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THERMAL AND HYDROLOGICAL CONDITIONS OF REPTILE SPECIES-AT-RISK HABITAT ALONG EASTERN GEORGIAN BAY DURING CRITICAL LIFE STAGES

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TITLE: Thermal and Hydrological Conditions of Reptile Species-at-Risk Habitat along Eastern Georgian Bay during Critical Life Stages

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ABSTRACT

Reptiles are the vertebrate taxon with the highest percentage of at-risk species in Canada, many of which exist at the northern limit of their species' home range in Ontario. Numerous reptiles are found in the Georgian Bay area; however, factors limiting their distribution in Ontario are poorly understood. It is likely that the thermal and hydrological conditions of a reptile's critical habitat are contributing factors. Specifically, peatlands serve as ideal hibernacula for the threatened Eastern Massasauga Rattlesnake (*Sistrurus catenatus*) while moss cushions may provide freshwater turtles, including the threatened Blanding's Turtle (*Emydoidea blandingii*) and endangered Spotted Turtle (*Clemmys guttata*), with nesting opportunities on open rock barrens. Although different in their functional purpose at opposite life stages for two separate orders of reptiles, these ecosystems provide suitable conditions to meet the physiological needs of the reptiles utilizing them. This analysis characterizes the thermal and hydrological conditions of moss-dominated ecosystems from a reptile species-at-risk perspective. The interaction between the water table and the frost line is important when assessing the winter survival of Eastern Massasauga Rattlesnakes hibernating in peatland hummocks. Larger hummocks are more ideal as they have a lower chance of becoming flooded in the winter while still providing protection from the advancing frost line. Ideally, hummocks that are 30-35 cm tall provide the greatest chance of survival when snakes hibernate 20-25 cm below the surface. Subsurface temperatures in relation to snow depth, as it is influenced by tree stand characteristics, was also assessed. This resulted in the conclusion that the presence, absence, timing, and frequency of freeze and thaw events is likely more important than snow depth when it comes to winter survival. Similar to rattlesnake hibernacula, turtle nests can be inundated for extended periods of time or exposed to extreme temperatures which reduces their chance of survival. The water storage dynamics of 22 hillslope and 12 hilltop moss cushions along with the temperature dynamics at nine locations were characterized. Although it was determined hilltop locations had greater water storage capabilities, continuously monitored moss cushions responded very quickly to rainfall events whereby sites were inundated for less than 12 hours. Average subsurface temperatures decreased with depth as did temperature fluctuations, both of which were positively correlated but not significantly affected by canopy openness. However, due to their tendency to grow on flat surfaces, not all moss cushions are suitable nesting sites. Moreover, temperatures that ensure proper development and equal ratios of male-to-female turtles were not achieved suggesting that this is a potential factor limiting the northern distribution of turtles in Ontario. In order to properly asses the vulnerability of these populations to threats including habitat loss and climate change, the habitat requirements of different species at critical life stages needs to be understood. Therefore, conservationists can use this study to implement mitigation strategies that consider impacts on the thermal and hydrological dynamics within reptile habitat.

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LIST OF ABBREVIATIONS

Carling	CAR
Diameter at Breast Height	DBH
Digital Elevation Model	DEM
Depression Storage	DS
Eastern Massasauga Rattlesnake	EMR
Georgian Bay Biosphere Reserve	GBBR
Gap Light Analyzer	GLA
Hibernacula Hummock Zone Index	HHZI
Magnetawan First Nations	MAG
Moss Volume	MV
Moss Water Storage Volume	MWSV
Northern Ontario Barrens Ecohydrological Landscape	NOBEL
Pointe au Baril	PAB
Species at Risk	SAR
Volumetric Water Content	VWC
Water Table	WT

DECLARATION OF ACADEMIC ACHIEVEMENT

The main chapters of this thesis (2 and 3) have been prepared as independent manuscripts for submission to peer-reviewed academic journals. Each chapter has an extensive introduction in order to address the separate topics that are the major focus of each.

The work presented in this thesis is the result of collaboration with biologists at Magentawan First Nations, initiated by Dr. Jackie Litzgus, and Ron Black, an advisor to the Massasauga Recovery Team and Principal Investigator of Massasaugas at Wildlife Preservation Canada. Dr. Paul Moore from McMaster University substantially contributed to various aspects of the analysis and photogrammetric methodology discussed in Chapter 3. Assistance with MATLAB analysis was provided by Eric Dyer and field assistance was provided by Alex Furukawa, Waverley Birch, Kyra Simone and Victoria Propp.

CHAPTER 1: INTRODUCTION

1.1 Reptiles in Canada

Ecosystems across Canada offer humans a suite of ecological services while serving as the home to an array of wildlife. However, there is concern that these ecosystems are at risk of development and land-use changes as well as climate disturbances. In Ontario, there is an area that hosts a wide range of biodiversity known as the Georgian Bay Biosphere Reserve (GBBR) which was designated by the United Nations Educational, Scientific and Cultural Organization as a world biosphere reserve in 2004 (UNESCO, 2015). Specifically, in southern Ontario, habitat along the eastern shores of Georgian Bay are threatened by highway development and cottage country expansion associated with rising numbers of summer cottagers, campers, and travellers. The direct loss of habitat and subsequent fragmentation that is associated with these land-use changes is detrimental to reptiles (Aresco, 2005; Gunson *et al.*, 2012). This activity threatens vagile animals with extensive home ranges that need various habitats to fulfill the life stage requirements associated with activities including nesting and overwintering. Known as critical habitat, these types of ecosystems are necessary to ensure the survival of a species within their home range. Reptiles, such as freshwater turtles and snakes, are particularly at a higher risk due to their movement patterns and critical habitat requirements. Many of these semi-aquatic reptiles are already listed as ‘at risk’ under the Canadian federal Species at Risk Act and the Provincial Endangered

Species Act. In fact, reptiles are the vertebrate taxon with the highest percentage of at-risk species in Canada (Lesbarrères *et al.*, 2014). Therefore, it is imperative that favourable habitat conditions of these species be understood in order to effectively re-evaluate conservation strategies in this area.

1.2 Temperature, a Limiting Factor to Reptile Survival

The majority of reptile species in Canada exist in ecosystems along the northern limit of their species' range where there is lower tolerance for any degree of environmental change or disturbance (Lesbarrères *et al.*, 2014). Reptiles are ectotherms meaning they rely on energy from the sun to meet their thermoregulated physiological needs. Therefore, suitable temperature conditions are a critical aspect of their habitat. For freshwater turtles, the temperature dynamics of nests during incubation can control sex determination and influence post-hatchling fitness characteristics (Freedberg *et al.*, 2001; Booth *et al.*, 2004). For instance, temperatures greater than 29°C yield all female hatchlings while temperatures lower than 28°C result in the majority of hatchlings becoming male (Freedberg *et al.*, 2001; Markle and Chow-Fraser, 2014). Moreover, the constraints of environmental factors such as temperature and moisture affect the spatial ecology (Andrews 1982; Brooks *et al.*, 1987) and distribution of species at their northern limit (Muth 1980; Lavigne *et al.*, 1989; Standing *et al.*, 1999). The slower development and longer incubation as a result of the cooler temperatures at their northern range limit almost certainly limits the northern distribution of turtles (Yntema, 1976; Packard *et al.*, 1987). Embryo development does not occur fast enough, if at all, due to low heat-unit accumulation during incubation (Obbard and Brooks 1981; Lavigne *et al.*, 1989). Overwintering snakes also face challenges in this thermally challenging landscape whereby cold overwintering conditions and a short period between parturition and hibernation result in high rates of mortality, limiting their extent in Ontario (Harvey and Weatherhead, 2006b). Moreover, in order to survive the long winters experienced at this latitude, snakes must hibernate below the frost line to avoid freezing but above the water table (WT) to avoid desiccation (Smith, 2009). These conditions are influenced by several factors including snowpack, hummock size, and substrate thermal conductivity (Zhang, 2005). Overall, in order to accurately assess habitat suitability for reptiles, thermodynamic properties need to be considered.

1.3 Spatial Ecology of Reptiles

1.3.1 Movement Patterns

Movement across highly variable landscapes allows species to actively seek areas that provide sufficient resources to meet their physiological needs and habitat requirements at particular moments in time (Moore and Gillingham, 2006). The spatial ecology of reptiles is a result of this which gender and reproductive status might provide a greater understanding of in terms of movement patterns (Johnson, 2000; Marshall *et al.*, 2006). In general, gravid female turtles and snakes are known to move greater distances than non-gravid females and males for two distinct reasons. On one hand, freshwater female turtles are known to make long-distance overland excursions to reach nesting sites that meet the necessary conditions for egg incubation (Millar and Blouin-Demers, 2011, 2012). Travel distances up to 4 km have been observed for Blanding's Turtles which tend to travel significantly longer distances than Spotted Turtles in search of nesting sites (Beaudry *et al.*, 2010). On the other hand, the increased movement of gravid female Eastern Massasauga Rattlesnakes (EMR) is a result of thermoregulatory behaviour changes to achieve warmer body temperatures and facilitate embryo development (Marshall *et al.*, 2006; Harvey and Weatherhead, 2010). Studies show variable data on average daily movement for these snakes ranging from 1.14 m d^{-1} for a population at Ives Road Fen Preserve, Michigan (Moore and Gillingham, 2006) to 56 m d^{-1} at Bruce Peninsula National Park, Ontario (Weatherhead and Prior, 1992). Nonetheless, the general consensus is that few differences in movement patterns exist between male and non-gravid females but there is substantial differences between these cohorts and gravid females (Marshall *et al.*, 2006).

1.3.2 Effects of Road Ecology on Reptiles

Networks of roads fragment the landscape, physically impeding migrating populations of reptiles, thus reducing the quality and quantity of species biodiversity (Gunson *et al*., 2012). In Southern Ontario, where 38.7% of Canada's population resides, there has consequently been a fivefold increase in major roads since 1935 (Gunson *et al*., 2012). These anthropogenic by-products of urban sprawl represent obstacles to migrating populations of reptiles which move across the landscape in search of nesting and hibernating sites. Although death by vehicle-animal collision is recognized as a direct threat to species, the realistic effects roads have on reptile populations are highly underestimated. However, construction of culverts and underpasses are an effective mitigation strategy (Aresco, 2005; Colley *et al*., 2017). Once mortality hot-spots have been identified, implementation of specific reptile crossing structures can effectively intercept reptile migration routes and reduce road mortality (Beaudry *et al*., 2008; Colley *et al*., 2017; Markle *et al*., 2017).

1.3.3 Habitat Selection and Species Management Requirements

Life history studies of populations in need of protection in Ontario indicate the importance of ensuring the survival of adults and juveniles alike to maintain population stability (Congdon *et al*., 1993; Enneson and Litzgus, 2008). This requires an understanding of habitat selection and associated overland migratory patterns to mediate the immediate threats to life history traits such as road mortality (Rasmussen and Litzgus, 2010). By observing habitat selection, macro- and micro-habitats that are critical to a specific species' needs can be identified, a crucial first step for conservation management (Litzgus and Brooks, 2000; Harvey and Weatherhead, 2006a; Rasmussen and Litzgus, 2010). Understanding habitat ecology of reptiles requires observation of the movement and seasonal patterns of such species over extensive spatial scales due to the variety of ecosystems within which these organisms exist (Beaudry *et al.*, 2008). Even if executed perfectly, extrapolating information across geographic regions to determine species habitat requirements does not effectively represent their habitat requirements as there are no universal site conditions. It is therefore important to note the significance of species-specific habitat selection, as opposed to generalized designation of critical habitat based on habitat characteristics common to all species (Harvey and Weatherhead, 2010). Management decisions can then be made regarding protection policies to sustain at risk populations based on specific knowledge of local populations of individual species (Moore and Gillingham, 2006). Management policies should also consider seasonal activity and habitat use of species since many reptiles relocate to find appropriate nesting, foraging, and hibernation sites (Litzgus and Brooks, 2000; Millar and Blouin-Demers, 2011). Even so, the focus should remain on local gestation and hibernation sites which often are selected based on specific conditions (Harvey and Weatherhead, 2006a).

1.4 Moss as Habitat Features

Eastern Georgian Bay is a mosaic of habitats including rock outcrops scattered amongst wetlands and densely treed forests (Jalava *et al.*, 2005). The variety of habitats encourage an array of wildlife to exist, many of which utilize specific environments at various life stages. One such habitat feature plays an important role in the ability of reptiles to exist on this otherwise rocky barren landscape. Whether it forms small cushions, shallow pools, or deep peatlands, moss provides extensive ground cover in both hydrologically-connected and isolated environments. The depth of these moss depressions range from a few centimeters on rock outcrops, to several meters in peatlands (National Wetlands Working Group, 1997; Lindo and Gonzalez, 2010). There are two dominant types of moss in the GBBR: *Polytrichum* and *Sphagnum*. Each have various subspecies that not only diversify the range of habitat types available for reptiles to use, but also have different hydrological traits.

1.4.1 *Sphagnum* and Peatlands

Boreal peatlands on the inlands of Eastern Georgian Bay are unique in the sense that their depth rarely exceeds a few meters and are always located on bedrock as opposed to glacial sediments often encountered in the boreal plains of central Canada (National Wetlands Working Group, 1997). Aside from being a critical overwintering habitat for reptiles within the GBBR, peatlands are an important carbon sink, accounting for roughly one third of the world’s terrestrial carbon stock

(Gorham, 1991). They also store approximately 10% of the world's surface freshwater and therefore are affected by the fluctuating WT associated with climate change (Holden, 2005). However, unlike an open body of water, boreal peatlands are dominated by *Sphagnum* mosses (Johnson *et al.*, 2015) which result in unique ecohydrological feedbacks (Moore *et al.*, 2015; Waddington *et al.*, 2015). *Sphagnum* controls the development of hummock-hollow ecosystems as a result of feedbacks between moisture retention (Granath *et al.*, 2010), decay rates, peat accumulation, and acidification (Hájek and Beckett, 2008; Johnson *et al.*, 2015). They are therefore suitably referred to as ecosystem engineers (Hájek, 2014; Johnson *et al.*, 2015). Minor fluctuations in WT position, specifically drawdowns, can have substantial impacts on the structure of the habitat via feedbacks (Waddington *et al.*, 2015). These feedbacks can be altered dramatically if thresholds are breached, a consequence often associated with climate change. Therefore, sites that reptiles exhibit high fidelity to are at risk of becoming less suitable due to expected climate and land-use change disturbances. Long-term changes associated with WT drawdown may include continued vegetation succession towards greater shrub density (Strack and Waddington, 2007), and potential expansion of hummock microforms due to their ability to grow high above the WT (Johnson *et al.*, 2015), to retain moisture more efficiently than hollow species (Hayward and Clymo, 1982), and to conduct water from the WT better than hollows under dry conditions (McCarter and Price, 2014). Although this shift towards hummock dominated systems in response to climate disturbance may increase the number of suitable overwintering locations in hibernacula, other factors including changes to snowpack, temperature, and shrub density may deter species from utilizing this habitat.

1.4.2 *Polytrichum* and Moss Cushions

Polytrichum is the dominant moss species colonizing bare rock on this landscape (Jalava *et al.*, 2005). It is also often incorporated into peatlands, serving as a nurse plant to hummock development (Groeneveld *et al.*, 2007). Specifically, *P. commune*, *P. strictum*, and *P. piliferum* are the most common species that grow both on bare rock and in forested areas (Jalava *et al.*, 2005). The thermodynamics of moss horizons are similarly dependent on moisture regimes but are also affected by species-specific traits such as albedo and evaporative cooling potential (Nichols and Brown, 1980; Stoy *et al.*, 2012). Whereas past studies on the impacts of mosses as regulators of surface and below-surface temperature regimes considered bryophyte mats as uniform entities (Soudzilovskaia *et al.*, 2013), several differences exist between species. For instance, Vogelmann and Moss (1993) found *S. cuspidatum* showed increasing reflectance during a drying experiment, thereby demonstrating that albedo is a function of moisture content. This explains why *Sphagnum* in general maintains a cooler maximum surface temperature than most *Polytrichum* species (Stoy *et al.*, 2012). Similarly, *Cladonia* species, particularly *C.*

rangiferina, maintains a relatively high albedo regardless of moisture content which reduces temperature fluctuations between the surface and 5 cm below ground (Stoy *et al.*, 2012). However, it is important to note that the canopy structure of these general moss types can influence the heat flux via air mixing. This component, attributed to surface roughness and wind, can have substantial impacts on the temperature dynamics near and just below the surface (Stoy *et al.*, 2012), which is important when considering nesting habitat suitability for freshwater turtles (Steen *et al.*, 2012). For example, Rice *et al.* (2014) demonstrated that with the relatively open canopy of *P. commune* (1.8 stems cm^{-2}), average temperature within the canopy dropped from 19.9°C in still air to 18.1°C with 1.1 m s^{-2} wind under laboratory conditions (25.5°C, 10% relative humidity). Unless being considered on a global scale whereby species composition has proven to be independent of soil temperature fluctuations (Soudzilovskaia *et al.*, 2013), models of heat transfer on smaller scales should account for species differences.

1.5 Fill and Spill

The Canadian Shield hosts a suite of different habitats which are characteristic of rock barrens in that they are often shallow organic deposits (Jalava *et al.*, 2005). Nonetheless, these bedrock features which range from small depressions to large wetlands play an important role in storing water in northern catchments (Spence and Woo, 2002, 2003, 2006). A “fill and spill” approach can be applied to this landscape to better understand the large cumulative storage capacity of these hydrologically connected systems (Spence and Woo, 2006). Essentially, once a depression's water retaining capacity is met, any additional water supplemented by rainfall or overland flow may contribute to fill depressions further downslope with the eventual discharge out of the catchment outlet (Spence and Woo, 2003, 2006). The amount and hydrophysical properties of material within the depressions - effective porosity, bulk density, and specific yield - will dictate the capacity to store water. However, although empty depressions may theoretically have the greatest potential to store water, it is unrealistic to expect their hydroperiods to last very long, especially in the summer (Altermatt *et al.*, 2009). Therefore, it is important to consider evaporative rates, hydrological demands of the vegetation, and moisture retention capabilities of the vegetation when assessing the water storage potential (Brooks and Hayashi, 2002). This has direct implications on habitat suitability for reptiles because their semi-aquatic nature requires that they avoid desiccation by using habitat with sufficient water availability.

1.6 Thesis Objectives

Although the carbon significance of these landscape features has been extensively studied over the years, there has yet to be the same research effort put forth for understanding these systems from a species-at-risk (SAR) perspective. These depression-filled landscape features are utilized by a surprisingly large number of different animals (De Meester *et al.*, 2005; Gala and Young, 2015), including reptiles (Johnson, 2000; Gibbons, 2003; Harvey and Weatherhead, 2006b; Markle and Chow-Fraser, 2014). Unfortunately, within plant ecology, bryophytes and lichens are often overlooked since the majority of research focuses on vascular plants (Cornelissen *et al.*, 2007). This fact is surprising considering these ecosystem engineers are dominant colonizers in various biomes, facilitate vegetative succession, contribute substantially to biogeochemical cycling, determine moisture and thermal regimes of surface and below-ground fluxes, and serve as habitat/foraging opportunities for numerous reptiles. Also, despite having an ecologically significant status, reptiles are not paid the same attention as large mammals or animals with cultural appeal. It is therefore not surprising that there is substantial disconnect between the study of reptiles and the physical dynamics of their habitat. In other words, the literature focuses either on the species, their movement patterns, and their general habitat or the hydrological, ecological, biogeochemical, and thermal dynamics of the landscape upon which they exist. Therefore, there is an obvious need to bridge the gap between these two inherently similar fields of research in order to better construct conservation strategies that can emphasize the importance of integrating both simultaneously.

In order to address this gap in knowledge, the temperature and moisture conditions of reptile SAR critical habitat was assessed at representative and known habitat locations across Eastern Georgian Bay. Temperature and water-table dynamics of *Sphagnum* dominated peatlands were assessed to understand the hibernation conditions required for EMRs which is the focus of Chapter 2. Shallow moss-filled depressions, or moss cushions, dominated by *Polytrichum* moss were also studied to determine their suitability as nesting locations for Blanding's and Spotted Turtles on rock barrens which is covered in Chapter 3.

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CHAPTER 2: HIBERNATION HABITAT

2.1 Introduction

2.1.1 Snakes in Ontario

In Canada, the Eastern Massasauga Rattlesnake (EMR; *Sistrurus catenatus*) is currently listed as threatened (COSEWIC, 2012). It is found in four general locations across southern Ontario: near the city of Windsor, on the northeast shore of Lake Erie by Wainfleet Bog, the Bruce Peninsula and along the shores of Georgian Bay (COSEWIC, 2012). In fact, the populations that exist along Eastern Georgian Bay and at the Bruce Peninsula are believed to be the largest in Ontario and therefore represent the most stable populations across the species' entire range (Rouse and Willson, 2002; Rouse *et al.*, 2011; Rogers, 2015). The variety of habitats utilized by these snakes – which includes meadows and peatlands (Yagi and Frohlich, 1999; Johnson, 2000), forests (Harvey and Weatherhead, 2010), marshes and fens (Hutchinson *et al.*, 1993; Marshall *et al.*, 2006), and rocky shorelines – makes it difficult to identify their critical habitat since they are known to move great distances across landscapes in search of suitable habitat. Throughout this species' entire range in North America, populations are declining as a result of direct persecution and habitat loss due to anthropogenic land-use changes (Szymanski, 1998; Johnson *et al.*, 2000; Pomara *et al.*, 2014). Sensitivities to various land-use changes varies between populations and amongst parts of their life history such as migration and hibernation (Small-Lorenz *et al.*, 2013; Pomara *et al.*, 2014). What is more complicating is the effect of these anthropogenic stressors in the context of rapidly changing climate, specifically in areas where the species is existing at their spatiotemporal limit (Pomara *et al*., 2014). Since 1911, the region surrounding the Great Lakes has been warming at a trend of 0.11°C per decade in the spring and 0.6°C in the winter (Magnuson *et al.*, 1997). Moreover, annual precipitation has also increased by 2.1% (Magnuson *et al.*, 1997). Therefore, understanding this species' vulnerability to land-use change, climate impacts, and their combined effect at various life stages is needed to formulate proper mitigation strategies.

EMRs spend a substantial amount of time - approximately six to seven months – overwintering (Harvey and Weatherhead, 2006a). As ectotherms, EMRs rely on energy from the sun to fulfill their thermoregulatory metabolic needs. However, in the winter, they must retreat underground to survive and reduce their metabolic activity to a level low enough to avoid starvation (Blem and Blem, 1995; Shine and Mason, 2004). Such sites which offer survivable conditions are limited along Eastern Georgian Bay, hence why individuals demonstrate high fidelity to hibernation sites (Harvey and Weatherhead, 2010; Parks Canada Agency, 2015; Rogers, 2015). Therefore, understanding this species' highly specific but poorly studied hibernation requirements is key to ensuring proper recovery strategies for declining populations in Ontario (Smith, 2009). Not only will it help in determining periods of greater sensitivity to impacts, but also assist in identifying suitable critical habitats that require greater protection. Although it is clear how important hibernation is as part of a snake's life history at these higher latitudes, a lack of understanding exists regarding the factors that influence overwintering success (Smith, 2009). Furthermore, despite spending a large portion of the year overwintering, there has been little research done to characterize the structural properties of snake hibernacula (Johnson, 2000; Harvey and Weatherhead, 2006a; Moore and Gillingham, 2006; Smith, 2009), prompting the identification of suitable hibernation sites to be based largely on surface features. This practise is not ideal since thermal properties are often attributed to differences in subsurface geomorphology and so identification of suitable hibernation sites cannot be solely based on superficial appearance (Macartney *et al.*, 1989).

