect to the controller's position.

The UAV was operated with the lens in the 90-degree position (NADIR) for the duration of the imaging process, and all images were acquired with a DJI FC200 sensor (110-degree field of view, 1/2.3" sensor, 14 megapixel, true colour) from a height of 120 m. This flight height was chosen to balance spatial resolution with the amount of flight time required to collect image data for the study area, with a goal of achieving spatial resolution <10 cm and capturing all image data in a single day. We opted to fly the UAV manually rather than use the built-in autopilot system because otherwise we would be

limited to a flight distance of 5 km, travelling no further than 500 m from the operator. When autopilot is engaged, the flight speed is 10 m/s which would only allow for an 8-minute flight plan and resulting in only 2 flights per battery. Since this severely limits the area of image data we can collect, we opted for manual operation, which allows us to fly a longer period and thereby capture the majority of the study site. We set the camera on the Phantom 2 Vision+ to take photos every 3 seconds (time lapse mode), and set the camera to auto white balance and auto exposure with no exposure compensation. Flight speeds were maintained between 10 and 15 km/h to allow for 60% overlap in post-processing (i.e., image stitching).

We processed the images in Adobe Photoshop Lightroom 5.0 (Adobe Systems Incorporated, San Jose, California, USA) using the lens-correction algorithm provided by DJI for the Vision camera. We cropped images to squares in order to remove the distortion inherent in the 140-degree fisheye Vision+ lens. No other modifications were made to the photos. We then used Microsoft ICE (Image Composite Editor; Microsoft Corporation, Redmond, Washington, USA) to stitch together the suite of photos and used the planar motion 1 option to avoid skewing and distortion. This treatment assumes that all of the photos were taken at the same angle, but may have differences in orientation or height above the ground. The mosaic was visually assessed for accuracy stitching before it was used in a GIS.

We manually geo-referenced the stitched image in ArcMap 10.2 (ESRI, Redlands, California, USA) and imported the available SWOOP image data into ArcMap as a base

layer. At first, we attempted to use the GPS coordinates directly from the image metadata for geo-referencing, but the accuracy was too low for this purpose. We had to use this method of processing because the GPS information in the geotagged image is not sufficiently accurate to be used in a software such as Pix4D or photoscan. While the GPS itself has an accuracy of 2.5 m (DJI, 2015), this is not stored in the image data. Even though the coordinates are recorded in degrees, minutes, seconds, no decimal places are recorded in the geotagged image, and this results in a grid-like orientation with 20 m accuracy. For example, if you have two images with different coordinates (43°15'40.19", 79°55'4.11" and 43°15'40.45", 79°55'4.49"), only the rounded coordinates are stored with the image (both geotagged images are now located at 43°15'40.00", 79°55'4.00"); hence, both images would be placed in the same location even though it is not necessarily the correct location for either image. This is an inherent data reporting issue with Phantom 2 Vision+ models and below, but has been rectified in the Phantom 3 Pro/Advanced models.

In total, we recorded and stitched over 800 images in the Microsoft ICE software. All computations were performed on a Lenovo desktop computer (equipped with Windows 7 64-bit, Intel Core i7-4770 CPU, 12.0 GB RAM, Intel HD Graphics 4600, and a 1TB hard drive), and the entire process took approximately 6-8 hours to create a TIFF file (4.02 GB).

# Fixed-wing image acquisition

The fixed-wing UAV used in this study was a senseFly eBee (Parrot, Cheseaux-Lausanne, Switzerland), herein referred to as eBee, with a 96 cm wingspan, 0.25 m<sup>2</sup> wing area, and electric brushless motor. Including the sensor (Canon ELPH 110 HS, true colour) the total weight was 800 g (Styrofoam body). The eBee is powered by a 3-cell Lithium-Polymer battery with each flight lasting approximately 50 minutes and is hand launched and cruises at about 27–31 knots, with a landing speed of 2–17 knots for either straight in or circular landing options. The flight plans are pre-programmed in eMotion 2.9 (Parrot, Cheseaux-Lausanne, Switzerland) and the image collection is controlled by autopilot. Onboard, the eBee is equipped with a GPS, barometric pressure sensor and wind speed sensor. The flight paths were pre-programmed to ensure that complete coverage of the study area is obtained. We conducted all post-processing in PostFlight Terra 3D (Parrot, Cheseaux-Lausanne, Switzerland) which downloads the image data and flight plan from the eBee to create a georeferenced orthomosaic. The eBee is aimed at commercial/industrial users and is the first 'compliant UAV' in Canada, meaning government authorities have approved its airworthiness. This also makes flight applications (called Special Flight Operating Certificates) easier and allows for a longer or broader scope of flight areas.