2.1.2 Overwintering Habitat

Unlike turtles which can survive anoxic conditions under water during the winter, EMRs select hibernaculum which allow them to remain above the water table (WT) but below the frost line to avoid death by drowning or freezing (Harvey and Weatherhead, 2006a; Parks Canada Agency, 2015). Another critical internal feature of overwintering sites includes the amount of space in the hibernaculum hummock that is accessible to the snakes in the event that they need to adjust to changing conditions such as temperature fluctuations and flooding (Sexton and Marion, 1981; Macartney *et al.*, 1989). Snakes have been shown to move overland within their overwintering sites to new holes during warm periods in autumn before the first snowfall after which the snakes retreat further into the hummock and may remain partially submerged next to the WT (Smith, 2009). Since annual fluctuations in precipitation patterns and temperature may change the microclimate conditions of specific hibernaculum each year, it is important to consider at least a 100 m radius around the hibernaculum when identifying overwintering habitat (Ministry of Natural Resources, 2007). It is this area that ERMs show high site fidelity to; such landscapes can encompass entire wetlands, fens, wet depressions, or any surficial landscape feature that supports *Sphagnum* moss hummocks which offer the ideal hibernacula conditions for snakes (EMRT 2002; Rouse and Willson, 2002; Ministry of Natural Resources, 2007). Across their range in southern and central Ontario, EMRs may choose to hibernate not only in peatland hummocks (Johnson, 1995) but also in burrows constructed by crayfish or small mammals (Seigel, 1986), rooted wetland systems (Smith, 2009), and rock crevices that provide sufficient protection from winter conditions and optimal hibernaculum settings (Wright, 1941; Harvey and Weatherhead, 2010).

2.1.3 Effects of Snow on Overwintering Habitat

Snow is important for facilitating the necessary conditions for overwintering of reptiles. The insulating effect of snow inhibits the extent of frost penetration, allowing overwintering sites of terrestrially hibernating reptile species to remain above freezing temperatures (Lesbarrères *et al.*, 2014). Snow acts as an insulator, reducing heat loss in the winter and maintaining higher temperatures belowground than the air, especially in cold regions (Zhang, 2005). This effect is attributed to several properties of snow, including surface albedo, emissivity, and low thermal conductivity. As such, factors including timing and duration of snow cover can strongly influence the insulating effect of snow (Nichols, 1998) and likely, by extension, rattlesnake hibernacula suitability. While a thick snowpack can benefit rattlesnake hibernacula, a rapid mid-winter or spring snowpack melt can increase peatland water levels (Johnson *et al*., 2000; Ketcheson *et al.*, 2012) and potentially flood critical overwintering habitat for long periods of time. In this sense, snow accumulation and ablation processes at the surface, as they are affected by forest canopies, are a key component to the success of these hummock hibernacula.

The amount of snow that reaches the surface as well as the duration of snow cover into the spring are factors affected by forest canopy characteristics (Bunnell *et al.*, 1985). As much as 60% of cumulative snowfall may be intercepted by boreal forests in mid-winter (Pomeroy and Schmidt, 1993; Hedstrom and Pomeroy, 1998), but the reduced incoming shortwave radiation by the forest canopy results in a slower melting rate (Sicart *et al.*, 2004; Essery *et al.*, 2008; Ni-Meister and Gao, 2011). Snow accumulation and snow melt as they are affected by air temperature fluctuations can impact conditions within rattlesnake hibernacula. The mixed coniferous and deciduous forest along Eastern Georgian Bay varies from open canopies characteristic of the rock barrens to heavily treed woods (Jalava *et al.*, 2005). Combined with the variable local weather conditions across the coast, differing snow conditions are experienced at various sites.

2.1.3.1 Freeze and Thaw Events

Snow accumulation in autumn and snowmelt in the spring are complex processes where the insulating effect of snow has yet to be established, thus causing unfavourable ground surface temperature fluctuations. In autumn, the snow is relatively thin as the air temperature fluctuates around 0°C, allowing the ground surface to cool. In the spring, the ground surface remains cooler than the air temperature since much of the incoming solar energy is consumed during snow melt (Zhang, 2005). Although these intermediate time periods of initial snow accumulation and snow melt are only short term, they can have severe effects on the hibernating conditions for EMRs depending on their occurrence and duration relative to the arrival or emergence of EMRs to the overwintering site. For example, a mass mortality event of EMRs at various sites across the GBBR in the winter of 2014-2015 (Parks Canada Agency, 2015) is thought to have occurred either during a cold period before the first significant snowfall, which may have caused the snakes to freeze, or after spring snowmelt whereby the EMRs may have drowned in the hummocks (Ron Black and Mike Colley, pers. comm).

2.1.4 Peatland Water-Table Fluctuations

2.1.4.1 Hibernacula Water Table Fluctuations

WT depth is an important habitat characteristic for EMRs during the winter. Most snakes will remain close to the WT throughout the winter to avoid desiccation and may have some physiological tolerance to anoxia if the hibernaculum were to become flooded (Storey, 1996; Sage, 2005; Smith, 2009). For instance, some species can exercise extrapulmonary respiration if they are not capable of tolerating anoxic conditions when temporarily submerged (Costanzo, 1989; Ultsch, 1989), and may also be capable of tolerating freezing conditions for short periods of time (Hermes-Lima and Storey, 1993). Although these physiological adaptations to anoxia and freezing have never been studied in EMRs, the high mortality rates as a result of flooding suggest a poor tolerance to anoxia, if at all (Harvey and Weatherhead, 2006a). It has also been demonstrated that both extreme high and low WT fluctuations due to flooding and drought, respectively, during the winter can adversely affect body condition the following spring, cause direct mortality within the hibernaculum, and may lead to early emergence when daily weather conditions are not suitable for survival outside the hummock (Prior and Shilton, 1996; Harvey and Weatherhead, 2006a; Smith, 2009; Pomara *et al.*, 2014). WT fluctuations as a result of climate change, or anthropogenic disturbance such as wetland draining for agricultural intensification, urbanization, or road development can adversely affect EMR populations, especially in the winter (Yagi and Frohlich, 1999; Smith, 2009).

2.1.4.2 Peatland Water Table Feedbacks

Peatlands are complex adaptive systems (Levin, 1998) that exist as a result of various hydrological feedbacks that dictate their structure and function (Strack *et al.*, 2008; Waddington *et al.*, 2015). In the winter, a number of the feedbacks that typically influence the WT in the summer are subdued since evaporation and albedo are not causing WT-drawdowns. The dominant factors affecting the WT in this season are water inputs from melting snow and potentially rainfall events. Flooding becomes more prevalent because of the lack of drawdowns that would otherwise occur in the summer. The high WT experienced in the winter limit the soil water storage of peatlands, therefore reducing their ability to attenuate floods and increasing surface runoff due to saturation excess (Burke, 1967; Holden and Burt, 2003a; Ballard *et al.*, 2011).

Depending on the depth of peat, size of the catchment, and additional water inputs from freeze and thaw events, the response of the WT can be very flashy (Evans *et al*., 1999; Holden *et al*., 2001; Bragg, 2002; Holden and Burt, 2003b; Ballard *et al*., 2011). Therefore, the position of the peat surface, and thus the hummocks, relative to the sill of the peatland is important, especially when it comes to flooding risks (Spence and Woo, 2003, 2006). Moreover, the depth and thickness of peat is a key determinant in the extent of the effect of WT fluctuations in the winter (Waddington *et al.*, 2015) and is highly variable across the GBBR (Jalava *et al.*, 2005). Therefore, since EMRs living along Eastern Georgian Bay may select sites based on the WT position (Johnson *et al.*, 2000; Smith, 2009; Pomara *et al.*, 2014), factors which dictate WT fluctuations may be used to identify suitable overwintering sites.

2.1.5 Hypotheses

To better understand the conditions within the hummock hibernacula that may have led to the aforementioned mortality event that caused the death of over 50% of the snakes in the area, we characterized the thermal and WT dynamics in several hummocks at three geographically distinct sites across the GBBR. We hypothesized that taller, and thus larger, hummocks are better equipped to buffer against the advancing frost line as well as the fluctuating WT. These sites were monitored continuously for two winters following the mortality event to identify areas within hummock hibernaculum that may experience freezing, flooding, combined freezing and flooding, and survivable conditions. Moreover, to address the effects of snow on temperature fluctuations within the hummocks, we monitored snow depths at various times throughout the winter and compared differences in the thermal profiles. We hypothesized that sites with greater tree density would result in smaller amounts of snow reaching the surface, and result in cooler temperatures within the hummocks.

2.2 Methods

2.2.1 Study Area and Site Selection

The research was carried out in the eastern Boreal Shield ecozone, at various locations along the east coast of the GBBR in the Northern Ontario Barrens Ecohydrological Landscape (NOBEL). Bedrock topography in the rocky outcrops of Eastern Georgian Bay is generally flat with low, rolling gneissic rocklands consisting of mafic to intermediate gneisses with quartzitic and politic rocks dipping slightly to the southwest (Jalava *et al.*, 2005). Due to their location, the sites experience lake effect precipitation throughout the year and therefore experience harsh winter conditions. Long-term climate data from three weather stations in the vicinity of Eastern Georgian Bay (Honey Harbour, French River Chaudiere Dam, and Dunchurch) from 1981 to 2010 shows that the average temperature in January is -10.5°C. Furthermore, the total snowfall for this region is roughly 2.83m (Environment Canada, 2017).

Eastern Georgian Bay is a mosaic of wetlands, shallow open water lakes and beaver ponds, forested valleys and rock barren hilltops. Mineral soil tends to be either thin or absent on the rock barren ridges, leaving a large proportion of exposed bedrock which is partially covered in certain areas by a thin layer of lichen and moss in the uplands. The wetlands are formed in poorly drained bedrock depressions on the rock barrens of the Canadian Shield with peatland depths ranging from 0.4 m to several meters deep (Zoltai and Vitt, 1995; Jalava *et al.*, 2005). The peatlands in the region are dominated by tall-shrubs, low-shrubs, rushes, ferns, and blueberry plants. A variety of tree species can also be found within the peatlands (see below).

Based on previous and current tracking of rattlesnakes by our partnering biologists, we chose three peatland sites that were interpreted as representative of rattlesnake hibernacula for the region (Ron Black and Mike Colley, pers. comm.; Figure 2.1). Two of our three sites were chosen based on current tracking and previous radio-telemetry projects that indicated populations of EMRs utilizing specific peatland overwintering sites. Our northernmost site, located on Magnetawan First Nations land (hereafter referred to as MAG), and our southernmost site, located near Carling Township (hereafter referred to as CAR) were generally open, poor fens surrounded by trees along their margins. Located between the MAG and CAR sites is a densely forested peatland near Pointe au Baril (hereafter referred to as PAB). Interactive range maps developed by Ontario Nature using information from the Reptile and Amphibian Atlas were also used to validate site selection based on presence/absence of this species at our study site (Ontario Nature, 2017). The geographical area surrounding each site based on the UTM grid system indicated sightings of these species before and after 1970 (Ontario Nature, 2017). Due to threats of the pet trade, the exact location of the sites cannot be disclosed.

2.2.2 Site Characterization

For each site, we characterized the peatland area, peat depth, hummock microtopography, vegetation cover, canopy openness, and stand density (Table 2.1). The perimeter, or margin, is the interface between the peatland and the upland, identified based on vegetation differences whereas the middle is the interior of the peatland. Site area was determined by walking the complete perimeter of the peatland using the ‘Area Calculation’ tool on a Garmin eTrex 10 GPS. Triplicate measurements were made and averaged. Peat depths at each site were measured every 0.5 to 1.0 m along three margin-to-middle transects per site using a rebar of known length that was pushed into the peat until it hit the underlying bedrock. There were some cases where the depth of peat was greater than the length of the rebar (>1.5 m) and so the actual depth at those points could not be determined. Peatland microtopography was measured at each site using a Smart Leveler (Digital Leveling Systems, Smyrna TN, USA) to detect minor changes in surface features. Hummock sizes were measured and referenced to the closest groundwater well (see below). The dominant vegetation (shrub, sedge, graminoid) and moss cover (*Polytrichum* or *Sphagnum*) at each site was assessed at each peat depth location (*i.e.* the three transects per site) using standard methods (Didemus, 2016) with a quadrat (0.4 m by 0.4 m).

Canopy openness was measured at each instrumented hummock by taking photographs using a Sunex 185° SuperFisheye 5.6 mm F/5.6 lens on a Canon EOS Rebel DSLR camera. The camera was pointed with a designated mark on the lens facing north and placed on the forest floor on a levelled surface. The photographs were then processed using the Gap Light Analyzer (GLA) software. The blue channel pixels were extracted from the image colour plane as plants do not reflect blue wavelengths well (Zhang *et al.,* 2005). A threshold value was then selected to convert to a 1-bit black-and-white image that best captured the surrounding canopy. The software then calculates the proportion of black and white pixels to determine the canopy cover and openness (Frazer *et al.,* 1999).

Tree surveys were conducted to collect information on the species abundances and stand density to complement the canopy openness measurements. A quadrat (15 m by 15 m) of the MAG and PAB research sites were surveyed and included a portion of the margin as well as the interior of the peatland whereas the entire CAR study site was surveyed. Measurements of every tree circumference-at-breast-height were recorded and converted to a diameter-at-breast-height (DBH). The tree heights for various species of trees were also measured. Tree heights were measured using the Smart Measure cellphone application (Smart Tools co. ver 1.6.6).

2.2.3 Thermal and Hydrological Data

The MAG and PAB sites were instrumented in the summer and fall of 2015 and continuous micrometeorological, soil temperature, and WT depth data was collected for the winters of 2015-2016 and 2016-2017. Our CAR site was instrumented for a separate ecohydrology study and rearranged according to the needs of this study in the fall of 2016. As such, the CAR thermal and hydrological data are only available for the winter of 2016-2017.

WT depth was measured every 10 minutes at all three sites in a 1-2 m deep groundwater well using self-logging Levelogger Junior pressure transducers (Solinst, Georgetown, ON (Solinst)) and was corrected for changes in atmospheric pressure using a Barologger Edge barometric logger (Solinst) or a Levelogger Junior pressure transducer that was mounted to the cap of the well and suspended in the top of the well. Air temperature and relative humidity were measured every 15 (CAR) to 30 (MAG and PAB) minutes using either a HMP35C (Vaisala Oyj, Helsinki, Finland) or HC2S3 relative humidity probe with an adjacent thermocouple wire to measure air temperature. This equipment was mounted in a radiation shield at a height of roughly 1.35 m at all wetland stations. Wind speed was measured using 03101-L Wind Sentry Cup Anemometer (Campbell Scientific, Inc., Logan, UT). Rainfall was measured using a tipping bucket rain gauge (TE525M-Campbell Scientific, Logan, Utah) mounted 1 m above the hummock surface. All data was recorded using either a Campbell Scientific CR10X or CR1000 data logger at meteorological stations that varied in number depending on the site.

Volumetric water content was measured at each of the sites using Campbell Scientific CS616 or CS655 moisture probes (Campbell Scientific, Inc., Logan, UT). The CS616 probe length was 30 cm, and the probes were installed horizontally at depths of 5, 15, and 25 cm relative to the peat surface. The CS655 moisture probes, which also measured soil temperature, had probes that were 12 cm long. Each moisture profile also had an adjacent temperature profile installed within the same hummock. Peat temperature profiles were measured in each hummock at each site using T-type thermocouple wire (Omega Engineering, CT, USA) inserted at various depths depending on the type of moisture probe installed in each profile. For hummocks which contained CS616 probes, thermocouple wire was installed at depths of 1, 5, 10, 15, 25, and 50 cm relative to the peat surface. At the CAR study site, there was no temperature measurement at 10 cm. For the profiles containing the CS655 probes which already measured temperature, thermocouple wire was installed to substitute the missing data at depths of 1, 10, and 50 cm below the surface of the hummock. Furthermore, the temperature probe installed at 1 cm was considered to represent the surface since exposure to sunlight and near-surface microclimate fluctuations at 0 m could occur and cause biased results.

The frost depth was estimated directly through interpolation of the 0°C isotherm from the soil temperature measurements (Lindström *et al.*, 2002). This coarse method for determining the frost line simply identifies points in time when the temperature at each depth falls below zero, which may be interpreted as a frost index (Maidment, 1993; Lindström *et al.*, 2002). Whenever a thermocouple detected a negative temperature, it would be assigned a nugatory value depending on the depth at which the negative temperature was recorded. Since the thermocouple was not a continuous profile, the depth of the frost line could only be observed according to the pre-set depths at which the thermocouple was installed. As the frost line advanced and negative temperatures were detected deeper in the profile, it was assumed that any point above that depth was also frozen. Therefore, this method does not take into account periods when the frost line was present despite the surface being thawed and deeper portions of the hummock remaining frozen. The number of instances that the frost line occurred was then divided by the total number of measurements that were taken within the specified time frame (October 1 to May 31) to get the proportion of time.

During October 2016, wooden meter sticks were inserted into each instrumented hummock to determine the depth of snow above the hummock surface at each site. Snow surveys were conducted in the winter of 2016-2017 on November 25, December 13 and 21, January 6 and 19, February 16 and 25, and March 1.

2.2.4 Hibernacula Hummock Zone Indices

Using the temperature and WT depth data (section 2.2.3) we determined the percent of time each 5 cm depth interval in each hummock was flooded, frozen, both, or neither (hereafter referred to as Hibernacula Hummock Zone Indices (HHZI)) for the period from October 1 to May 31. This period was chosen as this is generally the time when snake ingress and egress would be occurring at the overwintering site (Boulin-Demers *et al.*, 2000; Harvey and Weatherhead, 2006a; Harvey and Weatherhead, 2010; Parks Canada Agency, 2015). The snakes enter and emerge at their leisure and there has not yet been a method developed to determine when snakes choose to enter and emerge from overwintering site as it is likely a number of factors such as air temperature, phenotypic sex, or site specific variations (Boulin-Demers *et al.*, 2000; Lutterschmidt *et al.*, 2006). It is important to note that the PAB_004 and PAB_005 stations were installed in the middle of November and so the time frame for the 2015-2016 winter season starts on November 15 instead of October 1. Also, the only data that were available from the CAR_006 site was for the 2016-2017 winter season. Using aforementioned percent of time graphs, we used the trapezoidal numerical integration function in MATLAB (MATLAB R2017a, The MathWorks) to calculate the area in the graph occupied by each HHZI which was the HHZI score. For comparison purposes, the x-limit of each graph was set to 100% and the y-limit was set to 80 cm as the WT never dropped below this depth. The optimal depth where the maximum survival HHZI score was attained in each hummock was noted as the point of intersection between the frost line and WT lines, which were both monotonically decreasing and increasing lines, respectively.

2.3 Results

2.3.1 Site Characterizations

A summary of each overwintering site area and the instrumented hibernacula hummocks is provided in Table 2.1. Combined, MAG had six hummocks instrumented, PAB had ten, and CAR had two. The analysis that was performed may be improved by increasing the number of measured hummocks, specifically at the CAR study site. A one-way analysis of variance (ANOVA) was also performed, combined with a multiple comparison of the group means using the ANOVA results, which determined there was no significant difference between hummock sizes at each site (F(2) = 2.45, p-value = 0.12; Figure 2.2).

The average depth of peat in the interior portions of each overwintering site as well as the margins were calculated. All ± values are one standard deviation from the mean. The MAG study site was the shallowest with an average peat depth in the middle of 50.1 cm (± 17.4). The average margin depth at this site was 16.7 cm (± 11.3). The CAR study site was slightly deeper, with an average peat depth in the middle of 70.3 cm (± 35.3) and 25.5 cm (± 18.0) along the margin. All depths in the interior of the PAB site were greater than the length of the rebar (180 cm) and so the average depth in the middle of this site could not be accurately assessed. The average of the highly variable margin peat depth was 31.4 cm (± 30.6).

The PAB study area is densely forested. Calculated by stem count, the stand density including shrubs, is 0.44 trees m^{-2} with an average DBH of 10.9 cm. The average canopy openness was 32.5% (± 7.8). This peatland consists of roughly 23% tamarak (*Larix laricina*), 15% black spruce (*Picea mariana*), 4% each of jack pine (*Pinus banksiana*) and white pine (*Pinus strobus*), as well as roughly 48% shrubs which were mainly mountain holly (*Illex mucronata*). The MAG and CAR study sites are shallower peatlands with lower canopy cover, specifically in the middle of the peatlands. MAG has a stand density of 0.32 trees m^{-2} with an average DBH of 7.5 cm and average canopy openness of 47.7% (± 6.8). Over 80% of the trees at this site are jack pine and the remaining include paper birch (*Betula papyrifera*) and white pine. The CAR site has a stand density of 0.16 trees m^{-2} with an average DBH of 12.3 cm. The average canopy openness was 54.0% (± 7). Jack pine also dominates this site (54.4%) with white pine (17.8%), birch (10.0%), and maple (*Acer rubrum* and *saccharum*; 9.7%) as well as a few oak trees (*Quercus rubra*) and shrubs existing along the margins. It is worth noting that the few trees in the interior of the MAG study site were an average height of 2.9 m which are 46.3% shorter than those existing on the margins of the peatland which on average are 5.4m. Similarly to the MAG study site, the marginal trees at the CAR study site are larger (5.4 m average) than those in the interior which are approximately 22% smaller (4.7 m). There is no margin/middle differentiation of tree heights at the PAB site; the average tree height, excluding tall shrubs, was 11.0 m.

2.3.2 Water Table Analysis

The average WT depth was calculated for the months of October to May during both the winter of 2015-2016 and 2016-2017 (Figure 2.3). For consistency, the depths are relative to the hollow surface, as opposed to the hummock surface, and therefore only capture the fluctuations between years and months. It was assumed the hollows were a continuous, uniform surface which allowed the comparison of WT fluctuations across all sites. Depending on the size of the hummocks, the results would differ for each hummock. The results, summarized in Figure 2.3 show that the WT increased throughout both winters and in a few cases rose above the hollow surface. The WT was most variable at all sites later in the winter (February, March, April, May) for both years. It was also observed that the average WT depths at the CAR study site were significantly lower than those observed at MAG and PAB during the winter of 2016-2017.

2.3.3 Hibernacula Hummock Zone Indices

The percent-of-time that various depths within each hummock were frozen, flooded, both frozen and flooded, and survivable was calculated based on the depth of the frost line and WT. The shaded area for each represents the probability of time that at a specific depth, one of these four conditions occurred during the overwintering season since the frost line and WT change over time and depth (Figure 2.4). Each area was designated as the score for the survival HHZI, flood HHZI, frozen HHZI, or flooded and frozen HHZI. Figure 2.4 is an example of these results for a relatively small hummock (Figure 2.4a) and large hummock (Figure 2.4b). The larger the area, or score, represented on the graph by a specific condition, the greater amount of time that that condition occurred at the depth of interest. Therefore, the greater the percent-of-time that the hummock was survivable, the better the chances that an overwintering snake could survive.

Results from both winters showed a weak positive linear trend that the larger the hummock height, the greater the survival HHZI score (R^2 = 0.17, Figure 2.5). A negative linear relationship between hummock height and the drown HHZI score was also observed (R^2 = 0.25, Figure 2.5). No trend was observed for hummock height and the freeze and drown HHZI score and the freeze HHZI score. The substantial difference between the CAR hummocks and the hummocks at the other two sites is worth noting. They appear to be outliers within the entire data set and once removed, the relationship between hummock height and the HHZI improves. For the survival zone, the R^2 value increases from 0.17 to 0.36. The relationship between the drown HHZI and hummock height also improved from having an R^2 value of 0.25 to 0.72 once the CAR hummock data was removed. The depth within each hummock at which the greatest survival HHZI score was attained (*i.e.* the point of intersection between the frost line and WT line on the graph) showed a positive logarithmic relationship with hummock size, excluding CAR_006 HUM A, which appeared significantly different from the rest of the dataset ($R^2 = 0.59$, Figure 2.7). The results of the one-way ANOVA between the two winters showed no significant difference between each of the HHZIs between years (Figure 2.6). There was also no significant difference observed for hummock sizes between sites (Figure 2.2).

2.3.4 Snow Surveys

The results of all measured hummocks from each of the sites on various dates throughout the winter of 2016-2017 are shown in Figure 2.8. No measurements of snow were made at MAG or PAB on December 13 or January 6. When combined, the snow depths at MAG were significantly smaller than those at PAB and CAR ($F(2) = 10.18$, p-value <0.001) as determined after a multiple comparison of the means was performed on the one-way ANOVA results (Figure 2.9). However, the depths at the CAR study site were more variable than those at the PAB site.

The combined average daily temperatures on dates of the snow surveys at all depths is summarized in Figure 2.10. A multiple comparison of the means for each depth based on the results of a one-way ANOVA showed that there were differences between temperatures at similar depths across all three sites (Table 2.2). Except for the 0 and 50 cm depth, average daily temperatures at all other depths were significantly different between MAG and PAB sites (p-value <0.001, Table 2.2). Temperatures at the PAB and CAR study sites were generally statistically similar except at 0, 10, and 50 cm depths. There was no measurements taken at 10 cm depth for the CAR study site. At 0 cm, or the surface, the MAG and PAB sites were statistically similar. However, at 50 cm depth, all three sites experiences statistically different average daily temperatures (F(2) = 15.52, p-value <0.001, Table 2.2)

2.4 Discussion

2.4.1. The Survival Hibernacula Hummock Zone Index

For a species that spends almost half its life hibernating, the location and suitability of their overwintering habitat is critical to their survival. The sites used in this research were known overwintering sites meaning snakes chose these sites for a specific reason. We hypothesized that these peatlands were selected for because of the moss hummocks which provide suitable conditions conducive to a snake's winter survival. We did not assess proximity of these sites to other critical habitats as a reason for why snakes chose these particular sites, which may also likely dictate site selection. The temperature and WT dynamics were characterized to try and identify the conditions within various sized hummocks to determine if their size played a significant role in the snake's survival zone. Although there was a weak positive linear relationship with the survival HHZI score and hummock size (Figure 2.5, $R^2 = 0.17$), there was a strong logarithmic relationship between optimal depth for maximum survival HHZI and hummock size (Figure 2.7). This supports our hypothesis that larger hummocks are better for overwintering snakes and that there is an optimal depth that the snakes likely select to survive in. The data predicts that the depths where the survival HHZI is the greatest is 20-25 cm below the surface of each hummock in hummocks 30-35 cm high (Figure 2.7). Moreover, after a certain point, the size of the hummock likely will not matter as the optimal depth appears to reach a threshold around 25-30 cm. This is also highly dependent on the ecohydrological feedbacks controlling hummock growth (Waddington *et al.,* 2015).

The similarity between each HHZI for both winters indicates that despite weather variations between years, the peatlands are able to maintain appropriate survival conditions. However, because this analysis did not take into consideration timing and duration of freeze and thaw events in the fall and spring that may have caused increased snake mortality during ingress or egress, we still cannot make conclusions about what caused the mortality event in 2014. Further research that combines snake tracking with this type of data analysis would help to better understand the significance of these freeze thaw events in snake mortality.

2.4.2 Winter Water Table Fluctuations in Peatlands

The average WT calculations for both years at all sites (Figure 2.3) demonstrates how close the WT can be to the surface in the winter. Although the threat of drowning would depend on the size of the hummock (Figure 2.5), these average depths demonstrate the potential threat to EMRs which hibernate close to the WT. The differences between average WT throughout the winter is surprising considering the CAR site was much shallower (70.3 cm) than the PAB site (<180 cm) and was most similar to the MAG site, the shallowest hibernaculum (50.1 cm). One potential explanation could be the differences in land cover (*i.e.* tree density and canopy openness). Tree density and its effect on the WT can generally be explained by the WT depth – afforestation feedback which demonstrates how increased shrubification can lead to increased transpiration and eventual drop in the WT (Waddington *et al.*, 2015). However, this is the opposite for this study as the site with the smallest tree density, CAR (0.16 trees m^{-2}), had the deepest average WT. Therefore, other feedbacks or peatland characteristics must be contributing to the observed difference in WT.

Extreme fluctuations in the WT during hibernation can have catastrophic effects for EMRs (Johnson *et al.*, 2000). Past studies have shown that EMRs select habitats where the WT remains relatively high and constant but is far enough from the surface to prevent flooding (Smith, 2009). Peatlands with taller hummocks therefore should provide optimal conditions for overwintering snakes as they provide EMRs protection from freezing and drowning. However, rapid climate change and the associated variability in weather between years threatens this critical habitat to which EMRs show high fidelity to. The timing of flooding and drought within these peatland overwintering sites thus becomes increasingly important (Johnson *et al.*, 2000). Moreover, if the hydrological feedbacks that maintain the ideal WT conditions within the peatlands become disrupted by climate change or anthropogenic impacts (Waddington *et al.*, 2015), the already limited number of suitable hibernacula would no longer meet the needs of the EMRs. Intentional WT manipulations to raise or drop groundwater levels during the winter using levees or dikes has been suggested as a mitigation strategy (Johnson *et al.*, 2000; King *et al.*, 2004; Smith, 2009) but in the few cases where it has been practiced, populations of EMRs were extirpated due to inappropriate estimates of the impacts (King *et al.*, 2004). Moreover, the EMR Handbook for Land Managers states that these manipulations are not practical at all sites and may be prohibitively expensive unless structures are already in place (Johnson *et al.*, 2000). Therefore, conserving the current limited number of peatland overwintering sites is critical. However, projects which aim to restore or build appropriate hibernaculum should consider implementing WT manipulation strategies that minimize summer drought and winter flooding events. Levees and dikes can be incredibly expensive to construct. Therefore, use of natural vegetation to buffer hydrological fluctuations, which has historically proven successful, should be considered (Pomara *et al.*, 2014). Peatlands especially could benefit from this approach as the natural ground and moss cover already contribute greatly to the success of various feedbacks in maintaining a stable WT (Waddington *et al.*, 2015). Conservation strategies should therefore consider surrounding uplands, marginal vegetation, and the impacts of trees and ground cover as part of the mitigation plan.

Knowing the position of the WT relative to the sill is also critical in determining the potential impacts of winter flooding as peatlands are conceptually a fill and spill system. Similarly, the position of hummock hibernaculum relative to the sill should also be considered. The sill of natural, restored, or constructed overwintering sites should be monitored to ensure there is proper drainage throughout the winter. This management strategy would allow snakes to have access to the WT near the surface to avoid desiccation but reduce the likelihood of flooding in the case of increased inputs of water from snowmelt or precipitation. Intensive collection of accurate continuous snow depth, air temperature, and precipitation at scales small enough to capture minute changes within the hummock combined with monitoring EMR behavior is required to gain a better understanding of hibernacula suitability.

2.4.3 Snow Survey, Subsurface Temperatures, and Canopy Surveys

The Georgian Bay population of EMRs utilizes a variety of habitats for critical life stages such as hibernation. Peatlands are ideal overwintering sites, provided certain conditions are met within the hummock hibernacula and that they are within the home range of individual snakes. It is difficult to determine the suitability of sites simply by observing them. However, a distinguishing factor to characterize peatlands is tree density. Often, dominant tree species and abundances dictate the classification of a peatland. These same features may provide some information on the suitability of various peatlands as hibernation sites. It was hypothesized that the greater the tree density, the less amount of snow would be present, and thus the less suitable the hummocks would be for the snakes to overwinter in. This was not supported as the results did not indicate substantial differences between sites of differing tree densities and canopy openness. PAB had the greatest tree density and smallest canopy openness, but received no more or less snow than the CAR site which had the lowest tree density and greatest canopy openness. There was no significant difference between snow depths at the sites which indicates that despite being geographically different as well as having different tree densities and stand characteristics, the depth of snow was similar at all sites. However, despite all being located along Eastern Georgian Bay, the proximity of the sites to the Bay and their location relative to the entire Bay means the local weather could be extremely different at similar times throughout the winter. Therefore, the effect of tree density on snow depth may have been offset by increased or decreased amounts of snow observed for that specific location. However, in the spring, it was observed that sites with the greatest tree density tended to have snow present the longest which is likely due to the trees shading the site and preventing direct exposure to the sun for extended periods. This may be an important feature dictating the time of emergence for the snakes. Although the extended period of snow could delay their emergence, it may help to protect the snakes during periods of fluctuating temperatures throughout the spring. For instance, snakes which emerge at sites where snow melts faster may be exposed to periods of freezing when the air temperature remains unsuitably cold. This speaks to the insulating effect of the snow. Although the weather conditions outside may be suitable for snakes, the substantial fluctuations in daytime and nighttime temperatures in the spring may cause them to die as they venture out of the site. A longer snow cover may then extend their stay in the hummocks, and decrease the likelihood that they will freeze. A single behavioural or environmental factor has yet to be identified to determine the exact time of EMR emergence. It could be a combination of environmental variables such as air temperature, hours of sunlight, and/or WT rise from snowmelt. Despite not knowing these factors, the most important determinant of rattlesnake survival could be the timing of the first substantial snowfall in relation to the first freeze, and durations of freeze and thaw events during spring emergence.

2.4.4 Applications of Research

Implications of this research can be extended to areas of habitat conservation whereby strategies to mitigate the impacts of anthropogenic land-use changes and climate change can be more effective. Overwintering sites are critical habitat for species such as snakes which must find locations where they can survive below the frost line but above the WT during the winter. Few sites on this landscape offer this kind of survival zone, hence why snakes show high fidelity to their hibernaculum.

However, suitable sites are limited along Eastern Georgian Bay and may restrict the northern distribution of snakes (Harvey and Weatherhead, 2006a). Furthermore, as highway and cottage development is imminent, habitat loss threatens this population of snakes which is thought to be the primary cause of their decline (Szymanski, 1998). Efforts to prevent further habitat loss are hindered by a poor understanding of their habitat requirements which further highlights why this research is so important. This work provides insight into how to identify suitable overwintering sites that allow snakes to survive the harsh Canadian winters. Peatland sites with sufficiently large hummock hibernacula and proper drainage are ideal. However, the density of trees is not a useful indicator of overwintering site suitability as there was no significant evidence that tree density or height altered the snow depth and thus the insulating effect of the snow. It is also important to evaluate survival and habitat suitability based on population specific requirements. Although some populations with longer hibernation periods (*i.e.* more northerly locations) may have lower winter survival rates, their individuals may have greater overall longevity (Aldridge *et al.*, 2008; Jones *et al.*, 2012; Pomara *et al.*, 2014).

2.5 References

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2.6 Tables

Table 2.1: Summary table of rattlesnake hibernacula study sites

Site	**Area (m^2)**	**Tree Density (trees m^{-2})**	**Tree Height (m)**	**Canopy Openness (%)**	**Mean Depth of Peat (cm)**	**Station Name Within Site**	**Number of Instrumented Hummocks**	**Hummock Heights (cm) listed in order of smallest to largest**
CAR	2501.63	0.16	5.1	54	70.3	006	2	17.3, 21.8
PAB	10000 *	0.44	11.0	32.5	<180	004	5	22.1, 24.9, 26.7, 27.2, 29.2
						005	5	19.8, 26.4, 28.4, 29.7, 32.3
MAG	2323.35	0.32	4.2	47.7	50.1	001	2	24.6, 31.2
						002	2	13.2, 22.6
						003	2	21.6, 26.2

* Estimated (Rogers, 2015)

Table 2.2: ANOVA results for temperature comparisons at various depths among sites on dates of snow surveys. Letters designate differences between significantly different groups

	Significantly different groups at each depth			ANOVA results	
Depth (cm)	MAG	PAB	CAR	F statistic (df=2)	p-value (probability>F)
0	A	A	B	3.45	0.036
5	A	B	B	19.06	< 0.001
10	A	B	N/A	18.04	< 0.001
15	A	B	B	16.37	< 0.001
25	A	B	B	18.95	< 0.001
50	A	B	C	15.52	< 0.001

2.7 Figures

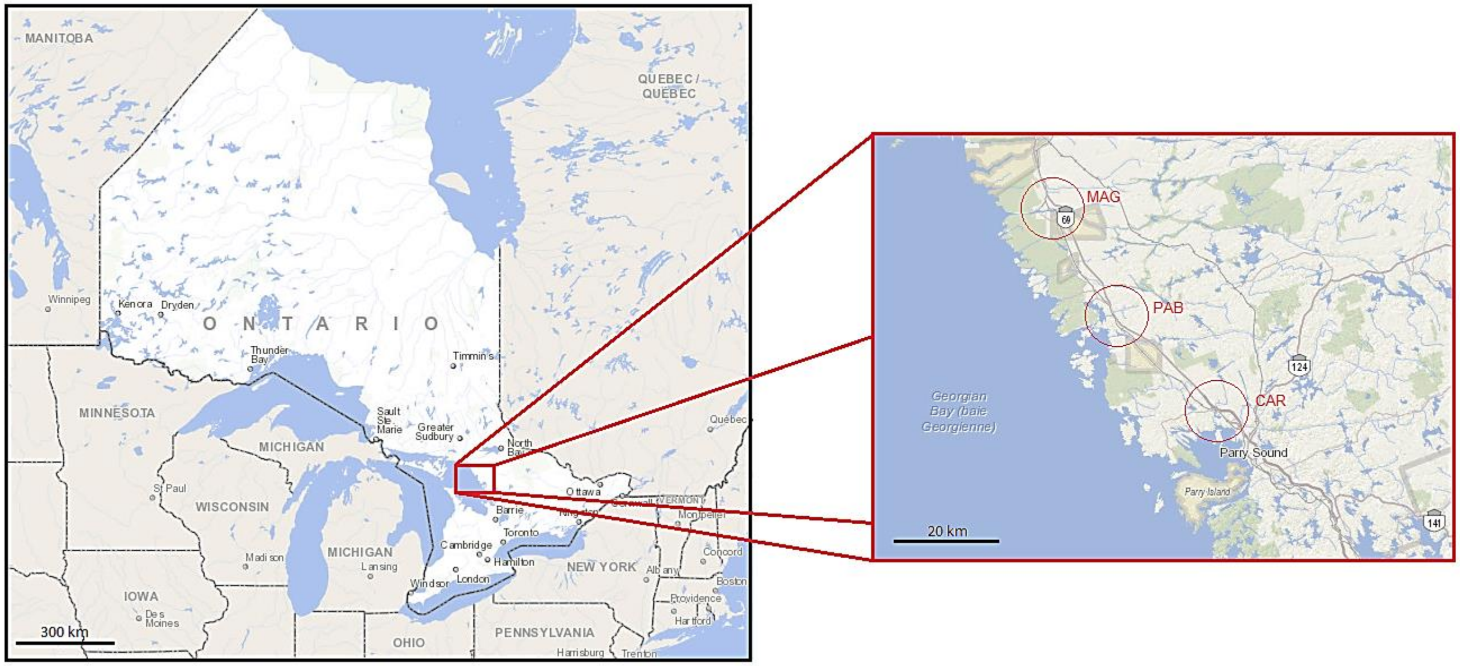


Figure 2.1: Location of study sites along the coastal inlands of Eastern Georgian Bay in the NOBEL

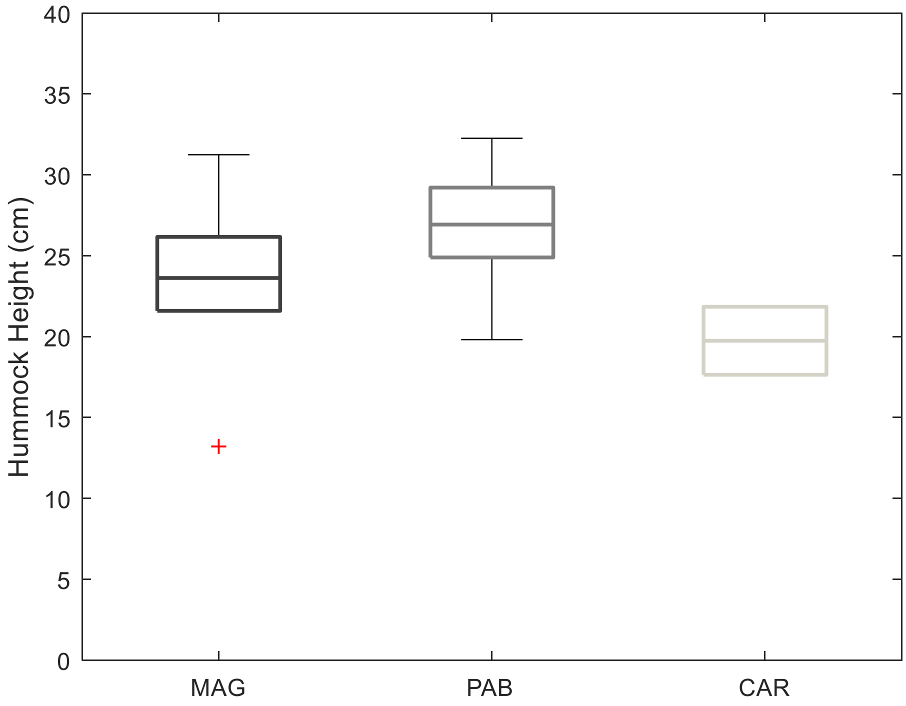


Figure 2.2: Distribution of hummock hibernacula sizes at each of the three rattlesnake overwintering sites

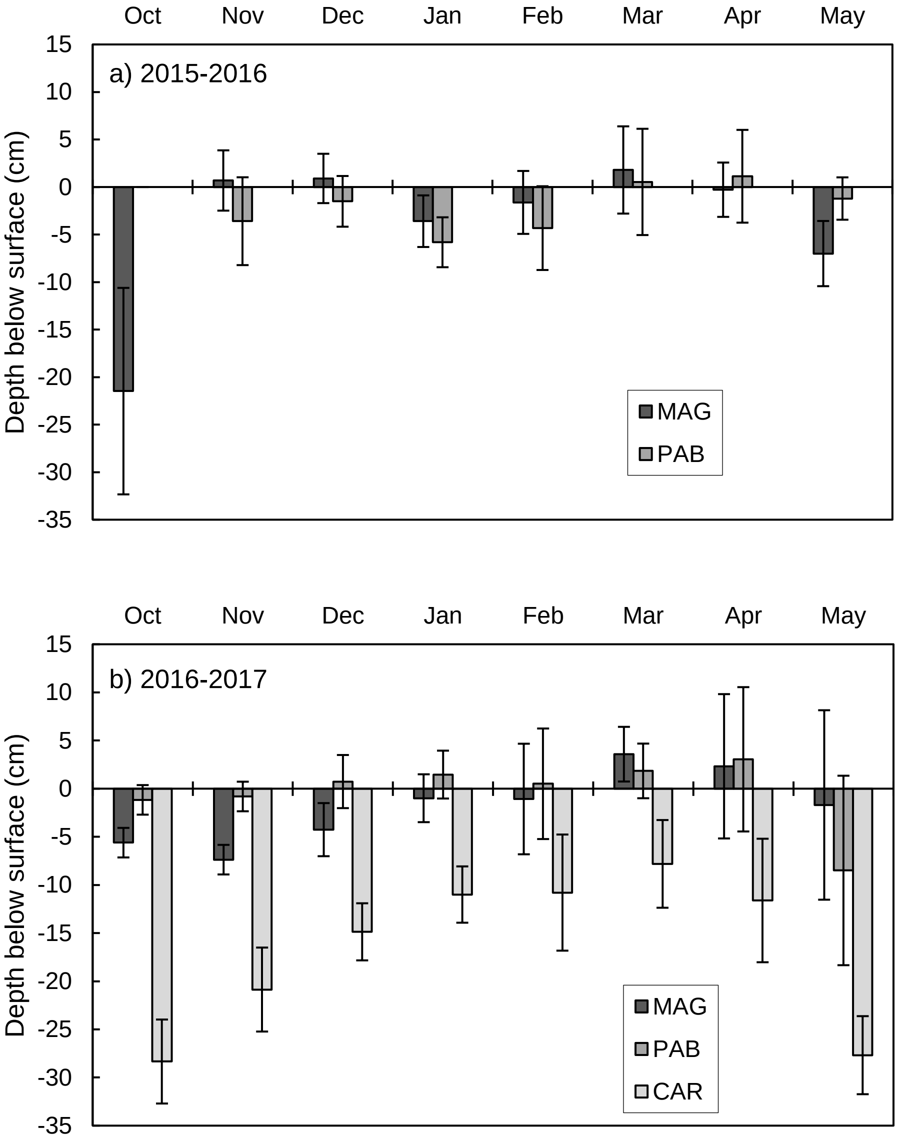
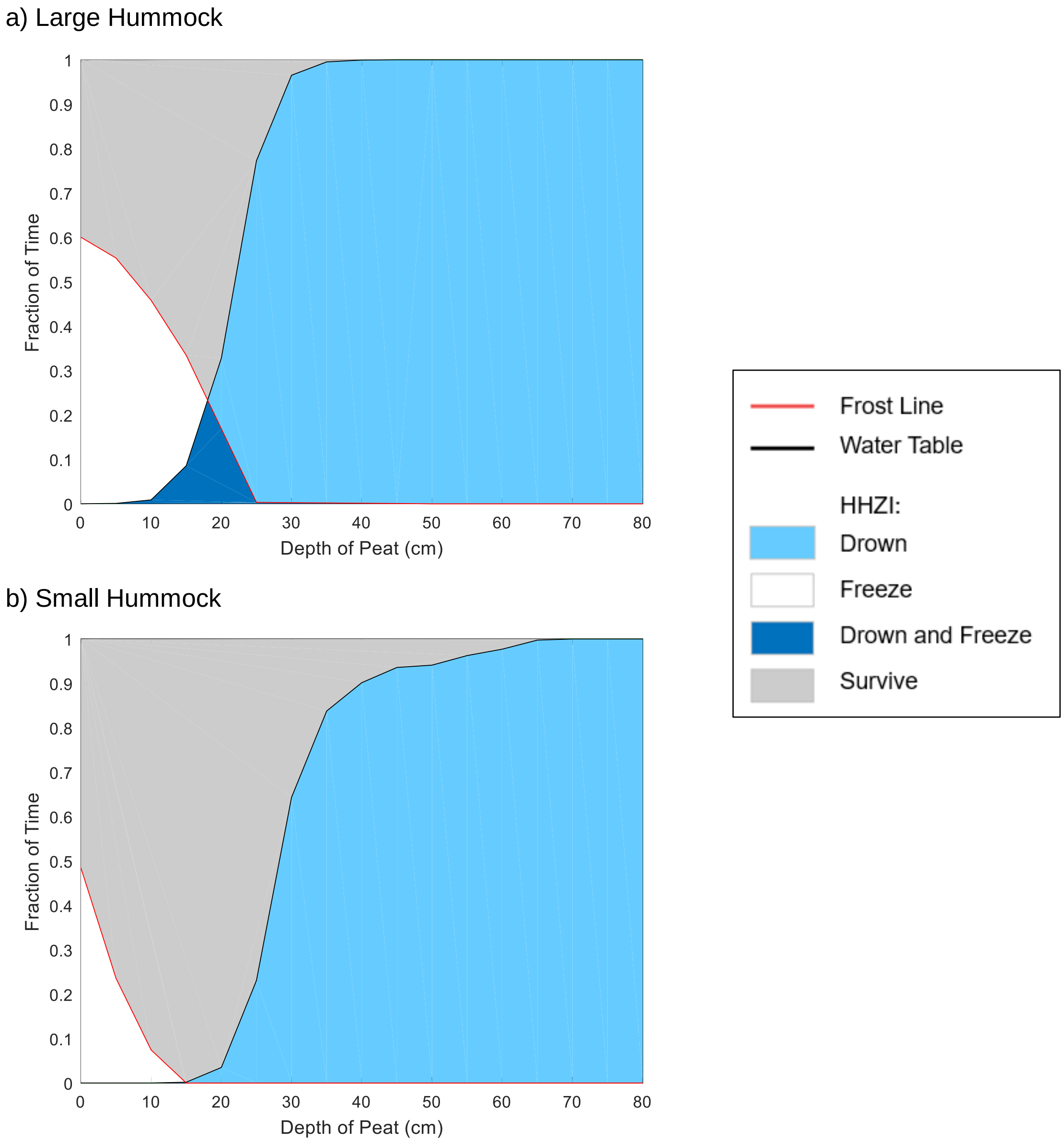