Image data from the eBee were collected on 4 September 2015 during clear-sky conditions and a wind speed of 5 km/hr. A total of 3 flights were completed between 1000 hrs to 1300 hrs, totalling 30 passes, and taking off and landing occurred in the same

spot each flight (Figure 11.3). The fixed-wing UAV collected 1319 images and were all pre-processed in PostFlight Terra 3D 3.2 (Figure 11.2b). All computation was performed on a custom-built desktop (Intel Core i7-4790K CPU, 32GB RAM, EVGA GeForce GT 730 (2GB GDDR5), Samsung 850 Pro 256GB SSD), and the entire process took approximately 24 hours to create a TIFF file (6.38 GB).

#### Field validation data

As part of a separate study on habitat use by several species at risk, we had conducted vegetation surveys of the impounded wetland between 14 July and 14 August 2014. Using a quadrat (2m x 2m), we estimated the percent cover of each of the three aquatic vegetation groups (i.e. emergent, submergent, and floating). Separately, each vegetation group was assigned to one of the 6 categories: 0-10%, 11-20%, 21-40%, 41-60%, 61-80%, 81-100%. If any vegetation was present within the quadrat, we determined the dominant vegetation as that with the highest cover. In total, we collected vegetation information in this way for 176 quadrats. To permit comparisons, we converted the data to three relative percent cover categories: none, <50% cover, or >50% cover. When percent cover was recorded as 41-60%, the result was counted as >50% if only that class existed, or another species of the same class (e.g., *Typha* and grasses are both emergent) was present in another category other than 0-10% so that total cover would be over 50%.

To determine dominant vegetation and percent cover from the collected image data, points from the field were plotted in ArcMap 10.2 (ESRI, Redlands, California,
USA). A quadrat (2m x 2m) was placed around the points to represent area surveyed in the field. These individual points were manually identified by remote sensing of each type of image data (i.e., SWOOP, Phantom, eBee). To calculate dominant vegetation type, the entire quadrat was considered and whichever vegetation (grasses, cattail, submergent, floating) occupied the greatest area was given this class. To calculate percent cover, the relative area which each vegetation type (emergent, submergent, floating) occupied was determined and then directly translated into one of the three classes (i.e. 0, >50%, <50%).

#### Accuracy analyses

We created 3 x 3 matrices to compare image data (SWOOP, Phantom, eBee) to the field classification separately for percent cover of emergent, submergent, and floating and dominant vegetation type. For each 3 x 3 matrix, we calculated producer and user accuracy in addition to overall identification accuracy. Producer's accuracy provides an estimate of precision, and is the proportion of plots correctly identified compared to all plots that contains the particular class, whereas user's accuracy, or reliability, is the probability that a plot identified as one class actually belongs to that class. These accuracy measurements were calculated for each class (percent cover: none, up to 50%, over 50%; dominant vegetation: grass, cattail, submerged, floating/open water). Finally, we provide the kappa estimate to provide a unitless measure of agreement between the image data and field data (Viera & Garrett 2005). It is reported on a scale of no agreement, poor, fair, moderate, good, to very good agreement.

# <u>Results</u>

### Image data

Using the multi-rotor DJI Phantom Vision 2+, we began flights at 0900 hrs on 8 August 2014 and ended at 1200 hrs. The UAV was operated from a small grassy patch located on the east side of the impoundment. We completed four flights, 19 passes in total, in favourable weather conditions with wind speeds below 15 km/h and limited cloud cover, with each flight lasting approximately 22 minutes in length. Although manual operation was required in order to achieve desired spatial resolution (<10 cm) and temporal resolution (all image data collected on a single day), image data for a section of the wetland were missing (Figure 11.2a). We were unable to obtain comprehensive coverage of the entire dyked impoundment because after changing the batteries and relaunching the UAV, it was difficult to ascertain where the previous flight path had stopped, and this led to missing data in the final mosaic. The UAV itself does not record its flight path and therefore we were unable to download this to view previously flown areas. This is a trade-off between manual operation and automatic operation for this multi-rotor platform. While manual operation permits longer flying times and further flying distances to maximize area of capture, it can result in sections of missing data as was the case in our study.