Figure 2.3: The location of the water table relative to the hollow surface of the peatland for a) Winter 2015-2016 and b) Winter 2016-2017. The CAR study site was not instrumented in the winter of 2015-2016


a) Large Hummock

b) Small Hummock

Figure 2.4: Delineation of freeze, drown and freeze, drown, and survival zones within a) a small, 19.8 cm (PAB_005 Hum D) and b) a large, 31.2 cm (MAG_001 Hum A), rattlesnake hummock hibernaculum using continuous changes in frost line and water table at specific depth intervals, during the winter of 2015-2016. Data between points was interpolated linearly

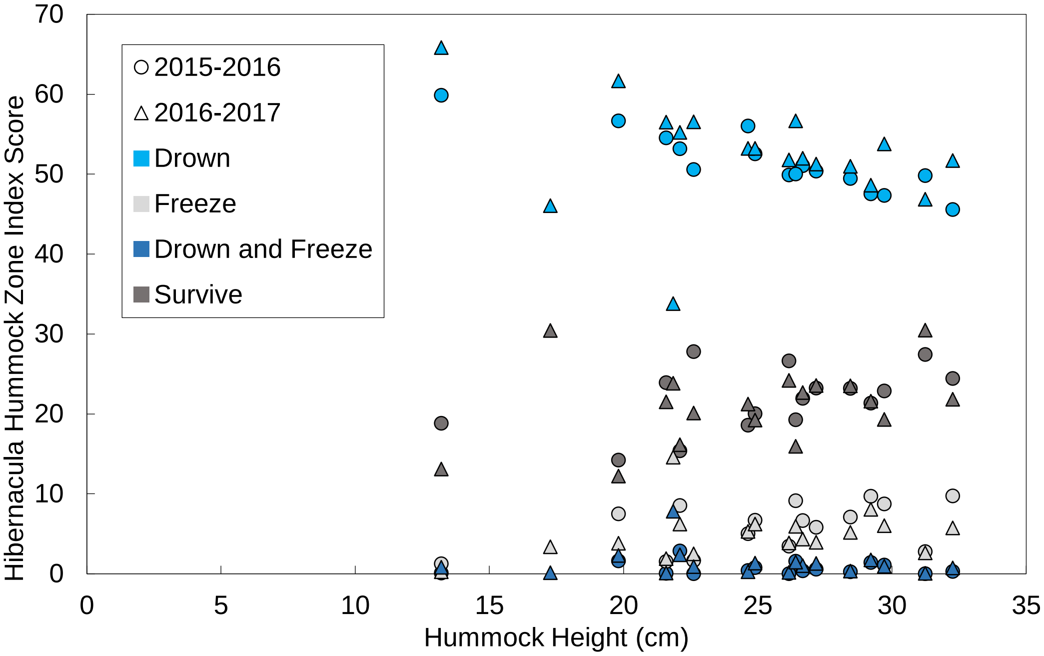


Figure 2.5: Freeze, Drown and Freeze, Drown, and Survival Hibernacula Hummock Zone Index scores for all instrumented hummocks during two winters. The Hibernacula Hummock Zone Index measures the area of each of the four zones defined by the probability-of-time graphs of each hummock which depicts the frost line and water table over time and depth

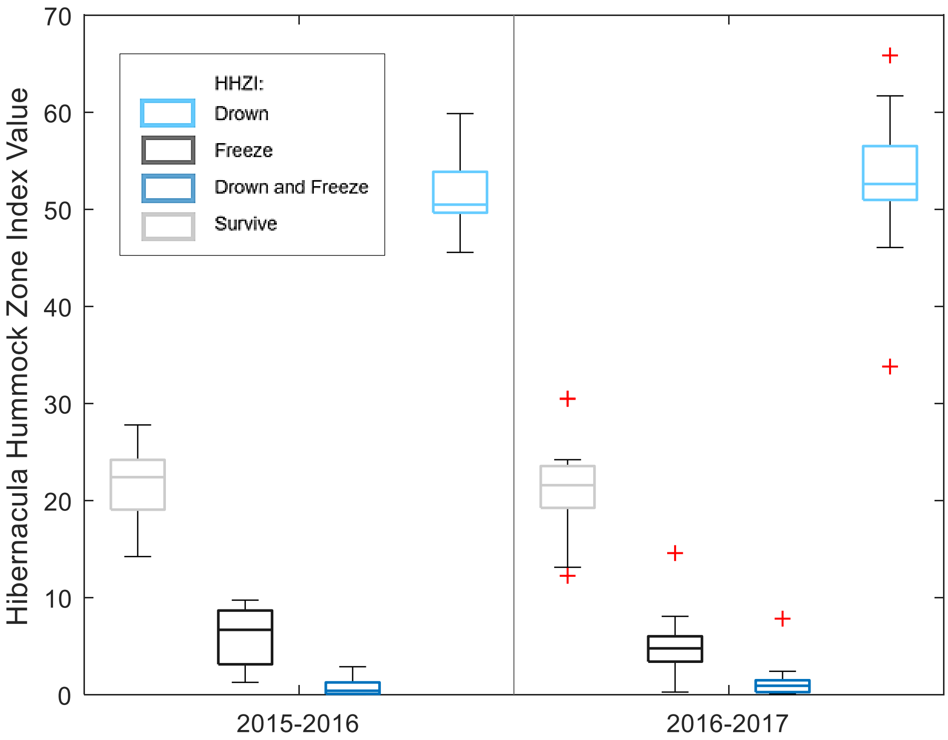


Figure 2.6: Comparison of Hibernacula Hummock Zone Indices for the two study winters at all sites. No significant difference within indices for each year

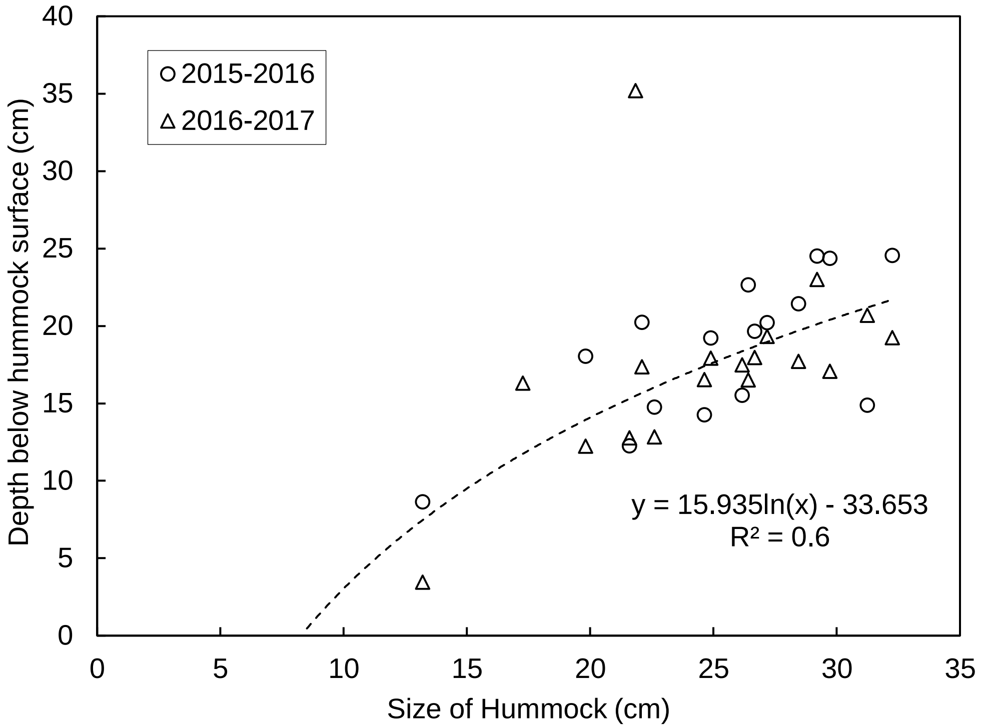


Figure 2.7: Depth corresponding to the optimum depth for maximum survival HHZI score versus the size of the hummock. The trendline is excluding CAR_006 Hum A (hummock size 21.8 cm) which was identified as an outlier

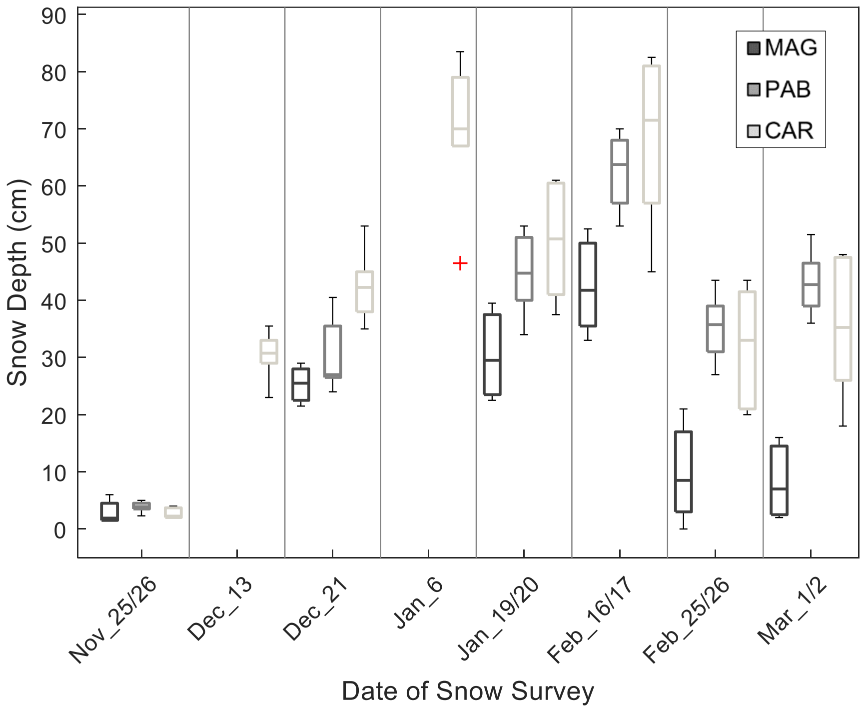


Figure 2.8: All snow depths above hummocks within each site during the second winter study season (2016-2017) and the dates when measurements were collected

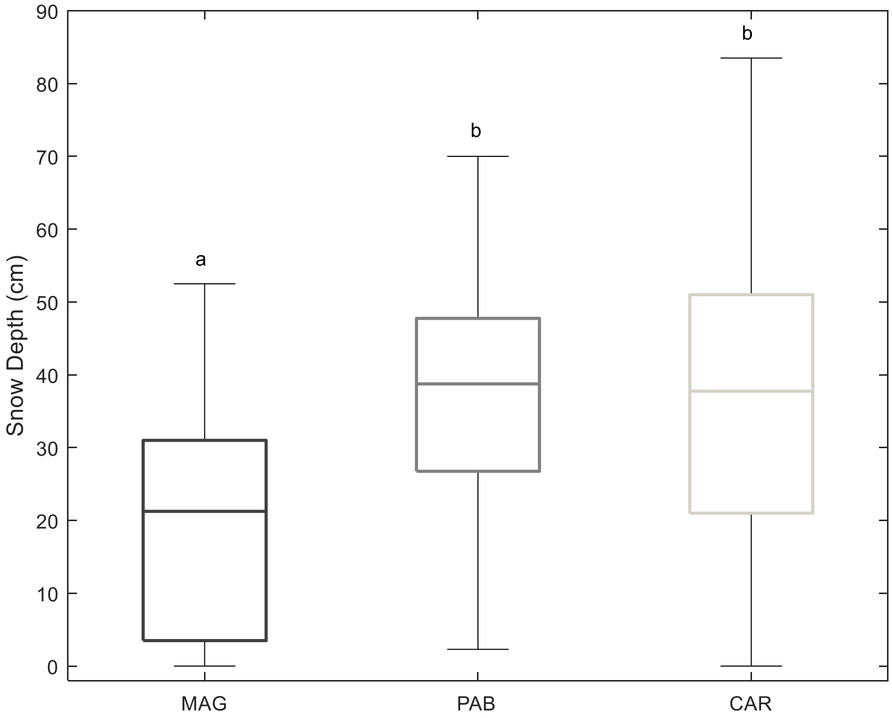


Figure 2.9: Summary of all snow depths during the second winter field season (2016-2017)

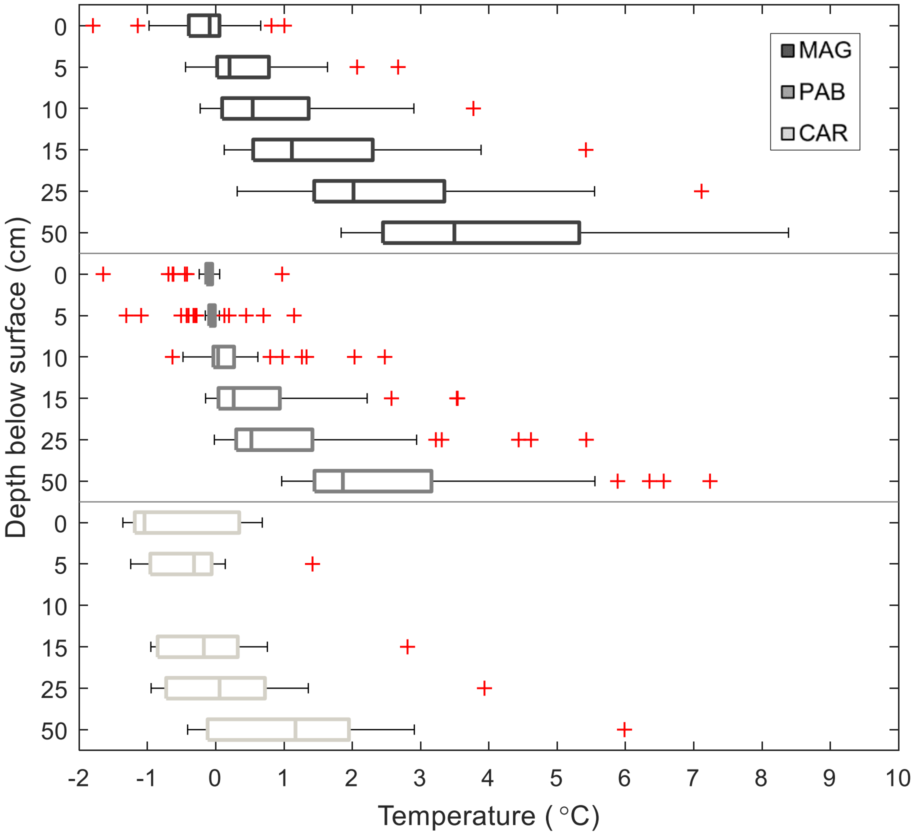


Figure 2.10: Distribution of average daily temperatures on dates when snow surveys were performed. Temperatures at various depths of measurement below the surface for each of the three study sites are depicted. Note: The CAR site did not have measurements of temperature at 10 cm depth

CHAPTER 3: NESTING HABITAT

3.1 Introduction

3.1.1 Turtles in Ontario

Georgian Bay is located in the southern-most part of Canada and therefore experiences weather that facilitates the existence of several species of reptiles, including turtles. Ontario has eight species of freshwater turtles, however, seven of these are listed as species-at-risk (SAR), under the Species at Risk Act (2007). Excluding the Spiny Softshell Turtle (*Apalone spinifera*), all other species utilize habitat in and around Eastern Georgian Bay making it a turtle biodiversity hotspot. While the mosaic of open rock barrens and wetland ecosystems provide optimal habitat for these species (Jalava *et al.*, 2005), few studies have explored the microhabitat characteristics of habitats used for turtle nesting, mating, overwintering, and foraging. As an animal that has both aquatic and terrestrial habitat preferences, freshwater turtles present a unique challenge of characterizing their habitat. Specifically, the variation of oviposition site types across numerous habitats makes it difficult to identify critical nesting habitat (Lesbarrères *et al.*, 2014). However, the general understanding is that temperature and moisture are two key components dictating the success of turtle egg incubation and embryo sex for those species which undergo temperature-dependent sex determination (Packard *et al.*, 1991). For those species which do not, temperature is still important in ensuring the development of the eggs which can only occur if incubated at higher temperatures. Generally, warm temperatures above a certain threshold tend to produce more females while temperature below this threshold result in males (Freedberg *et al.*, 2001; Morjan and Valenzuela, 2001). Such research is critical since these studies may be useful for determining conservation management strategies of reptile SAR (Millar and Blouin-Demers, 2011; Markle and Chow-Fraser, 2014).

The Blanding’s Turtle (*Emydoidea blandingii*) is currently listed as threatened in Canada (COSEWIC, 2005), whereas the Spotted Turtle (*Clemmys guttata*) is considered endangered in Canada (COSEWIC, 2011). The population of Blanding’s Turtles in Canada extends from the southern Great Lakes region to some parts of Quebec. However, a population in Nova Scotia exists separate from the Great Lakes/St. Lawrence population. These turtles can also be found in New York, Ohio, Nebraska, Michigan, New Hampshire and Maine (COSEWIC, 2005). In preparation for nesting, females spend most of the pre-nesting season basking and conserving energy in their resident wetlands, unlike non-gravid females and males which have lower energetic needs at this time (Millar and Blouin-Demers, 2011; Markle and Chow-Fraser, 2014). A number of different nesting sites have been recorded for populations of Blanding’s Turtles. Recently, it was confirmed that moss-filled cracks in bedrock act as nesting sites for Blanding’s Turtles which show high fidelity to specific locations (Millar and Blouin-Demers, 2011; Markle and

Chow-Fraser, 2014). At the same time, they have also been known to select marsh and wet forests for nesting habitat (Beaudry *et al.*, 2009; Millar and Blouin-Demers, 2011; Markle and Chow-Fraser, 2014). In comparison, Spotted Turtles frequently nest on open rock outcrops which also serve as nocturnal habitat during the rest of the active season (Litzgus and Brooks, 1998b; Litzgus and Brooks, 2000). The Musk Turtle has also been observed nesting on rock outcrops (Litzgus and Brooks, 2000) while several depredated Snapping Turtle (*Chelydra serpentina*) nests have been seen in similar habitats (Litzgus and Brooks, 2000). Spotted Turtles also nest in *Sphagnum* hummocks within their resident wetlands (Beaudry *et al.*, 2010) whereas only a few cases of Blanding's Turtles associated with *Sphagnum* moss have been observed (Beaudry *et al.*, 2009). The appeal of the rock outcrops as nesting sites is likely the warmth provided by direct sunlight and residual heat maintained by the large outcrops which also act as natural incubation systems for turtle eggs (Litzgus and Brooks, 2000; Millar and Blouin-Demers, 2011). Granite rock outcrops are able to store sensible heat and maintain warm temperatures throughout the day and into the evening as a result of the high heat capacity (Clauser, 2009; Clauser, 2011). Although it has been identified that these species utilize the relatively shallow organic deposits on the rock barrens as nest sites, little is known about the subsurface conditions at these sites which determines the success of this type of habitat as nesting sites.

3.1.2 Turtle Nest Incubation Conditions

The thermally and physically challenging environment that is the rock barrens of Eastern Georgian Bay begs the question, how do freshwater turtles nest on this landscape? There are few types of habitat that offer suitable temperature and moisture conditions to support the development of embryos. One such landscape feature are the moss-filled depressions, or moss cushions, that are often isolated from expansive moss carpets covering the forest floor and located in the open where tree cover is limited. Depending on the species, their size, and the average clutch size, turtles can nests in substrates that range from a few to several centimeters deep. For example, Spotted Turtles lay eggs in substrates as shallow as 5 cm, (Ernst *et al.*, 1994; Conant *et al.*, 2016) whereas Painted Turtle (*Chrysemys picta*) nest cavities in substrate are around 9.6 cm (Riley and Litzgus, 2013). The slightly larger Blanding's Turtles dig cavities approximately 12 cm deep (Standing *et al.*, 1999) while Snapping Turtles can dig cavities roughly 30 cm deep (Riley and Litzgus, 2013). Depending on the depth of nest these turtles typically dig, the incubation conditions can vary greatly within the nest cavities.

The influence of temperature fluctuations on reptile egg development has received more attention in recent years with countless studies focusing on a wide array of species around the world. Oviparous reptiles often select sites with specific incubation conditions that are suited to facilitate the development of their eggs

(Brooks *et al.*, 1991; Bodensteiner *et al.*, 2015). Sensitivity to variations in temperature and moisture that are often associated with different substrates can impact hatchling success and their long term development (Packard *et al.*, 1987; Janzen, 1990; Miller and Packard, 1992; Bodensteiner *et al.*, 2015). Species-specific traits such as temperature-dependent sex determination and soft- versus hard-shelled eggs are important factors to consider when understanding the effects of temperature and moisture on turtle eggs, as well as the interactions between the two. For instance, when the soil moisture is high, the solar heat input is dispersed deeper into the soil resulting in a cooler nest as well as reduced temperature fluctuations due to the high heat capacity of water and thus wet substrate (Al Nakshabandi and Kohnke, 1965; Bodensteiner *et al.*, 2015). Although the effects of temperature have been well documented amongst species, substrate moisture conditions have not been assessed in as much detail. Moreover, the majority of studies have been based on laboratory experiments whereby the environmental conditions are strictly controlled. Therefore, not only is further laboratory research needed that better reflects natural environmental variability, but field research that investigates the importance of temperature and moisture on nest-site choice by females in a variety of substrates is also warranted (Delmas *et al.*, 2008; Warner and Shine, 2009; Bodensteiner *et al.*, 2015). Further studies performed under ecologically realistic scenarios which document changes in moisture and temperature during fluctuating hydric regimes are needed (Delmas *et al.*, 2008).

3.1.2.1 Temperature

Not only does temperature dictate the timing and length of turtle nesting season in Ontario (Gibbons and Nelson, 1978; Obbard and Brooks 1981; Galbraith *et al.*, 1988; Bobyn and Brooks, 1994a; Litzgus and Brooks, 1998b), it is also an important incubation condition that affects the rate of development and metabolism of embryos (Ewert, 1985). The lower temperatures experienced further north in Ontario are assumed to limit the northern extent of several species' range although, there is no evidence that the survival and reproductive output of adult turtles is affected by cooler ambient temperatures experienced further north (Bobyn and Brooks, 1994a). In areas with relatively higher temperatures, embryos grow faster and the length of incubation decreases (Yntema, 1976; Packard *et al.*, 1987). Therefore, the cooler conditions and shorter growing season along Eastern Georgian Bay probably limit turtle species in Ontario by reducing recruitment via unfavourable incubation conditions (Bobyn and Brooks, 1994a). In a study comparing a northern (Algonquin Park) and southern (Cootes Paradise) population of Snapping Turtles, eggs collected further north were not better adapted to the lower incubation temperatures as was hypothesized (Bobyn and Brooks, 1994a). However, differing growth rates of hatchlings from populations further south in the United States reared in the same conditions suggest there is a genetic component to hatchling success.

Subsurface temperatures experienced during incubation not only determine successful development of turtle embryos, but also the sex of the hatchlings for those species which experience temperature-dependent sex determination during embryonic development (Mrosovsky and Pieau, 1991). Pivotal temperatures, or temperatures that when held at a constant during the thermosensitive period of embryo development produce 50% individuals of each sexual phenotype, vary among species (Mrosovsky and Yntema, 1980; Mrosovsky and Pieau, 1991). A constant temperature of 28.9°C is estimated to yield both sexes in equivalent proportions for species of red-eared sliders (Godfrey *et al.*, 2003; Delmas *et al.*, 2008). For Snapping Turtles, it has been shown that temperatures of 25.6-26°C produce entirely male clutches (Packard *et al.*, 1987; Brooks *et al.*, 1991) while temperatures less than 22°C, or greater than 28.6°C produce entirely female clutches (Packard *et al.*, 1987; Brooks *et al.*, 1991). Incubation temperatures less than 21.1°C result in stunted development or hatchling death (Bobyn and Brooks, 1994a). It is suggested that the pivotal temperature of Blanding's Turtles is 29°C (Packard *et al.*, 1982a; Gutzke and Packard, 1987) as well as for Northern Map Turtles (Janzen *et al.*, 1995). Although this is important, daily fluctuations in temperature are often experienced in the field and may lead to contradicting results. For instance, a study by Packard *et al.* (1991) increased temperatures 1°C per hour during the day to attain a daily maximum near 30.5°C and lowered temperatures at a similar rate in the evening to a minimum near 20°C during a laboratory experiment on clutches of Painted Turtles. All the surviving hatchlings were identified as male which suggests daily temperature fluctuations in the natural environment may yield unexpected results, despite spanning a wide range of pivotal temperatures.

3.1.2.2 Moisture

Turtles are oviparous reptiles but variations in type of eggshell is widespread, particularly amongst those which exist in Ontario (Iverson and Ewert, 1991; Janzen *et al.*, 1995). Rigid shelled eggs of some turtles, such as the Spiny Softshell Turtle, are minimally affected by differences in substrate moisture levels as their hard mineral exterior is less permeable than flexible-shelled eggs (Packard 1999; Thompson and Speake, 2004; Zhao *et al.*, 2013). Consequently, embryonic development in hard-shelled eggs relies on the availability of water in the eggs at the time of oviposition and is generally insensitive to the external hydric environment (Packard 1999; Booth, 2002; Zhao *et al.*, 2013). In comparison, embryo development in pliable-shelled eggs is affected by changes in the hydric environment during incubation (Packard, 1999; Zhao *et al.*, 2013). The moderately flexible shelled eggs of Blanding's Turtles and truly soft-shelled eggs of Painted Turtles and the common Snapping Turtle are particularly sensitive to environmental moisture conditions (Packard, 1991). Moreover, the shallow nest cavities turtles dig (Ernst *et al.*, 1994; Riley and Litzgus, 2013) are susceptible to the generally unpredictable drought and flooding events during a given incubation period

(Plummer, 1976; Cagle *et al.*, 1993; Kam, 1994; Delmas *et al.*, 2008). Studies show that substantial water uptake occurred during the first third of development but was independent of soil moisture (Packard, 1999; Delmas *et al.*, 2008) but became particularly sensitive to the changes in the hydric environment during the last third (Gutzke and Packard, 1987; Rimkus *et al.*, 2002; Delmas *et al.*, 2008). The results of these studies are varied depending on study species and whether they were conducted in the lab or the field. It has been found that moister nests induced longer incubation, as did extremely dry nests (Cagle *et al.*, 1993; Zhao *et al.*, 2013; Bodensteiner *et al.*, 2015), but yielded higher hatching success and larger hatchlings than drier nests (Cagle *et al.*, 1993, Packard, 1999; Packard *et al.*, 1982a, 1982b, 1987; Morris *et al.*, 1983; Packard and Packard, 1988; Brooks *et al.*, 1991). Kam (1994) demonstrated that hatching success of the Florida Red-Bellied Turtle (*Pseudemys nelsoni*) decreased and incubation time increased as the duration of submergence in water increased from one to three days. They found that 26% of the eggs submerged for 1 day and 4% of eggs submerged for three days survived whereas no eggs hatched from the 6-day submergence group. Similar studies also suggest short periods of inundation (2-6h) yield greater hatching success in reptile eggs at all ages and greater mortality for older embryos exposed to 12h of inundation (Joanen *et al.*, 1977; Kam, 1994). Although some studies found that some features such as egg size were not influenced by water potential (Janzen *et al.*, 1995), genetic variation amongst the eggs collected for the experiment may have caused biased results. Differences in hatchling size have also been attributed to genetic factors and parental effects in other studies, independent to substrate moisture (Janzen, 1993; Bobyn and Brooks, 1994b). Janzen (1993) also demonstrated that although larger hatchlings of Snapping Turtles had greater survival in the wild, the hydric environment during incubation did not affect hatchling survival independently of its effects on hatchling size (Bobyn and Brooks, 1994b). Furthermore, evidence that larger eggs reflect better 'quality' (*i.e.* hatchlings have greater fitness, growth patterns, and prospects for survival) is highly debated (Froese and Burghardt, 1974; Brooks *et al.*, 1991; Congdon *et al.*, 1999; Delmas *et al.*, 2008). A study by Bobyn and Brooks (1994b) on Snapping Turtle eggs in Ontario demonstrated how although hatching success was higher in moist substrates, posthatching survival and growth were not affected by the hydric environment of the nest during incubation. On the other hand, Finkler (1999) found that Snapping Turtle hatchlings incubated at greater moisture contents could withstand exposure to desiccating conditions longer than their dry-treatment counterparts suggesting wetter conditions during incubation may enhance their ability to survive prolonged exposure to drought conditions in the field, a likely situation depending on the proximity of the nest relative to cover or water. Sexual determination based on the hydric conditions during incubation has been proven both true (Paukstis *et al.*, 1984; Packard *et al.*, 1991) and untrue (Packard *et al.*, 1991). However, this remains an unanswered question as discrepancies between these studies such as mis-identified sexual phenotype and inability to replicate conditions exactly may have occurred.

3.1.3 Factors Affecting Incubation Conditions

3.1.3.1 Depression Shape Characteristics as it relates to Fill and Spill

The characteristic rock barrens of the Canadian Shield are speckled with shallow microhabitat features known as moss-filled depressions, dispersed amongst portions of deeper organic deposits and forests (Jalava *et al.*, 2005). These organic filled bedrock features, which can range from small depressions to large wetlands, play an important role in storing water in northern catchments (Spence and Woo, 2002, 2003, 2006). A "fill and spill" approach can be applied to this landscape to better understand the large cumulative storage capacity of these hydrologically connected systems (Spence and Woo, 2006). Essentially, once a depression's water retaining capacity is met, any additional water supplied by rainfall or overland flow may contribute to fill depressions further downslope with the eventual discharge out of the catchment outlet (Spence and Woo, 2003, 2006). The amount and hydrophysical properties - effective porosity, bulk density, and specific yield - of material within the depressions will dictate the capacity to store water. However, although empty depressions may theoretically have the greatest potential to store water, it is unrealistic to expect their hydroperiods to last very long, especially in the summer (Altermatt *et al.*, 2009). Therefore, it is important to consider evaporative rates, water demands of the vegetation, and moisture retention capabilities of the vegetation when assessing the water storage potential (Brooks and Hayashi, 2002). This has direct implications on habitat suitability for reptiles because their semi-aquatic nature requires that they avoid desiccation by using habitat with sufficient water availability.

3.1.3.2 Canopy Openness

In oviparous reptiles, such as turtles, there have been a number of laboratory based studies that have demonstrated how variable thermal and hydric conditions can influence embryo development and offspring size, shape, sex, and performance (Bull, 1980; Burger, 1989; Qualls and Andrews, 1999; Pike *et al.*, 2010). However, there has not been much attention paid to the variability in thermal and hydric conditions experienced during natural incubation and what factors may influence a female's decision to select certain nesting sites over others (Pike *et al.*, 2010). Canopy openness is one such natural variable which affects the amount of solar radiation reaching the ground surface and thus affects the subsurface temperatures of nesting microhabitat (Pringle *et al.,* 2003; Elzer *et al.*, 2013). Past research on maternal site selection has shown preferences based on temperature (Weisrock and Janzen, 1999; Kolbe and Janzen, 2002) and moisture (Packard, 1991). However, these studies often focus on characteristics of nest sites that likely explain less variation in nest temperatures than canopy openness, including slope, substrate type and distance from vegetation (Doody *et al.*, 2006a). Furthermore, when canopy structure is assessed as a habitat trait, it is often estimated by eye and categorized subjectively as ‘shaded’ or ‘unshaded’ (Pringle *et al.*, 2003). This qualitative assessment of habitat provides little to no insight on the effect of canopy openness on nests of reptiles with temperature-dependent sex determination. For these species, unnoticeable differences in canopy openness can have substantial effects on hatchling development and sex determination (Doody *et al.*, 2006a). Furthermore, the distribution of canopy gaps relative to the location of the sun throughout the day can lead to substantial differences in solar radiation which reaches the surface for sites with identical canopy openness (Pringle *et al.*, 2003). Studies by Pringle *et al.* (2003) and Doody *et al.* (2006b) have demonstrated that canopy openness is a successful predictor of incident radiation intensity at the nest site and thus temperature differences experienced between sites. Furthermore, it was demonstrated that openness and radiation intensity had a stronger influence on daily temperature fluctuations in the nest as opposed to mean nest temperatures (Doody *et al.*, 2006b).

3.1.4 Hypotheses

Using a process-based approach, various moss-filled depressions, or moss cushions, within the Northern Ontario Barrens Ecohydrological Landscape (NOBEL) were characterized in 2016 to assess the suitability of this type of microhabitat as potential nesting locations for turtle SAR on open rock barrens. The differences between microtopographic features of hillslope and hilltop locations were examined using photogrammetry. It was hypothesized that hillslope locations would be more prone to flooding due to a greater contribution of water from the upland catchment area as well as direct rainfall. Due to the concern of extended periods of saturation causing death of developing embryos, the moisture dynamics of two continuously monitored sites was assessed to gain a better understanding of the responsiveness of this type of habitat to rain events. This analysis was coupled with continuous temperature measurements over realistic incubation periods for turtles in this region to characterize the temperature dynamics of moss-filled depressions. Canopy openness and temperature measurements were compared to determine the effect of shading on the organic-filled depressions. It was hypothesized that regardless of canopy openness, the organic filled depressions would experience similar temperatures at the same depths due to the advection from the surrounding rock.

3.2 Methods

3.2.1 Study Area

The research for this study was completed during the spring and summer of 2016 within the NOBEL near Dinner Lake in Carling Township (CAR; Figure 2.1). Aside from being located relatively close to a road (HWY 69), the sites throughout this area are generally isolated from human disturbance. Perhaps most striking about this site is the extensive open rock barrens that have since remained exposed since the last ice age, when the Laurentide glacier scoured the surface of the Canadian Shield, scraping vast amounts of glacial deposits from the surface of the shield and depositing them in areas further south (Kor *et al.*, 1991; Kor and Cowell, 1998; Jalava *et al.*, 2005). The bedrock topography in the rocky outcrops of Eastern Georgian Bay is generally flat with low, rolling gneissic rocklands dipping slightly to the southwest (Jalava *et al.*, 2005). The thin or non-existent soils on this wind and ice-scoured landscape is contrasted by the dense forest often encountered further inland (Jalava *et al.*, 2005). The open rock barrens along the coast and interior of the Georgian Bay Biosphere Reserve (GBBR) consist of a mosaic of open rock, covered with pioneer bryophytes, low shrubs and graminoids such as lichen, moss, blueberry plants, juniper bushes, and other shrubs. Varying abundances of treed rock barrens are also common in the interior rocklands, dominated almost entirely by Jack Pine (*Pinus banksiana*). Conditions on the coastal and interior rocklands are harsh due to the frequent high winds and greater temperature fluctuations resulting from higher rates of evapotranspiration than in the surrounding forests.

These rockland habitats are beneficial to reptiles which require energy from the sun to fulfill their physiological needs. Not only do the open rock barrens provide sufficient areas for reptiles to bask, but they also retain heat from solar radiation which is absorbed by the granitic bedrock and can slowly release warmth to allow reptiles to thermoregulate more efficiently when open basking is not ideal, for instance in the spring, fall, and cool summer nights (Clauser, 2011). Moreover, the shallow rock depressions and rockland pools become saturated after rainfall or during the spring when meltwater is abundant (Didemus, 2016). This creates small ‘islands’ of suitable habitat which vagile reptiles can use during the active season as they move in search of mates, appropriate nesting and gestation sites, and staging areas. Similar to the metapopulation theory, these organic islands connect an otherwise barren landscape and allow these species to extend their home range (Attum *et al.*, 2008). However, later in the summer, these rockland pools dry out substantially when drought desiccates the rock barrens. Few plant species are adapted to such seasonal fluctuations of temperature and water availability and have become a characteristic feature of these rock barrens.

Polytrichum species comprise the majority of moss cushions at this site and were observed to be the dominant genus on this landscape. However, *Sphagnum* and lichen of the genus *Cladonia* are also well represented on the characteristic rock barren landscape of the GBBR where the Canadian Shield is exposed at the surface. The barren rock surface is also dominated by crustose lichen which colonizes in small patches but collectively covers vast areas on granite rock surfaces. The rock barren surface at CAR is relatively flat and smooth. The open rock landscape is divided by patches of forest habitat which act as vegetative ‘islands’ either directly on the rock surface or located in depressions infilled with soil of variable thickness.

Climate normals for the last 30 years (1981-2010) at two weather stations in the vicinity of Eastern Georgian Bay (Parry Sound Coast Guard and Dunchurch) demonstrate an average temperature in this region for the summer months of June, July and August is 17.6°C and an average precipitation throughout the summer is approximately 80.1 mm (Environment Canada, 2017). The summer of 2015 was an extreme drought year when the region only received approximately 40mm of rain throughout the summer and an average temperature of 19.5°C. The year this research was conducted was slightly warmer, 21.6°C on average, and received slightly more rain (56 mm).

3.2.2 Site Selection

Sites were selected based on openness, and isolation from connected organic deposits and larger hydrological units. The interactive range maps developed by Ontario Nature using information from the Reptile and Amphibian Atlas were used to validate site selection based on presence/absence of this species at our study site (Ontario Nature, 2017). The geographical area surrounding each site based on the UTM grid system indicates sightings of these species before and after 1970 (Ontario Nature, 2017). We also randomly encountered multiple Painted Turtles and one

Blanding's Turtle during the 2016 and 2017 field seasons. Due to threats of the pet trade, the exact location of the sites cannot be disclosed.

Two sites were instrumented to collect continuous data on temperature and moisture conditions. Manual measurements of temperature were conducted at five other sites. Including these locations, 34 other small organic filled depressions (<10 m^2) scattered within a 1 km radius from the center of the CAR study area were selected to have photogrammetry analysis performed on them. All sites were entirely composed of *Polytrichum* moss and *Cladonia* lichen. Using photogrammetric methods, the microtopography was assessed to determine the water storage dynamics of the representative nest sites. Continuous temperature was collected to capture temporal changes in subsurface conditions at a few sites while manual weekly temperatures captured spatial differences. The canopy openness was also assessed at each site.

3.2.3 Microtopography Photogrammetry Analysis

Close-range digital photogrammetry has been used in a number of applications to collect data on the three-dimensional properties of surface microtopography (Westoby *et al.*, 2012). The methods described here have been modified from Westoby *et al.* (2012) but follow the same basic principles of both field and post-processing analysis. The general workflow of photogrammetry applied here starts with acquiring a photoset for each site, then processing the images using 'Structure-from-Motion' methodology to create a 3-D structure from the series of overlapping, offset images collected in the field (Westoby *et al.*, 2012). Image processing occurs in two stages, utilizing two types of software that take the image data and reconstruct the 3-D image to create a Digital Elevation Model (DEM).

3.2.3.1 Photogrammetric Survey Methods

The effectiveness of this technique of close-range photogrammetry is dependent on the small scale of sites for which this type of analysis can be performed on. Therefore, sites were selected that were less than 10 m^2. Before images were taken, the dimensions of the square or rectangle that encompassed the entire moss depression were measured and the four corners were marked with a red poker chip. Coloured poker chips were selected to serve as 'keypoints' because they could be automatically identified over all scales and locations in each image during photo analysis (Westoby *et al.*, 2012). 20-30 coloured poker chips were evenly distributed within the identified area and placed flat on the moss surface. A small box of known dimensions was also placed on the surrounding rock but within the area of interest. The box was also levelled and orientated with one designated side facing north for use during post-image processing.

Once finished prepping the site, photos were taken with a Nikon CoolPix P600 camera using automatic settings by walking around the square/rectangular plot two times, at two different angles; the camera was held at an angle of approximately 45 degrees during the first set of photos and at a more vertical and downward angle the second time around. The photos were taken from a standing distance of approximately two meters from the edge of the plot which allowed the entire area of interest to remain in the middle of the frame. In total, approximately 100-150 photos were taken at each site.

In order to determine the bedrock topography below the moss cushions, a manual depth survey was conducted at each site. Using the same plot designated by the four corners of the photogrammetry survey, yard-tapes were used to measure 10 cm intervals along the x and y axis of the plot. The NW corner of the plot was designated as point (0, 0) while the NE side of the plot was the x-axis. At every 10 cm interval along the x-axis, starting at (0, 0), the depth of organic matter, or moss, was recorded at each 10 cm interval along the y axis, while the exact distances of all the moss edges from the y-axis were recorded as well.

3.2.3.2 Post Image Processing and MATLAB Analysis

The photos of each site were loaded into the VisualSFM software. The ‘keypoints’ are simultaneously detected and matched in all the images using approximate pairwise image matching (Wu, 2011). During this process, images that were not suitable for point-cloud reconstruction were automatically discarded before 3-D reconstruction begins. Once finished, a sparse reconstruction point-cloud was generated followed by an enhanced density point-cloud derived by applying the

Clustering View for Multi-view Stereo (CMVS) and Patch-based Multi-view Stero (PMVS) (Furukawa and Ponce, 2007; Furukawa *et al.*, 2010) algorithms which need to be downloaded as a separate package. The final point-cloud that was produced from this additional processing had a higher point density that increased the final DEM resolution.

The dense reconstruction model created from VisualSFM for each site was loaded into MeshLab, a point-cloud processing software (available for free download from www.meshlab.net/#download). During this processing stage, the point-cloud was cropped based on the location of the four red poker chips, representing the four corners of the plot. A copy of the cropped point-cloud was saved along with a cropped point-cloud of just the box of known dimensions that was levelled and placed within the plot during the field photogrammetry survey. Using the measure tool, the shape adjustment factor of the point-cloud was calculated from the box which was utilized during later analysis (Cignoni *et al.*, 2008). The cropped point-cloud was then loaded into the processing software CloudCompare (available for free download from www.danielgm.net/cc/). Here, the cloud was scaled appropriately to reflect the actual dimensions captured in the field (Girardeau-Montaut, 2011). Once transformed accordingly the x, y, and z data for each of the four corners were recorded to be used later during DEM analyses.

The next stages of analysis utilized the MATLAB software to create DEMs of the surface and underlying bedrock topography (MATLAB R2015a; R2017a, The Mathworks). First, a MATLAB function was used to create a levelled surface DEM from the point-clouds created in Meshlab. Data from the manual depth surveys was then loaded into the program and essentially subtracted from the photogrammetric-derived DEMs to create a 'rock' DEM of just the bedrock topography. At this stage, three separate DEM's had been created for each site: a photogrammetric-derived 'pit' DEM of the entire plot, a 'moss' DEM created from the manual depth survey data collected in the field, and a 'rock' DEM from the subtraction of the 'moss' from the 'pit' DEM. An example of these DEMs for a hillslope site are shown in Figure 3.1.1, and a similar example for a hilltop site is provided in Figure 3.1.2. The hillslope locations were identifiable by a decrease in elevation from one side of the plot to the other (Figure 3.1.1) whereas hilltop locations were surrounded by areas of equally high elevation (Figure 3.1.2). In order to determine storage characteristics of the moss-filled depressions, the TopoToolbox - which includes a set of functions for topographic analysis of DEMs - was utilized in MATLAB (Schwanghart and Kuhn, 2010). This enabled the calculation of depression storage (DS) of the moss-filled depression as well as moss volume (MV). The surface area of each moss-filled depression was also calculated using MATLAB.

Post-processing calculations were performed on the values of DS and MV in order to determine volume of water storage within the moss, or moss water storage volume (MWSV), below the pit sill and the depth of water required to fill up, or flood, the moss-filled depression. To calculate MWSV, the average specific yield of *Polytrichum* moss samples, 0.38, was applied to the DS. The specific yield was measured from samples collected at each site. The MWSV was then divided by the area of the moss-filled depression to calculate the depth of water required to fill the depression up to the sill, assuming it was at field capacity and the ratio of water input from rainfall and runoff was equal to one.