The total root mean square error (of the georectification process) for the completed image from the multi-rotor UAV was below 5.0 m, and visual observations confirmed a good fit of the UAV-acquired image to the SWOOP dataset. The image had

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a resolution of 8.0 cm/pixel as defined in ArcGIS (Table 11.1). The final image data from the fixed-wing UAV had a spatial resolution of 4 cm/pixel as defined in ArcGIS (Table 11.1). In addition, a digital elevation model was created by PostFlight Terra 3D in areas where sufficient image overlap existed, although this data was not used in this study.

#### Accuracy analyses

Both the Phantom and eBee were comparable when used to identify dominant vegetation, with an accuracy of 62–65% (Table 11.2). Both image data sources were in moderate agreement with field data, with the lowest identification accuracies for floating vegetation. The Phantom and eBee were both able to identify grass and cattail as the dominant habitat class with accuracies ranging from 60–80 % (Table 11.2). In comparison, the SWOOP image data were in poor agreement with the field data due to the difference in timing between the field survey and image data capture and had an overall identification accuracy of 35% (Figure 11.2c; Table 11.2). This source of image data failed to accurately identify any of the dominant vegetation classes.

Identification accuracies varied among image data collection method when used to determine the percent cover of emergent, submerged, and floating vegetation. When determining percent cover of emergent vegetation, the eBee produced a 64% accurate identification and was a fair match with the field data (Table 11.3). The majority of the confusion occurred when identifying an area with less than 50% cover. The Phantom had a similar problem with this class which resulted in a slightly lower overall accuracy of 55%, but was still a fair match to the field data (Table 11.3). The SWOOP image data were only able to identify percent cover of emergent vegetation with an accuracy of 39%, and had the poorest agreement with ground truth data of the three methods evaluated (Table 11.3).

All methods had high overall accuracy when used to identify submerged vegetation; however, we must interpret these cautiously because none of the field plots had over 50% submergent vegetation cover, and this meant that only two classes (no submergent vegetation and less than 50% submergent vegetation) had been identified. Between these two remaining classes, user accuracy was quite low for the below 50% cover class (Phantom = 0.52; SWOOP = 0; eBee = 0.44; Table 11.3). This indicates that image data were very good at interpreting locations with no submergent vegetation, but not as good at identifying the amount of cover. For example, SWOOP was unable to identify the cover of submerged vegetation, had 0% reliability and 0% precision, and consequently no agreement with the field data (Table 11.3). In comparison, the Phantom and eBee methods had moderate to fair agreement, respectively, with the field data (Table 11.3).

The identification accuracy of floating vegetation cover ranged from 18% for SWOOP, 35% for the Phantom and 58% for the eBee (Table 11.3). The SWOOP image data were completely unable to identify floating vegetation, and yielded 0% producer and user accuracy for both cover classes (Table 11.3). Both the Phantom and SWOOP image data had poor agreement with field data, whereas the eBee was in fair agreement (Table 11.3).

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## Discussion

Use of a multi-rotor or fixed-wing UAV is of particular interest for mapping coastal wetlands because these ecosystems are dynamic, and experience seasonal and interannual fluctuations in water levels that greatly influence the vegetation community (Midwood and Chow-Fraser 2012). As a result, during the growing season, coastal wetlands can often appear as large open bodies of water in the spring, and undergo seasonal succession to a completely vegetated habitat towards late summer (See Figure 11.4). This characteristic is one of the main reasons why coastal wetlands can support high biodiversity, and provide unique, sometimes critical habitat for many species at risk. This dynamic nature of coastal marshes means that a single image acquired at the beginning of the season (such as SWOOP) is inappropriate for mapping habitat that is used by species later in the season. This situation is challenging for most researchers who lack funds to acquire their own image data at the most appropriate time of the season, and who must use publicly available orthophotoimagery. This may also explain the lightning speed at which UAVs have become adopted by wetland ecologists over the past year.

We found that the eBee system produced the most effective data for determining percent cover of floating and emergent vegetation compared to the SWOOP and Phantom image data. For submergent vegetation identification, all methods had high accuracy (75– 83%), although this is likely inflated because plots with no submergent vegetation are almost impossible to identify incorrectly. Logically, when determining percent cover of emergent and floating vegetation, image data in the summer season with high spatial accuracy is best. But, if the goal is to determine where submergent vegetation will or will not colonize, publicly available spring images were able to identify this just as well as the UAV acquired image data. For both UAV platforms, percent cover of vegetation was identified with 55–83 % accuracy (eBee 58–75%; Phantom 55–83%) and dominant vegetation type with 62–65% accuracy. This large range underscores how image data can vary in a dynamic ecosystem. Even though the two UAV images were acquired at roughly the same time of year over two consecutive years, there were marked differences between them (Figure 11.2; Figure 11.4).

Both multi-rotor and fixed-wing platforms can allow researchers to acquire aerial images of their study sites at a time in the year that is most relevant to their study objectives. When compared to aerial image data acquired by mounting cameras on an airplane, the Phantom and eBee were much more cost-effective. For example, for a wetland of the size in this study (approximately 90 ha), it would have taken two researchers six to eight days to complete all of the field work in order to generate a habitat map. By comparison, acquiring images with the UAV only took 6-24 hours (Table 11.1). While up to \$5,000 CAD would be required to map even a small area by plane, the DJI Phantom 2 Vision+, with extra batteries, case, and a tablet or phone for viewing, would cost less than \$3,000 CAD. If the desire mapping area is a few hundred hectares in size, the eBee would be more effective, but involve a higher cost of \$30,000 CAD. The benefit in both cases, however, is that these are one-time costs, and

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maintenance/operation costs are relatively low (Phantom spare propellers, the most frequently broken part, can be obtained for \$5 CAD each).

While the Phantom can be useful for mapping small areas (<100 ha), restrictions in data reporting (coordinates, flight plans) capabilities limited its functionality. For instance, we attempted automatic geo-rectification to reduce the time required, but the GPS accuracy on the DJI Phantom 2 Vision+ was too low for this purpose. Recently, Pix4D have released an Android application to improve mapping and geo-rectification called Pix4DMapper (Pix4D, Xuhui District, Shanghai, China), but it requires the use of their own software and can only map relatively small areas at one time (maximum 200 m by 120 m; 2.4 hectares) compared to manual flight (with 60% overlap, approximately 20 hectares). In total, using autopilot would have garnered less than 20% of the area obtained during our 3 flights (65 ha; Table 11.1). This being said, the Phantom Vision 3 Series does provide the GPS coordinate accuracy required to overcome these challenges.

Even though we found SWOOP to be inferior to the UAV-acquired image data, it is freely available for research and are ideal for other research applications (e.g., planning and agriculture). Limitations discussed in this study are more of a reflection of the image data being collected in the spring, long before floating and submerged vegetation are fully established (Figure 11.4). Overall, our comparison highlights how technological advances can improve our ability to map dynamic systems like coastal wetlands.

# **Conclusion**

The flexibility of UAVs for research and monitoring will revolutionize the way we address and solve ecological problems, especially in dynamic coastal wetlands. The resulting high spatial and temporal resolution image data will permit investigators to ask questions previously limited by traditional imaging technologies. We confirmed that the UAV-acquired images could be used to estimate the percent cover of three broad classes of wetland vegetation (submerged aquatic vegetation, floating aquatic vegetation, and emergent vegetation) with fair to moderate agreement with field data. To achieve a more exact picture of vegetation communities, we recommend using a UAV platform to acquire image data precisely when desired. By comparison, image data from SWOOP was unable to determine dominant vegetation type and percent cover for emergent and floating aquatic vegetation, which comprise a large portion of the study site in the summer season.