3.2.4 Continuous Data Collection

Two sites, 425 and 426, were instrumented on May 29, 2016, with continuous logging equipment to collect data on subsurface temperature and moisture conditions. Data was recorded using a Campbell Scientific CR10X data logger which was programmed to collect data every 15 minutes. Rainfall was measured at a nearby site using a tipping bucket rain gauge (TE525M – Campbell Scientific, Logan, Utah) mounted 1 m above the moss surface.

Moisture and temperature were measured at two locations within the same site, totalling four profiles between the two sites. Volumetric water content was measured using Campbell Scientific CS616 moisture probes (Campbell Scientific, Inc., Logan, UT). The CS616 probe length was 30 cm, and the probes were installed horizontally at depths of 5 and 15 cm relative to the surface. Each moisture profile also had an adjacent temperature profile installed. Temperature profiles were measured in each moss profile at each site using T-type thermocouple wire (Omega Engineering, CT, USA) inserted at depths of 1, 5, and 15 cm. The depth of each profile varied but generally represented the maximum depth of the entire moss-filled depression. The shallowest location was at 426B with a depth of 25.5 cm and the deepest was 425D at 35.2 cm. 426A was 29.4 cm and 425C was 25.7 cm deep. Air temperature was recorded using the same type of thermocouple attached to the underside of the datalogger enclosure to ensure it would not receive direct sunlight. Volumetric water content (VWC) was calculated using the same methodology outlined in Hansson and Lundin (2006). A porosity value of 0.73 (Didemus, 2016) was applied to the deeper moisture measurements (15 cm) whereas a porosity value of 0.97 (Redding and Devito, 2006) was applied to the near surface measurements (5 cm). The percent saturation was calculated by dividing VWC by the porosity value associated with each depth.

3.2.5 Canopy Openness and Manual Temperature Measurements

3.2.5.1 Field Methods

Analysis of canopy structure and the effect on subsurface temperature required the collection of hemispherical photographs of canopy openness and measurements of temperatures at each location. Temperature measurements were collected on a weekly basis and concentrated after rainfall events for simultaneous collection of moisture measurements. Measurements were conducted throughout the day sometime between 9:30-14:00 depending on when the sites could be visited. To try and keep measurement times consistent, the same sites were measured in the same order each day to try and best represent a similar time frame from previous days of measurement. Temperature was measured using an Oakton WD-35626-10 handheld thermometer with a thermistor temperature probe that was inserted vertically into the ground at 1, 5 and 15 cm. Some sites were less than 10 cm deep and therefore no data were collected at depths greater than 10 cm. A flag was used to mark the exact location that the measurements were taken to ensure the same spot was measured each week. Furthermore, this was also used to designate where to take the hemispherical photographs to assess canopy openness. Canopy openness was measured by taking photographs using a Sunex 185° SuperFisheye 5.6 mm F/5.6 lens on a Canon EOS Rebel DSLR camera. The camera was pointed with a designated mark on the lens facing north and placed on the forest floor on a levelled surface. For detailed methodology, see Doody *et al.* (2006a).

3.2.5.2 Gap Light Analyzer Image Processing

The hemispherical photographs were processed manually using the Gap Light Analyzer (GLA) 2.0 software. User defined configurations were created for each site whereby the latitude, longitude, and elevation of each site was manually inputted using data from a Garmin eTrex 10 GPS unit. The growing season length was set from May 31 until September 1 to approximate the length of incubation (Doody *et al.*, 2006a). Following the same methodology as Doody *et al.* (2006a) which adopted the same properties as Iqbal, 1983, the cloudiness index (Kt) was calculated for each month (Current Results, 2017; National Research Council Canada, 2017). However, there was no statistical difference between the value we calculated and the program's default settings (Kt = 0.5). The default settings of all other inputs within each site configuration were used (Doody *et al.*, 2006a). Once the image was configured according to the appropriate settings, the blue channel pixels were extracted from the image colour plane as plants do not reflect the blue wavelengths well whereas the sky does (Zhang *et al.*, 2005). A threshold value was then selected to convert to a 1-bit black-and-white image that best captured the surrounding canopy. The software then calculated the proportion of black and white pixels to determine the canopy cover and openness (Frazer *et al.*, 1999).

3.3 Results

3.3.1 Photogrammetry

Results from the MATLAB analysis of the photogrammetry performed on the 22 hillslope and 12 hilltop locations are summarized in Table 3.2. All ± values are one standard deviation from the mean. On average, the hillslope locations were smaller in area (1.38 m^2 ± 1.24) and therefore had lower values of DS (3.92 E-03 m^3 ± 7.80E-03) and MV (7.14E-02 m^3 ± 1.35E-01) than the hilltop locations, with respective values of area, DS and MV as follows: 3.80 m^2 ± 3.72; 8.01E-03 m^3 ± 6.39E-03; 1.89E-01 m^3 ± 2.31E-01. Results of those parameters mentioned previously were also more variable for the hilltop locations than those of the hillslope locations, shown in Figure 3.2. The results of all other parameters were generally greater for hilltop sites than hillslope sites, including the volume of moss water storage below the sill, moss above the sill, and depth of rain required to flood the organic filled depression (See Table 3.2). The only variable for which this was not true was the depths of moss, both measured and calculated, at the hilltop sites which were shallower than those of the hillslope sites. Some discrepancies between the average depth of moss calculated from the MATLAB analysis and those measured in the field were observed due to the interpolation of depths between points to create a continuous moss DEM.

3.3.2 Rainfall and Continuous Moisture

Climate normals of the last 30 years (1981-2010) show that the historical average precipitation for the months of June, July and August is 80.1 mm. Including September and October, the average historical precipitation is 94.4 mm (Environment Canada, 2017). The rainfall data collected this summer was slightly less than the 30 year climate normals. The average monthly precipitation in June, July and August was 76 mm. Including September and October, the average is roughly 75.4 mm. There was a large rainfall event on August 30, totalling 65.1 mm which is why the average monthly precipitation calculated for June, July, and August is so high. The historical 30 year average precipitation in August is only 87.7 mm whereas in the summer of 2016, 139.5 mm of rain fell in August, the greatest monthly precipitation from June to October. Otherwise, the summer of 2016 could be considered a drought year. Including the single large rainfall event on August 30 of 65.1 mm, there were 67 days in total that received rain between June 1 and October 31. 44.8% of these rainfall events were less than or equal to 1 mm. 31% were between 1 and 5 mm. 11.9% of the total days of precipitation received between 5 and 20 mm of rain whereas 10.4% received between 20 and 30 mm of rain (Figure 3.3.1a; 3.3.2a).

The average percent saturation of each profile was greater at 15 cm depth than at 5 cm depth (Figure 3.3.1b, c; Figure 3.3.2b, c). More frequent and severe rainfall events were observed during the months of September and October which led to an increase in average percent saturation at all sites at both depths of 5 and 15 cm. Furthermore, the average difference between the percent saturation at 5 and 15 cm throughout the entire study period increased as depth of profile increased meaning the shallower the profile, the more similar the moisture at increasing depths. In other words, the percent saturation at 5 and 15 cm were closer in the shallowest profile (average difference of 0.14 percent saturation ± 0.13) than the deepest profile which had the greatest difference in values at 5 and 15 cm (average difference of 0.45

percent saturation ±0.21). Another observed pattern was the responsiveness to rainfall events which is more apparent during the months of September and October. In this context, responsiveness refers to the change in high and low percent saturation values experienced at each depth separately following a rainfall event. Excluding profile 426B (Figure 3.3.2b) which was located near a small tree, the shallower site 425C was much more responsive to rainfall events in September and October (Figure 3.3.1c) than the deeper profile, 425D (Figure 3.3.1b). The moderately deep profile 426A (Figure 3.3.2c) appeared less responsive than 425C but more responsive than 425D.

The percent saturation of each profile at depths of 5 and 15 cm were assessed regarding their response to rainfall events during dry and wet time periods. A dry period occurred between June 11 and July 1, 2016 (20 days) whereas the wet period was between September 11 and September 26, 2016 (9 days). These time periods are designated by light and dark sections, respectively, in Figures 3.3.1 and 3.3.2. The difference between the maximum and minimum percent saturation during this time was then divided by the number of days between both dates to determine the rate of change during each period (Figure 3.4). In all cases, the rate of change of percent saturation was higher at 15 cm depth than 5 cm for each site during both wet and dry periods. At sites 426B, 425C, and 426A, the rates of change at 5 and 15 cm depth during the wet period were greater than those during the dry period

(Figure 3.4). Profile 425D was the only location where the rate of change during the dry period at each depth was greater than those experienced at the same depth during the wet period (Figure 3.4). The only pattern that emerged with increasing profile depth was during the dry period at 15 cm depth. During this time, percent saturation rate of change per day increased as the overall depth of the profile increased. For example, the shallowest profile, 426B (25.5 cm), had the smallest rate of change at 0.015 day^{-1} whereas the deepest profile, 425D (35.2 cm), had the largest percent saturation rate of change at 0.035 day^{-1}.

3.3.3 Continuous Temperature

The average temperature experienced at various depths within four profiles at two sites was analyzed based on start date and the number of days that eggs would hypothetically be incubated. June 1 to July 15 were selected as incubation start dates based on a review of the literature whereby other populations of turtles nested between similar time periods in northern Ontario (Litzgus and Brooks, 2000; Edge *et al.*, 2010; Rasmussen and Litzgus, 2010; Millar and Blouin-Demers, 2011; Markle and Chow-Fraser, 2014). The maximum date of incubation was set at October 31. We did not assess the overwintering conditions within the representative nest sites although we do acknowledge that some turtles remain in the nest once hatched over winter if conditions outside the nest are not favourable for their emergence and survival (Packard *et al.*, 1999). Of these four profiles, 425D and 426B had the greatest number of days with the warmest temperatures and the greatest number of days with coolest temperatures, respectively. Figure 3.8.1 depicts the average temperatures experienced from June 1 (day number 152), until July 15 (day number 197) at site 425D for the specified depths. Similarly, Figure 3.8.2 depicts average temperatures experienced over the same time period and depths at site 426B. Within each profile, the warmest and coolest daily temperatures were experienced at 1 cm depth. The maximum temperature at 425D was 24.0°C, which occurred on July 13, and 23.9°C at 426B on July 7. The minimum temperature at 425D was 12.9°C and 9.9°C at 426B which both occurred on June 8. The maximum daily temperatures (duration of 1 day) decreased with depth whereas the minimum daily temperatures increased with depth.

All profiles had the same pattern whereby incubation lengths with higher average temperatures over the entire incubation period decreased as the day of year increased. That is, mean temperature during the incubation period decreased as the summer progressed. In general, there was a greater variety of temperatures and fluctuations that occurred at 1 cm depth. This variability decreased as depth increased which is shown in the respective isotherms for each profile in Figure 3.8.1 and 3.8.2.

Although extremely variable depending on species, microclimate conditions, and substrate, the general length of incubation for freshwater turtles is 90 days

(Congdon *et al.*, 1987; Doody *et al.*, 2006a). Furthermore, depths of nest cavities are realistically greater than the depths measured here. However, eggs deposited last will be closer to the surface and may experience temperatures at the depths specified here. Within all four profiles, the maximum average temperatures experienced at 5 cm and 15 cm were consistently below 22°C. At both sites, the smallest period of time where such maximum temperatures were reached occurred at 15 cm depth.

The daily maximum and minimum values were identified from each of the four profiles as well as the difference between the two to determine how variable temperatures at each depth were. A one-way analysis of variance (ANOVA) was performed after which a multiple comparison of the means was applied to the results to identify differences between depths and months. For the months of June, July and August, maximum daily temperatures of the air and at 5 cm depth were not significantly different from each other, but differed from temperatures at 1 cm and 15 cm depths (F(19) = 155.98, p-value <0.001). All temperatures were statistically similar in the months of September and October (Figure 3.9a). As depth increased, the variability of temperatures each month also decreased with depth, demonstrating how consistent temperature conditions are experienced at greater depths (Figure 3.9a). Regarding the minimum daily temperatures, there was no significant difference between the air, 1 cm and 5 cm depths for the months of June, July and August (Figure 3.9b; F(19) = 86.15, p-value <0.001). Furthermore, the minimum daily temperatures for each month generally increased with depth suggesting that higher temperatures are maintained at depth. Similar to the maximum daily temperatures, variability in minimum daily temperature decreased with depth. With both the maximum and minimum temperatures, the greatest temperatures were experienced in August, as demonstrated in Figure 3.9(a) and (b).

Regarding the differences in maximum and minimum temperatures each day at sites 425 and 426, the averages are presented in Table 3.3. Generally, as depth increased, the differences between daily maximum and minimum temperature decreased, which has been documented in other cases (Packard *et al.*, 1985). This same pattern is true for variability of average differences between maximum and minimum temperatures, evident by the decreasing standard deviations with depth for each month (Table 3.3). Furthermore, the smallest differences between maximum and minimum temperatures at all depths occurred in October (Table 3.3). The greatest average difference between maximum and minimum temperature occurred at 1 cm depth in June (26.9°C ± 5.9). The smallest average difference between maximum and minimum temperature occurred at 15 cm depth in October (1.7°C ± 0.26). The mean differences between the maximum and the minimum of the air most similarly reflect those at 5 cm depth (Table 3.3).

3.3.4 Canopy and Manual Temperature

Combined with four continuously monitored profiles at sites 425 and 426, canopy openness and temperature was assessed at nine locations in total. Following the methodology specified by Doody *et al*., (2006a), the total transmitted radiation incident on the surface (TTR) (MJ m^{-2} day^{-1}) was determined for each location using the GLA software and compared to the canopy openness (Figure 3.5). Similarly to Doody *et al*. (2006a), we also observed an increasing trend of TTR with canopy openness based on a second order polynomial (R^2=0.962). Since it could be measured directly, canopy openness was used to address differences between temperatures at depth, as opposed to TTR. Each site, and their respective canopy openness measurements, are summarized in Table 3.1. There were positive linear correlations between canopy openness and average temperature at each depth (Figure 3.7). The greatest variability was experienced at 1 cm depth (R^2 = 0.14), and decreased at 5 cm and 15 cm depth (R^2 = 0.47 for both). This is also evident in Figure 3.6 which shows the temperatures for each depth (1, 5 and 15 cm) in order of increasing canopy openness. Sites 304 and 319 were not deep enough to obtain a 15 cm depth measurement and therefore are not show. A one-way ANOVA performed on the results from the multiple comparison of the means showed that there was no significant difference between temperatures at the same depths for all sites except at 1 cm. Sites 426A and 425C were statistically different from 426B (F(24) = 6.49, p-value <0.001; Figure 3.6).

3.4 Discussion

3.4.1 Hilltop and Hillslope Moss-Filled Depressions

The moss-filled depressions used for this study were selected based on their isolation on the rock barrens and the increased accuracy that could be achieved using the post-processing methodology. Therefore, the characterizations were biased towards isolated and relatively small moss cushions as opposed to expansive carpets. Although these relatively small and shallow moss-filled depressions may not support turtle nests, this research provides a necessary first step to understanding the geomorphology of this type of habitat on hilltop and hillslope locations. Few studies have elaborated on the physical characteristics of freshwater turtle nests beyond simple descriptors of substrate type and depth. Moreover, even fewer have identified key nest site characteristics on open rock barrens along Eastern Georgian Bay.

The initial hypothesis was that moss cushions located on the hillslope would be more susceptible to flooding following rainfall events due to the contribution of water from rainfall as well as overland flow. On the other hand, hilltop locations would have a small catchment contributing to the water input and therefore would be less susceptible to flooding. We found that the amount of water required to flood the hillslope locations was smaller than that of the hilltop locations, thus supporting our hypothesis. The average DS of the hilltop locations was greater than that of the hillslope locations which explains why the hillslope locations are more susceptible to flooding. There were no differences in substrate type among all the sites meaning the difference in geomorphology was the only predictor of differences in the depression characteristics, summarized in Table 3.2. The average ratio of moss volume to pit area amongst all the sites was 5.07 which speaks to the fact that depression storage at the sub-meter scale of which these depressions were surveyed at is quite small. In other words, it appears the moss cushions are not situated in bowl like depressions as previously hypothesized. Therefore, due to the lack of substantial variation in the bedrock topography at the sub-meter scale, and the nature of the moss to colonize on flat surfaces, the majority of isolated cushions are not suitable nesting sites. Of the few documented cases of turtles nesting on rock outcrops, it may be that turtles select more crevasse like depressions for nesting (Litzgus and Brooks, 1998b; Litzgus and Brooks, 2000; Markle and Chow-Fraser, 2014). However, the hillslope or hilltop classification of such sites and differences in their ability to drain depending on depth and horizontal extent is important when considering the flooding potential of these sites. Future studies should document geomorphological features of nests sites and the surrounding topographic characteristics such as slope and aspect to gain a better understanding of the limited type of nesting habitat used on rock barrens.

3.4.2 Subsurface Moisture Dynamics

Studies have shown that varying degrees of exposure to flooding events and moisture conditions can have significant impacts on the survival of the developing turtle embryos (Cagle *et al.*, 1993; Packard *et al.*, 1999; Delmas *et al.*, 2008). Periods of saturation ranging from 6 to 12 hours have been shown to alter the hatching success of turtles (Joanen *et al.*, 1977). Moreover, extended periods of 100% saturation at and beyond 24 hours leads to death of the embryos (Kam, 1994). Therefore, understanding the subsurface moisture conditions within nesting habitat is key to understanding response to rainfall and potential flooding. Except for 426B, all locations experienced times when the site was close or equal to 100% saturation for short periods of time.

Initial moisture conditions within the cushions would also impact their response to rainfall events due to the differences in amount of water required to flood. In all cases, rainfall events equal to or greater than 10 mm caused the percent saturation at 15 cm at all sites to increase to 100%. Of the 67 rainfall events that occurred throughout the summer of 2016, only 16% were greater than 10 mm. However, for those sites which did reach 100% saturation at 15 cm depth, the values dropped immediately following the rainfall event. At 5 cm and 15 cm depth, the average rate of change in percent saturation was around 2% and 4% per day. Therefore, there was no instance where the sites were flooded at 15 cm depth for periods longer than

24 hours. This may have been caused by drainage or evaporation and demonstrates how quickly these systems respond to rainfall events. Moreover, it was found that when the moss cushions were wet, the rate of change per day of percent saturation between rainfall events was greater than when they were dry. This is likely because of the initial drainage that occurs when the cushion is already wet. Any addition of rain is quickly runoff and the percent saturation drops more quickly than if the moss were to have been dry. Furthermore, the rate of change during the dry period was calculated over 20 days versus only 9 days for the wet period. These results may be different if only the first few days following the rainfall event were considered instead of the entire period between rainfall events. However, smaller rainfall events, less than 5 mm, appeared to cause greater response in the moss cushions during the dry period. When conditions became wetter later in September and October, addition of water from rainfall events of any size did not impact the moisture regime as significantly.

The depth of each profile also appeared to have an impact on the moisture regimes throughout the summer. Deeper profiles showed a greater difference in percent saturation between 5 and 15 cm. This is likely a result of the deeper profiles having greater water storage which takes longer to evaporate completely and can therefore maintain higher moisture conditions throughout the entire profile. Moreover, the greater proportion of highly decomposed organic matter in the deeper profiles also increases the ability of the substrate to hold onto moisture longer. At the same time, the higher bulk density of highly decomposed organic matter means less pore space, or reduced porosity, which has a higher moisture retention (Smolarz, 2014). In the case of turtle nests, it is more important to retain moisture for longer periods of time as opposed to being able to hold greater amounts of moisture.

3.4.3 Subsurface Thermal Dynamics

Depending on the species, turtle nests vary greatly in depth from a few (Ernst *et al.*, 1994; Riley and Litzgus, 2013; Conant *et al.*, 2016) to several centimeters deep (Standing *et al.*, 1999; Riley and Litzgus, 2013). Therefore, temperatures at 5 and 15 cm are most representative of depths that eggs would be oviposited at in real nests. Our general finding was that even the warmest site, 426B, experienced temperatures that are relatively cool for incubating turtle eggs. In order to develop, turtle embryos require consistent temperatures above 22°C. Nests which achieve higher temperatures also require a shorter incubation time than those which may experience cooler temperatures which is important when considering the relatively short growing season that Eastern Georgian Bay experiences relative to sites further south (Bobyn and Brooks, 1994a). Moreover, for turtles which undergo temperature-dependent sex determination, higher temperatures are required to produce higher numbers of females (Ewert, 1985). For a species that is already affected by skewed sex ratios due to higher road mortality amongst females (Gibbs *et al.*, 2005; Steen *et al.*, 2006), it is essential that greater temperatures are achieved in the nests to produce more females. The sites we instrumented did not achieve high average temperatures throughout the incubation periods at realistic depths of nests. Furthermore, even when slightly higher temperatures were achieved, the duration was much too short to have any impact on the minimum incubation period. Therefore, in accordance with similar studies on populations at their northern limit in Ontario, we suggest that temperature is likely a limiting factor of the distribution of freshwater turtles on this landscape (Bobyn and Brooks, 1994a). Maximum temperatures required for development, let alone equal ratios of male to female, are not achieved. It was also observed that the fluctuations between daily maximum and minimum temperatures was reduced at greater depths (Packard *et al.*, 1985). This reduction in variability provides more stable temperatures which may benefit egg development if the maintained temperature is high enough.

The entire realistic incubation period was assessed in this study as a necessary first step to understanding the temperature conditions of this specific type of habitat and substrate. However, whether or not it is true that temperatures experienced during the thermosensitive period of embryo development is more important than the average temperature over the entire incubation period remains unknown. If this were the case, there may be short periods later in embryo development that experience higher temperatures. There were periods throughout the summer where maximum temperatures at 15 cm depth were high enough to produce female turtle (Figure 3.9a). Therefore, although the average temperatures may not have reached suitable levels (Figure 3.8.1, 3.8.2), shorter time periods of high temperatures were achieved that could facilitate the development of embryos. A better understanding of temperature fluctuations during the thermosensitive period, their impact on embryo development, and at what point during incubation embryos in natural sites experience this critical period needs to occur. However, it is difficult to do so when conditions fluctuate significantly year to year. Even so, the maximum and minimum temperatures measured for these locations demonstrate higher temperature are achieved during the summer (Figure 3.9a, b), but whether or not these temperatures are achieved during the thermosensitive period and if the average temperature is more important needs to be understood. The small number of sites used in this study limit the number of factors that could be tested for as potential reasons for differences amongst temperatures. For instance, canopy cover is a critical habitat feature that can cause significant differences in subsurface temperatures.