As demonstrated, the timing of aerial image acquisition can limit the extent of our research. Seasonal image data can greatly improve our mapping of dynamic wetland ecosystems and allow managers to develop more effective recovery strategies for species at risk. Acquiring images multiple times during a single season would have been prohibitively expensive with traditional large plane or satellite platforms, but with low-cost UAVs, this is no longer an obstacle. Researchers no longer need to use commercially available image data that are out-of-date or taken at the wrong season, and instead, learn to create their own. We hope that this study will affirm the use of UAVs in

ecological coastal wetland research while encouraging more research into this emerging and inexpensive remote sensing platform.

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	Multi-rotor:	Fixed-wing:	Piloted Aircraft:		
Parameter	DJI Phantom 2 Vision+	sensefly eBee	(SWOOP*)		
Time of data acquisition	User determined	User determined	Spring only every 4-5 years		
	This study: Aug 2014	This study: Sept 2015	This study: spring 2010		
Sensor	DJI FC200 sensor	Canon ELPH 110 HS	Leica geosystems ADS80 SH82 sensor		
Spatial resolution	8 cm	4 cm	20 cm		
Cost to researcher	\$1,500 CAD	\$30,000 CAD	No cost to university researchers under existing data-sharing agreement		
Coverage	65 ha	281 ha	4,500,000 ha		
	16 ha/flight	94 ha/flight	(throughout Southwestern Ontario)		
Operator	User	User			
	Manual or automated	Automated			
Post-processing type and duration	Manual (6-8 hours)	Automated (24 hours)			
Lag time			1 to 1.5 years after image acquisition		

**Table 11.1:** Comparison of 3 methods for image data collection. \*Southwestern OntarioOrthophotography Project (spring 2010 edition).

Method	Accuracy Type	Class			Kappa	Overall	
		Grass	Cattail	Submerged	Floating	Assessment	Accuracy
Phantom	Producer	0.693	0.565	0.910	0.465		
	User	0.658	0.667	0.910	0.435	moderate	62%
eBee	Producer	0.813	0.630	0.910	0.302		
	User	0.656	0.690	1.000	0.433	moderate	65%
SWOOP	Producer	0.750	0.475	0.002	0		
	User	0.300	0.463	0.334	n/a	poor	35%

**Table 11.2:** Accuracy values calculated for each method when image data are compared to field data for respective types of dominant vegetation.

			Class (Percent Cover)				
Vegetation Type	Method	Accuracy Type	None	Up to 50% cover	Over 50% cover	Kappa Assessment	Overall Accuracy
Emergent	Phantom	Producer	0.733	0.226	0.8	Fair	55%
		User	0.379	0.459	0.623		
	eBee	Producer	0.666	0.387	0.869	Fair	64%
		User	0.625	0.690	0.624		
	SWOOP	Producer	0.666	0.480	0.259	Poor	39%
		User	0.172	0.444	0.611		
Culture encod	Dhanton	Producer	0.796	0.833		Madanata	820/
Submerged	Phantom		0.786		n/a	Moderate	83%
		User	0.983	0.521	n/a		
	eBee	Producer	0.765	0.667	n/a	Fair	75%
		User	0.941	0.444	n/a		
	SWOOP	Producer	0.979	0	n/a	No agreement	81%
		User	0.826	0	n/a		
Floating	Phantom	Producer	0.677	0.316	0.148	Poor	35%
-		User	0.236	0.649	0.138		
	eBee	Producer	0.581	0.675	0.148	Fair	58%
		User	0.327	0.693	0.667		
	SWOOP	Producer	1.000	0	0	Poor	18%
		User	0.177	0	0		

**Table 11.3:** Accuracy values calculated for each method when image data are compared to field data. n/a indicates that no field plots exist for this class.



Figure 11.1: Location of study site. Impoundment along the northern shore of Lake Erie.







**Figure 11.3:** Flight path taken by the senseFly eBee. Each red dot represents the location of a photo and green lines show the connecting flight path.



**Figure 11.4:** Comparison of (a) mosaic image acquired with multi-rotor UAV (b) mosaic image acquired with the fixed-wing UAV and (c) SWOOP image. Details associated with the floating and submersed aquatic vegetation in (a) and (b) are absent in (c).