3.4.4 Canopy Openness and Temperature

We assessed canopy cover at various sites throughout the summer to understand the impacts of differing canopies on subsurface temperatures. We found that total incident radiation on the surface was positively correlated to canopy openness but not linearly. Since total incident radiation is not linearly correlated to canopy openness, after a certain point, TTR will not increase at the same rate as canopy openness (Figure 3.5). Therefore, it appears as though sites with a canopy openness greater than 75% may receive the same total incident radiation. However, we found that canopy openness was linearly correlated to temperature at 1, 5 and 15 cm depth (Figure 3.7). These differences suggest that a greater canopy openness may lead to longer exposures to sunlight during the sun's path throughout the day which may be more important that total incident radiation on the surface that has to do with the sun's strength. In other words, the strength of the sun reaches a maximum after which it can no longer increase the warming effect on the surface whereas the canopy structure and gap fraction relative to the sun's path can lead to longer periods of exposure. Generally, we found that nests with higher canopy openness had higher average temperatures at all depths (Figure 3.7) and that temperature fluctuations decreased with depth (Figure 3.6). However, except for two moderately shaded sites (426A, 425A) being statistically different from the site with the lowest canopy openness (426B), there was no significant difference between temperatures at each depth between sites (Figure 3.6). More frequent measurements at an increased number of sites would clarify these differences since nesting locations in the shade almost certainly would not receive enough sunlight to warm the nests sufficiently in this cool northern climate (Bobyn and Brooks, 1994a; Litzgus and Brooks, 2000). Moreover, on days when there are no clouds which can cause surface temperatures to increase to dangerously high levels, canopy openness likely plays an essential role in moderating the subsurface conditions.

The open rock barrens are an ideal natural incubator for turtle nests due to the high heat capacity of the granite outcrops and the slow release of latent heat throughout the night. Although the rock surfaces, and even some moss surfaces, can reach lethal temperatures for turtle eggs, temperatures below the surface in moss-filled depressions remain cool and stable with increasing depth. Future insights into the warming effect of the rocks throughout the day and evening would be valuable in understanding the effect of rock barrens on critical nesting habitat. For instance, could the rocks surrounding the nest site warm moss-filled depressions from the bottom? Or do the nests needs to be in direct contact with the rock surface to uniformly incubate the entire nest, for instance, in a rock crevasse?

3.4.5 Applications of Research

Variations in temperature and moisture can have differing effects on turtle eggs during incubation. Notably, the interaction between both factors and variations of wet, dry, warm, and cool conditions yield differing results. However, the general finding is that extremely high temperatures in drier media and low temperatures in wetter conditions result in reduced hatching success. Generally, duration of incubation and size of hatchlings are positively correlated with wetness of substrates and negatively correlated with temperature (Packard *et al.*, 1987). This may be concerning as extreme temperature fluctuations, drought periods, and rainfall events are expected in the future as a result of climate change (Bodensteiner *et al.*, 2014; IPCC, 2014). The soil moisture, temperature, and the interaction between these conditions is important to understand when considering the impacts of climate change on oviparous organisms with subterranean nests, particularly those which experience temperature-dependent sex determination (Bodensteiner *et al.*, 2015). This is why research focused on quantifying water content, soil temperature, and their interactions in various substrates is essential to understand the biological implications on hatchling phenotype, success, and survivability.

3.5 References

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3.5 Tables

Table 3.1: Summary of study sites and type of data analysis performed

	Temperature Data	**Site**	**Number of Profiles**	**Canopy Openness (%)**
	Continuous Temperature	425	2	74.75 (C), 65.28 (D)
		426	2	67.32 (A), 49.3 (B)
Photogrammetry	Manual Temperature	324	1	70.12
		304	1	72.88
		330	1	75.68
		419	1	78.17
		403	1	82.36
	Temperature Data	**Location**	**Number of Sites**	**Canopy Openness (%)**
	none	Hilltop	12	None
	none	Hillslope	22	None

Table 3.2: Results of photogrammetry and subsequent MATLAB analysis of 22 Hillslope and 12 Hilltop moss-filled depressions

	Hillslope		Hilltop	
	Mean	StDev	Mean	StDev
Moss Volume (m^3)	7.14E-02	1.35E-01	1.89E-01	2.31E-01
Depression Storage (m^3)	3.92E-03	7.80E-03	8.01E-03	6.39E-03
Volume of moss water storage below sill (m^3)	1.49 E-03	3.04 E-03	2.97 E-03	2.43 E-03
Moss above sill (m^3)	6.75E-02	1.27E-01	1.81E-01	2.26E-01
Pit area (m^2) (sum of mask)	1.38	1.24	3.80	3.72
Avg. depth of moss (cm; moss volume/area)	3.87	2.18	3.85	1.37
Avg. depth of moss (cm; from measurements)	5.30	4.82	4.82	1.05
Depth of rain (mm) required to flood *	0.78	0.56	1.07	0.71

* This parameter assumes that the initial moisture conditions of the moss/organic matter in the depression are at field capacity

Table 3.3: Average difference between the maximum and minimum daily temperatures (°C) of the air and in all continuously monitored sites at 1, 5, and 15 cm depths in June, July, August, September, and October

	Air		1 cm		5 cm		15 cm	
	Mean	StDev	Mean	StDev	Mean	Mean	Mean	StDev
June	15.90	4.97	26.89	5.90	13.64	3.35	4.39	1.13
July	14.22	3.31	21.40	5.56	11.41	3.69	4.22	0.77
August	14.68	5.00	24.90	5.11	13.50	4.19	4.09	0.43
September	11.26	3.19	11.05	3.24	5.40	2.17	2.36	0.49
October	9.74	3.69	8.35	1.83	4.28	1.43	1.73	0.26

3.6 Figures

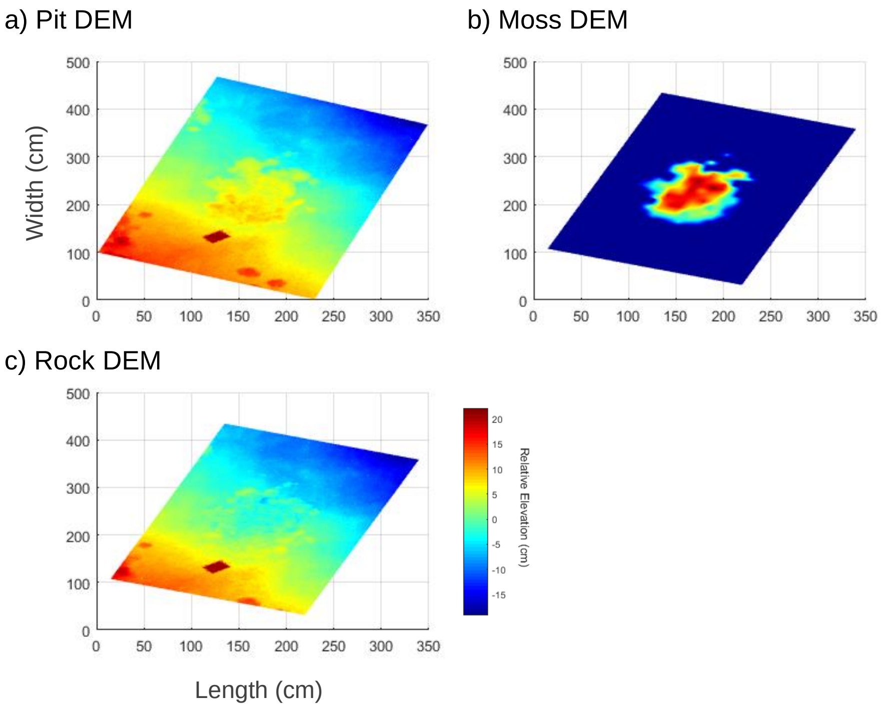
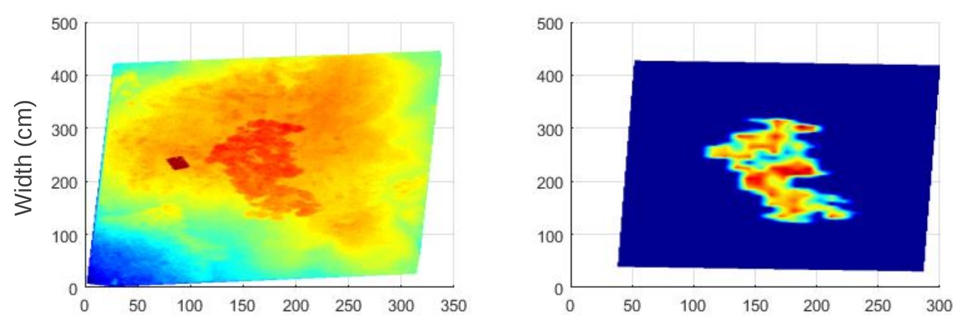


Figure 3.1.1: Example of an organic filled depression on a hillslope. (a) pit DEM developed from photogrammetry (b) moss DEM developed from manual survey of moss measurements (c) rock DEM created by subtracting the moss DEM from the pit DEM.


a) Pit DEM

b) Moss DEM

c) Rock DEM

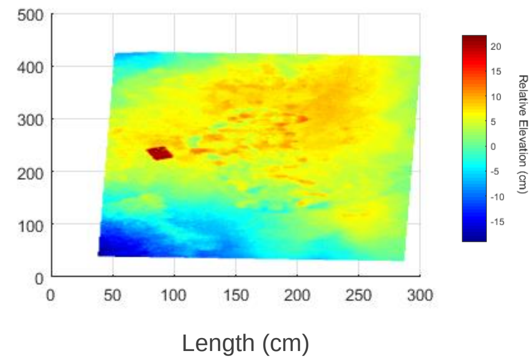
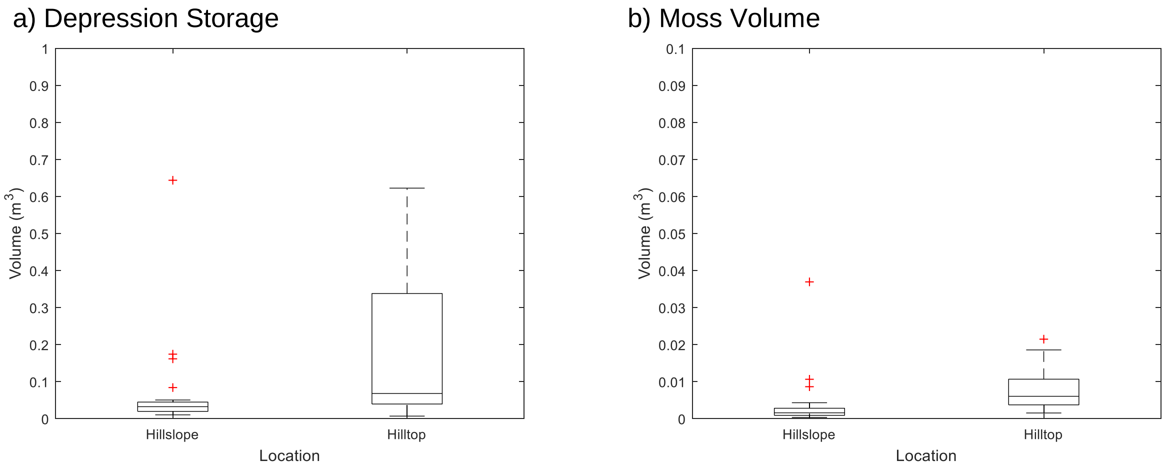


Figure 3.1.2 Example of an organic filled depression on a hilltop. (a) pit DEM developed from photogrammetry (b) moss DEM developed from manual survey of moss measurements (c) rock DEM created by subtracting the moss DEM from the pit DEM



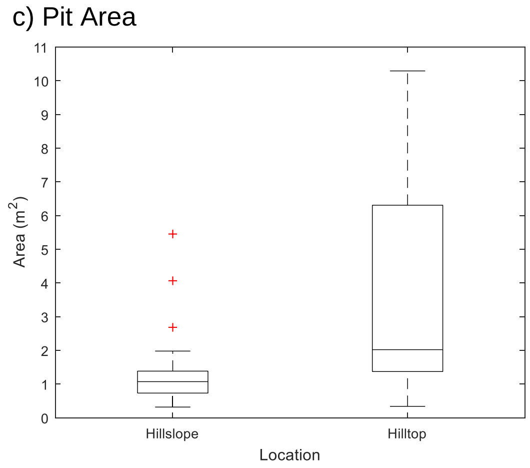


Figure 3.2 (a) Depression storage (b) moss volume and (c) pit area of 22 Hilltop and 12 Hillslope organic filled depressions

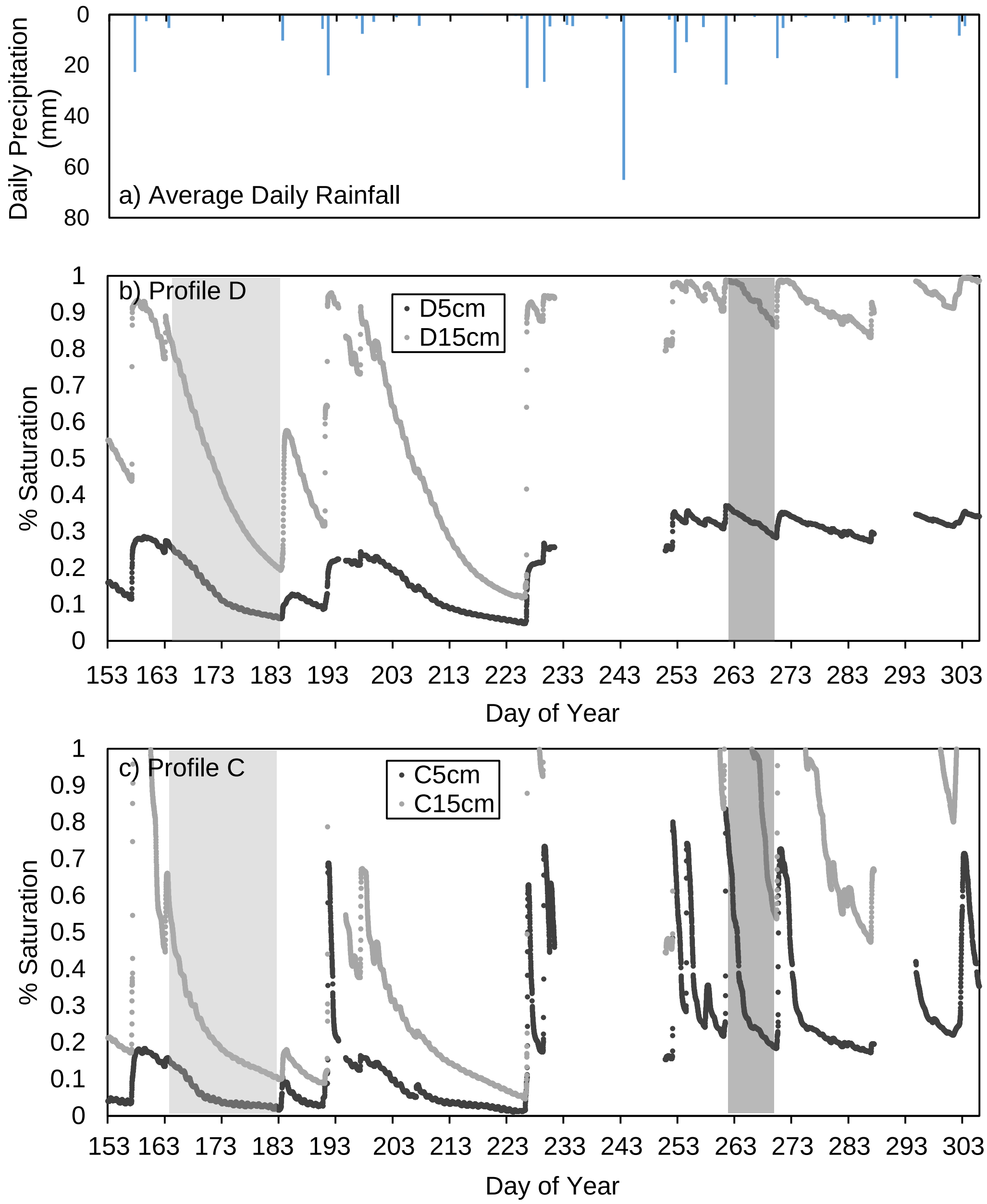


Figure 3.3.1: (a) average daily rainfall (mm) and percent saturation at two depths from (b) profile D and (c) profile C. Percent saturation was calculated from moisture probes installed at 5 cm and 15 cm below the surface. Each profile was located within site 425. Light and dark grey bars represent time periods where evaporation rates were assessed during dry (light) and wet (dark) conditions

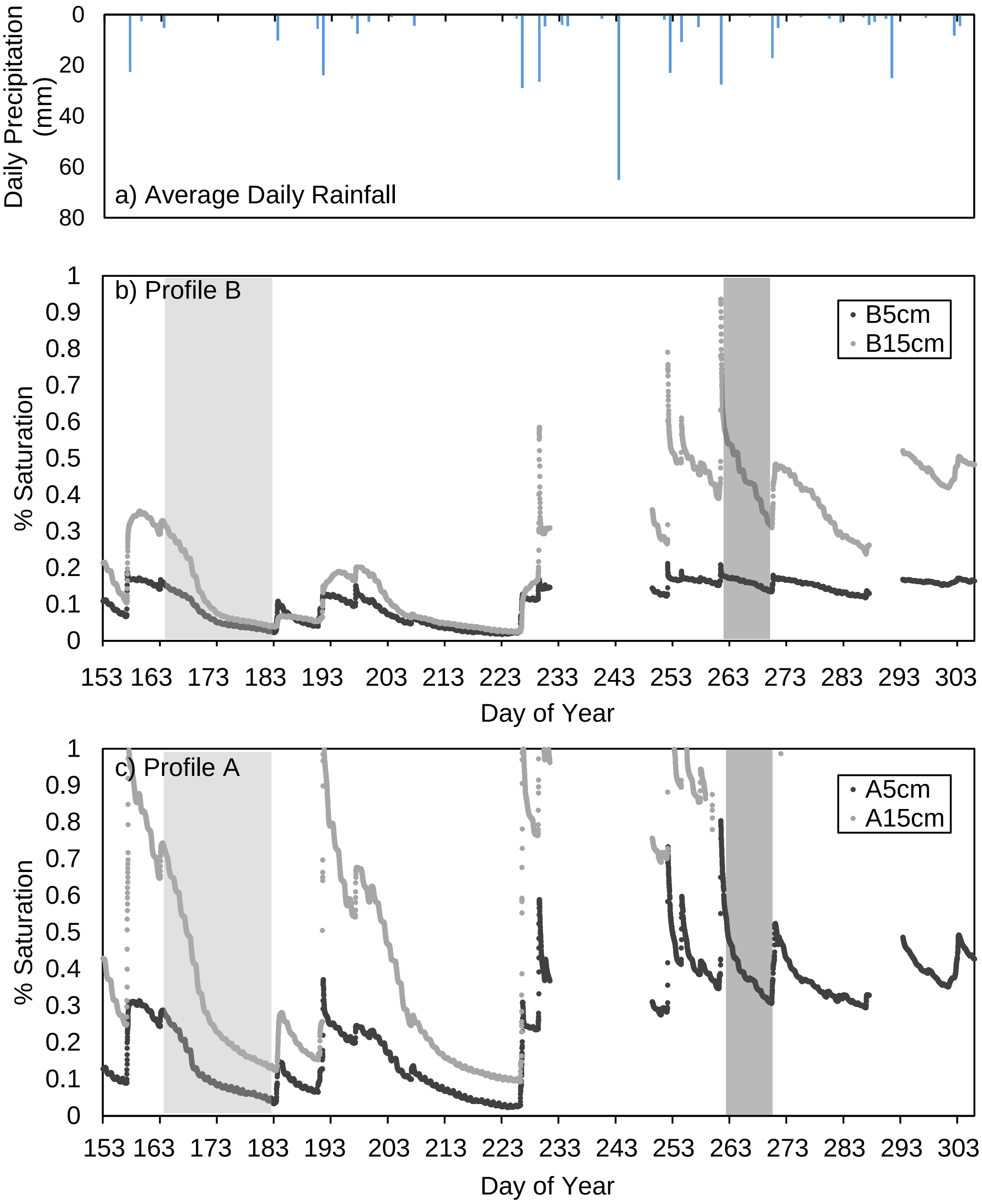


Figure 3.3.2: (a) average daily rainfall (mm) and percent saturation at two depths from (b) profile B and (c) profile A. Percent saturation was calculated from moisture probes installed at 5 cm and 15 cm below the surface. Each profile was located within site 426. Light and dark grey bars represent time periods where evaporation rates were assessed during dry (light) and wet (dark) conditions

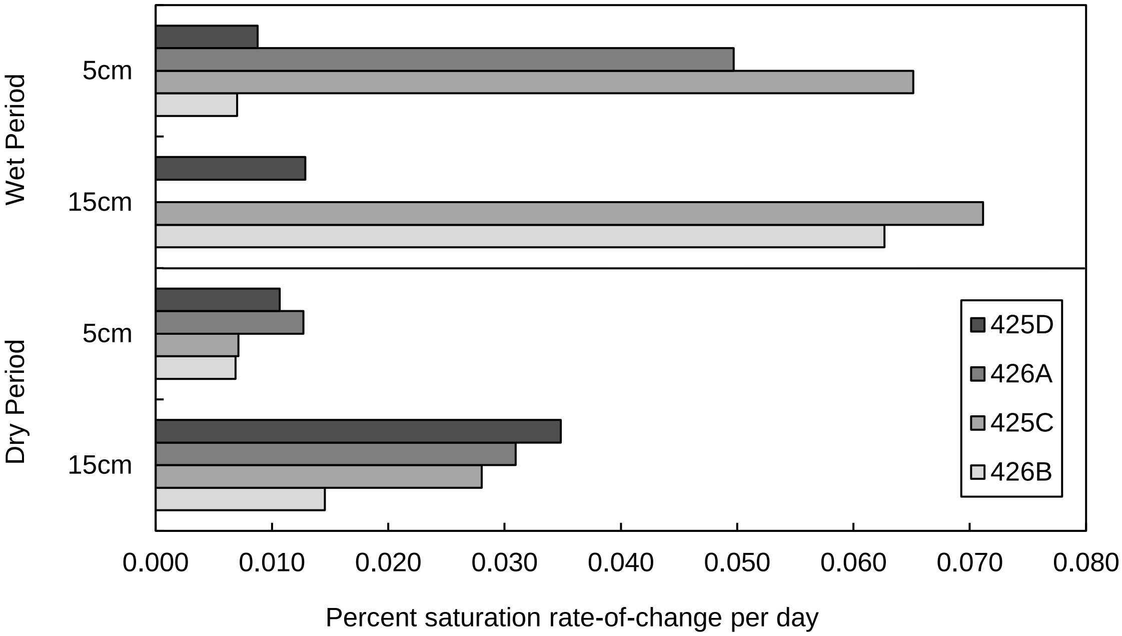


Figure 3.4: Percent saturation rate of change between 5 and 15 cm depths during a wet (top panel) and dry (bottom panel) period at continuously monitored moss-filled depressions. Sites are listed from top to bottom in order of increasing depth of entire profile

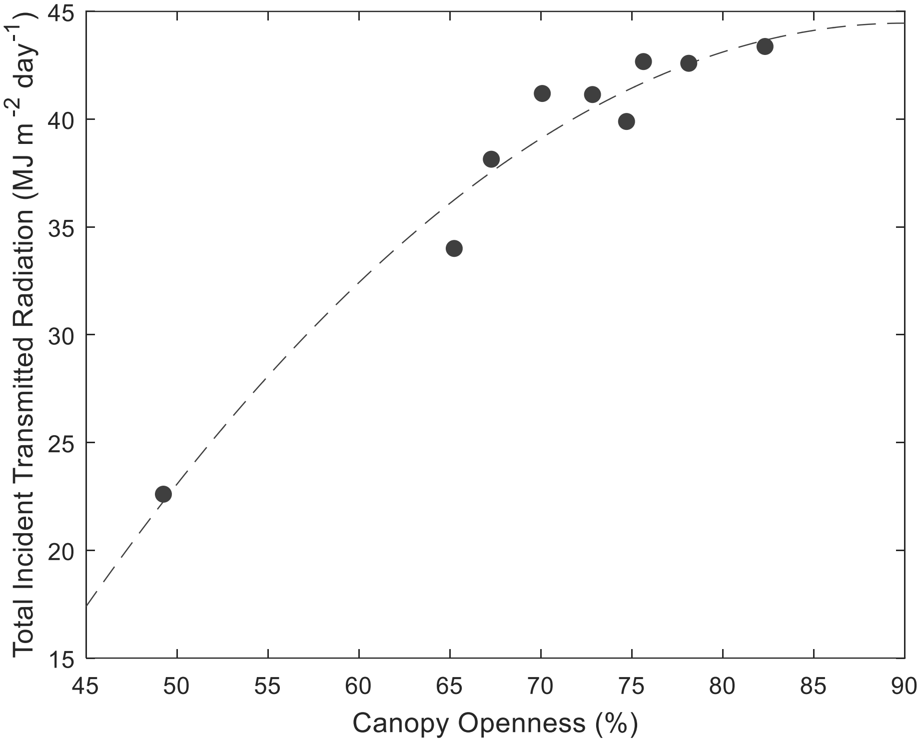


Figure 3.5: Canopy openness and total radiation incident on the surface at nine profiles

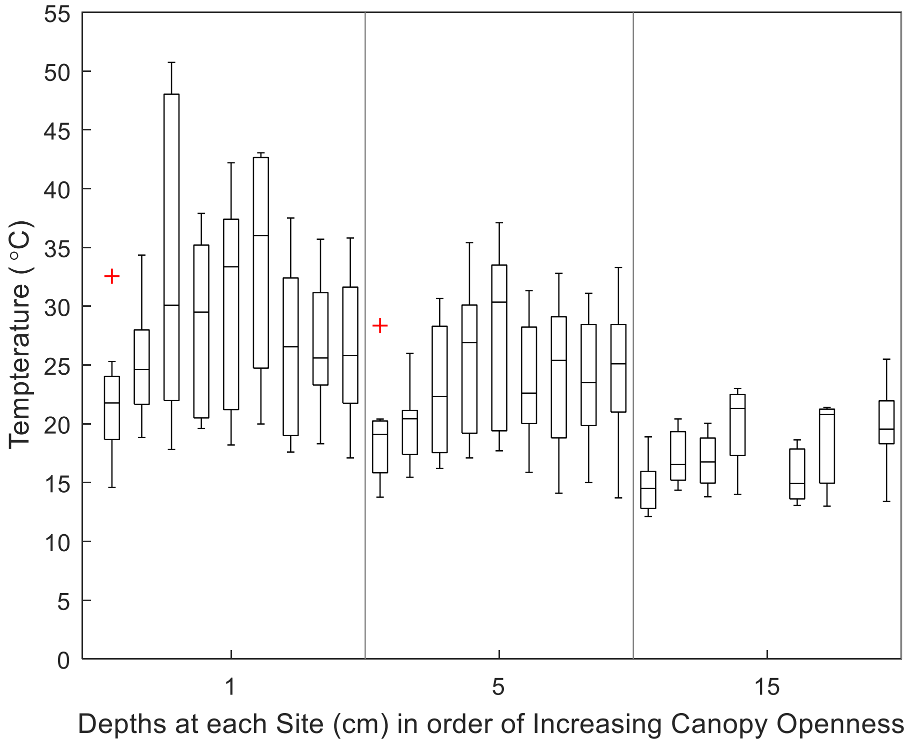


Figure 3.6: Weekly temperatures at nine locations taken at three depths (1, 5, and 15 cm). Box plots are listed in order of increasing canopy openness between sites. From left to right, in each depth category, they are 426B, 425D, 426A, 324, 304, 425C, 330, 419, and 403

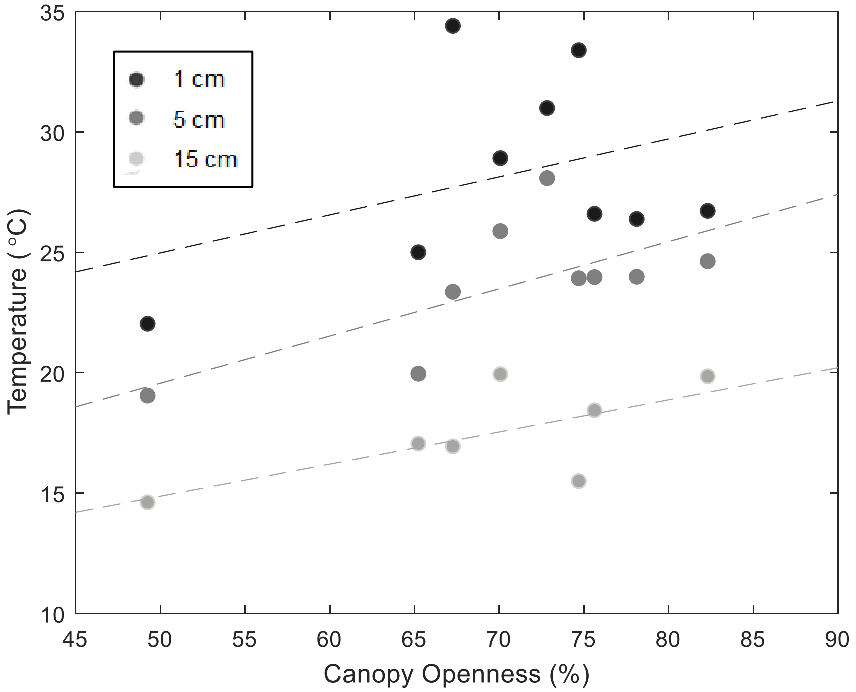


Figure 3.7: Canopy openness and average temperatures collected once a day, every week, throughout the summer from manual and continuous temperature profiles at nine locations

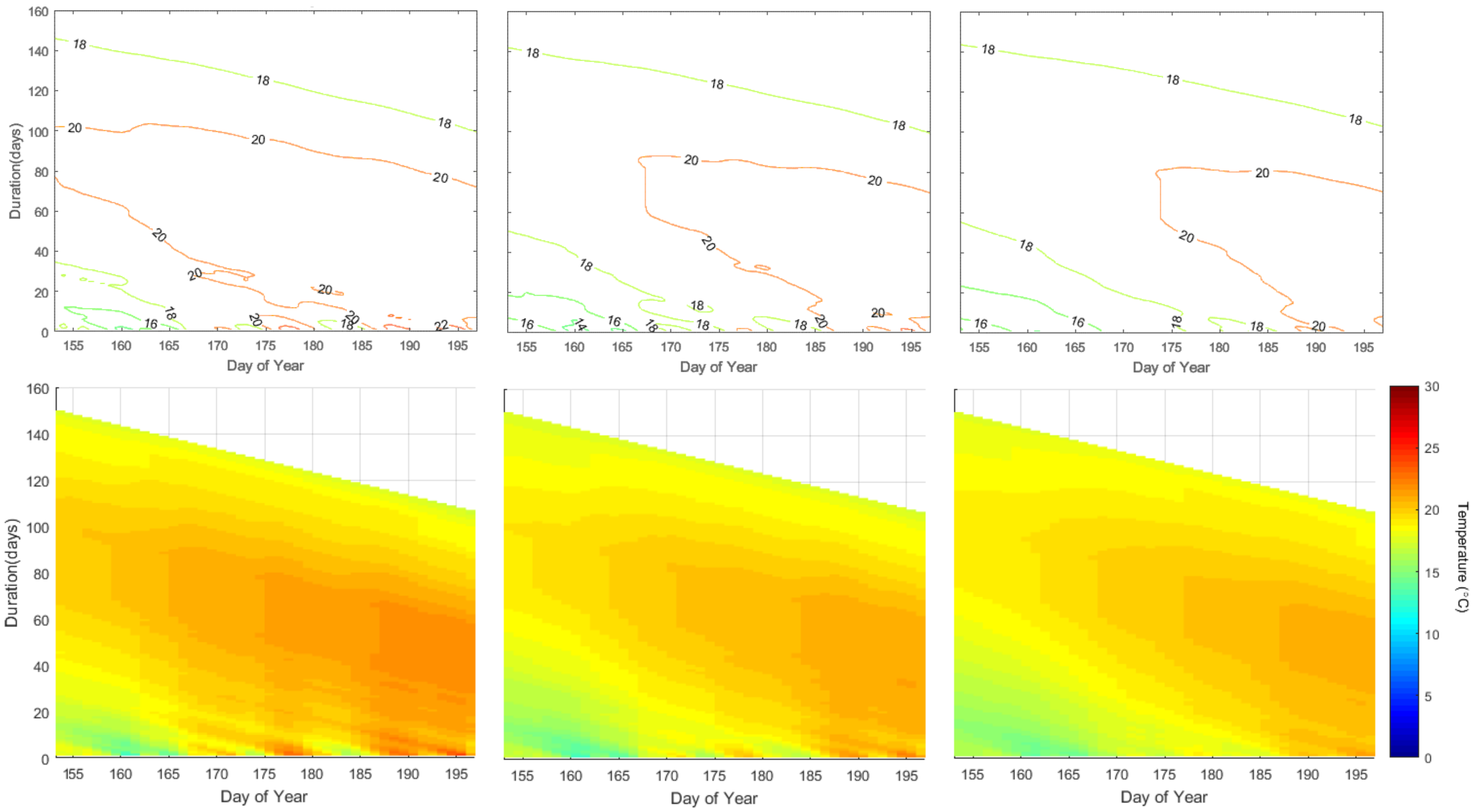


Figure 3.8.1: Average daily temperatures in profile D at site 425 at 1, 5, and 15 cm depths below the surface based on start date and duration of incubation. This location experienced the warmest temperatures

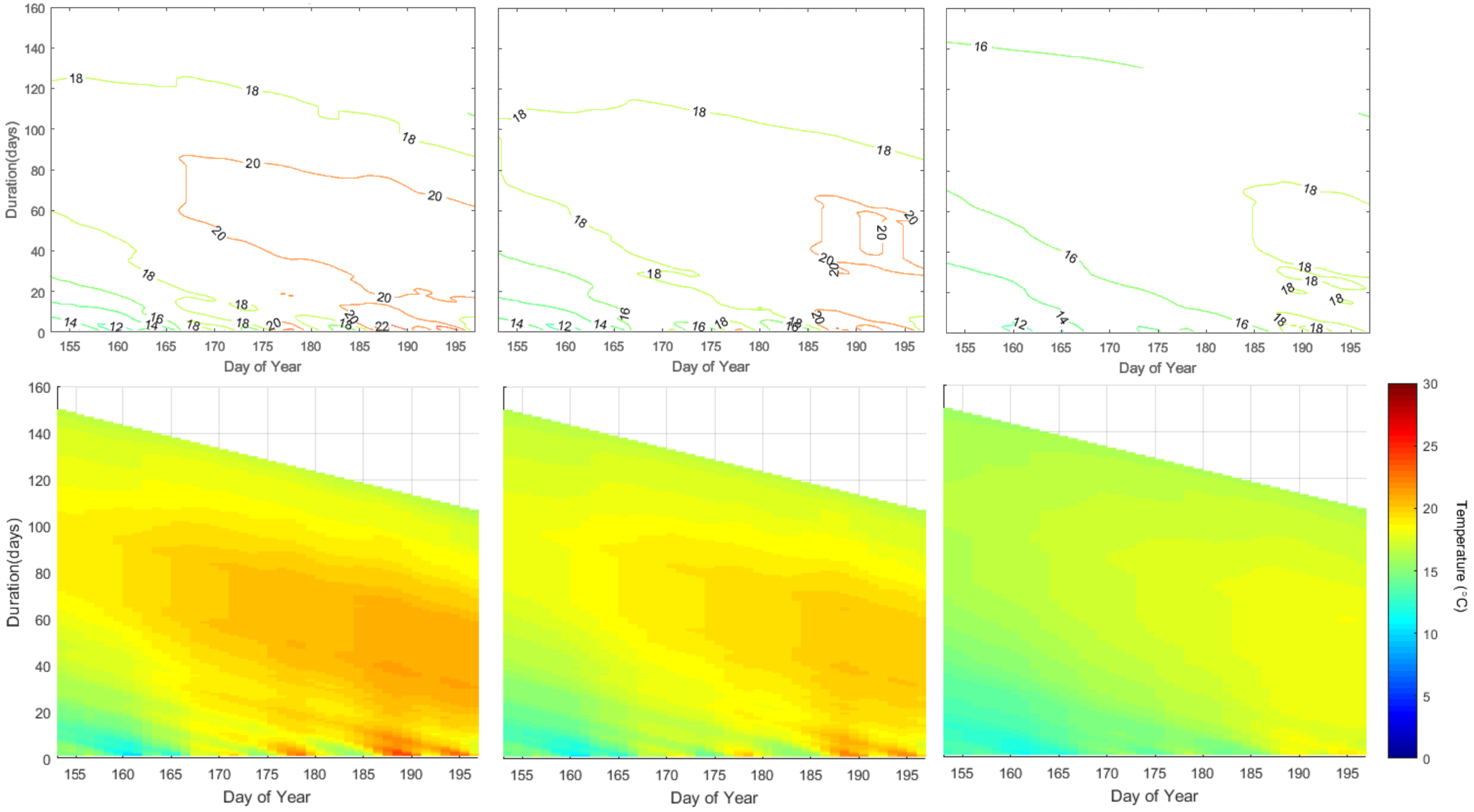
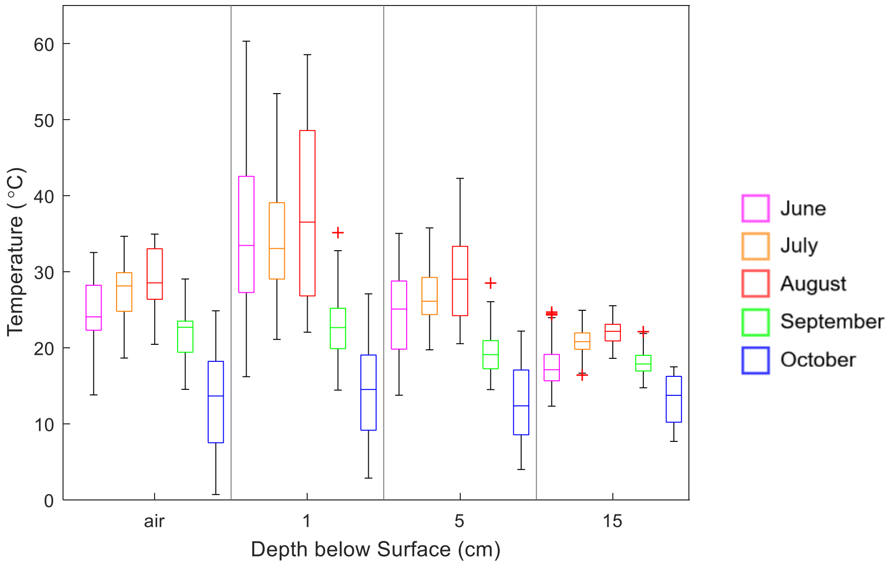


Figure 3.8.2: Average daily temperatures in profile B at site 426 at 1, 5, and 15 cm depths below the surface based on start date and duration of incubation. This location experienced the coolest temperatures

a) Maximum Daily Temperatures



b) Minimum Daily Temperatures

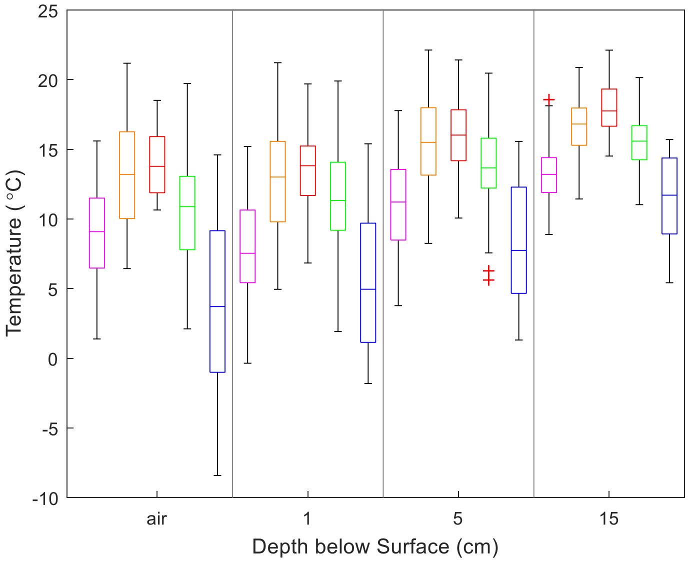


Figure 3.9 (a) Maximum daily temperatures (b) minimum daily temperatures (°C) of the air and at all continuously monitored sites at 1, 5, and 15 cm below the surface during the months of June, July, August, September and October

CHAPTER 4: CONCLUSION

Eastern Georgian Bay is one of the most pristine landscapes in Ontario which is characterized by exposed portions of the Canadian Shield amongst densely treed forests and an abundance of wetlands (Jalava *et al.*, 2005). The variety of habitats here meet the needs of an incredible array of reptiles at various life stages, many of whom are existing at the northern extent of their range limit. Amphibians and reptiles are the most at risk vertebrate group in the world (Gibbon *et al*., 2000; Lesbarrères *et al.,* 2014). In Canada, 65% of the reptiles have been listed as a species-at-risk by COSEWIC as of December, 2016 (Canadian Herpetological Society, 2017). Therefore, understanding the spatial ecology and ecohydrological habitat requirements of these at risk species is key to their sustained existence in Canada. More specifically, characterizing the critical habitat of these reptiles is of great importance when it comes to developing conservation and mitigation strategies. The various types of critical habitat, including snake hibernation and turtle nesting sites, are limited along the rock barren landscape that comprises the majority of the Georgian Bay Biosphere Reserve. Therefore, habitat requirements on this landscape need to be assessed for specific populations which may utilize a variety of habitats across their entire species' range.

The few isolated populations of Eastern Massasauga Rattlesnakes (EMR) in Ontario makes conservation efforts and habitat assessments difficult. Often times, these strategies cannot be generalized across all populations (Pomara *et al.,* 2014). The population along Eastern Georgian Bay utilize peatlands to overwinter (Rogers, 2015; Harvey and Weatherhead, 2006) since the snakes are able to survive below the frost line but above the water table (WT; Smith, 2009) in the *Sphagnum* hummock structures. Peatlands are a well-known carbon storing ecosystem (Gorham, 1991) but are poorly studied regarding their role as EMR hibernacula. Similarly, the hibernation requirements and behavior of EMRs is poorly studied despite being highly specific and a critical component of their life history (Johnson *et al.*, 2000; Smith, 2009). This research addresses this gap in knowledge by characterizing the hydrological and thermal dynamics of peatland hibernacula. There are various feedbacks controlling the WT position within the peatlands (Waddington *et al.*, 2015). However, during the winter, these feedbacks that normally maintain or reduce the WT position are weakened and the WT rises which is not uncommon (Smith, 2009) as we have shown here (Figure 2.3). This is where the size of hummock hibernaculum becomes important. Large hummocks (Figure 2.4a) provide protection from potential floods as well as the advancing frost line as opposed to small hummocks (Figure 2.4b). Hummocks between 30-35 cm provide the greatest chance for survival (*i.e.* have the highest survival hibernacula hummock zone index) when snakes hibernation 20-25 cm below the surface of the hummock (Figure 2.7). Snow is also an important component to consider when determining overwintering success as it provides necessary insulation during the harsh winters.

However, we found that so long as snow was present, temperature differences between different sized hummocks were not significantly different (Figure 2.10). Therefore, timing of the first frost in relation to the first snow, as well as freeze/thaw events in the spring and fall is likely more important. The next steps would be to model the effects of snowfall events, temperatures, wind, canopy cover/tree density, and WT. To predict catastrophes like the mortality event of 2014 which affected populations of EMRs along Eastern Georgian Bay (Rogers, 2015; Parks Canada Agency, 2015), future studies should assess the vulnerability of critical overwintering sites to climate or land-use change.

Nesting sites on the open rock barrens of Eastern Georgian Bay are a unique habitat type. Numerous freshwater turtle species, including the threatened Blanding's Turtle (*Emydoidea blandingii*) and endangered Spotted Turtle (*Clemmys guttata*) rely on this habitat to incubate their eggs during the relatively short growing season experienced at this latitude (Litzgus and Brooks, 2000; Markle and Chow-Fraser 2014). However, little is known about the temperature and moisture dynamics of the available habitat on rock barrens since the various types of potential nesting habitat are not well documented. Based on evidence from the literature, personal observation, and field surveys, the common landscape feature available to turtles as nesting habitat are small moss-filled depressions (Litzgus and Brooks, 2000; Markle and Chow-Fraser 2014). Using a process-based approach, this research focused on characterizing the moisture and thermal dynamics of moss cushions as a potential type of nesting habitat for turtles on rock barrens. We found that suitable nesting sites for turtles do not include many of the moss cushions that exist on the rock barrens because many of these cushions do not form in depressions of appropriate depths for a nest, but rather the moss tends to grow on the rock surface. However, the responsiveness of the moss cushions to rainfall events demonstrates the importance of substrates and nesting sites with appropriate drainage (Joanen *et al.*, 1977; Kam, 1994). The representative nest sites on hilltop and hillslopes had relatively small depression storage values (Table 3.2), meaning minor rainfall events of less than 2 mm could flood them. However, the moss did not remain saturated for periods longer than 12 hours (Figure 3.1.1, 3.1.2). Moreover, we demonstrated how cooler temperatures experienced in representative nest sites (Figure 3.8.1, 3.8.2), may also be a limiting factor to the existence of turtle species at this northern limit of their home range (Packard *et al.*, 1985; Bobyn and Brooks, 1994). Even when the canopy openness is high, average temperatures at realistic depths of turtle nests are not high enough to ensure successful development although temperature fluctuations become more stable with depth (Figure 3.6). Given that other factors such as catchment area, tree density, hydrological connectivity, evapotranspiration, slope and aspect, and canopy all affect the microclimate variability of nests sites, we suggest that more interdisciplinary research combining biological, ecological, and hydrological aspects of this reptile's behavior and habitat is needed.

The purpose of this research was to demonstrate the importance of linking biology and ecohydrology when studying reptiles and their critical habitat to improve conservation efforts and mitigation strategies. The habitat requirements at critical life stages including hibernation and nesting are different for each species. Further research should therefore focus on characterizing the conditions that make various habitats optimal for hibernation or nesting. This will foster better conservation strategies and provide insight into the vulnerability of these habitats to climate and land-use change.

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