

CHARACTERIZING TURTLE NESTING HABITAT IN THE EASTERN  
GEORGIAN BAY REGION

CHARACTERIZING TURTLE NESTING HABITAT IN THE EASTERN  
GEORGIAN BAY REGION

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## **ABSTRACT**

Reptile populations are decreasing throughout the globe, holding the highest proportion of species listed as threatened worldwide. To ensure proper management and conservation for reptile species at risk, it is essential to identify, characterize, and understand the microhabitat environment at critical life stages. Nesting is a critical life stage for turtles, and nest site selection can affect successful hatching. As Ontario is at the northern range limit for native freshwater turtles in the province, selection of nest sites that have high quality thermal and hydrological conditions may be particularly important in this region. Soil-filled bedrock depressions are known nesting habitats of turtles in the Eastern Georgian Bay region and vary in soil texture from the sand environment of turtle nests in more southern populations. Due to the difference in soil from more commonly studied regions, little is known about the nesting habitat in the eastern Georgian Bay area and how landscape characteristics and soil properties further influence the conditions during incubation. We surveyed 48 300m transects in a 660 ha study area, classifying land cover type for all surveyed points, and soil depth, canopy openness, ground cover type, moss and lichen height, slope, and aspect for all points classified as available habitat. Only 22.1% of the surveyed landscape was considered available turtle nesting habitat, and of this, only 2.6-10% of these points (dependent on species) were suitable for nesting (equal to 0.57-2.21% of total points surveyed). Our results demonstrate that suitable turtle nesting habitat in this environment is extremely limited (both by soil depth and canopy cover),

with canopy openness being the most limiting factor. Due to the already limited habitat, it is even more important to understand the thermal and hydrological characteristics of nesting habitat in the Eastern Georgian Bay region, so that soil properties can be used to inform creation of habitat. We also determined that a majority of the soil on the landscape is sandy clay loam, with a low organic matter content. The information gained through characterization of the soil can be used to determine appropriate soils for artificial nest construction and ensure more successful methods of conservation for turtle species at risk. We recommend that artificial nesting habitat must have sufficient depth (at least 10cm) and canopy cover requirements (90-100% openness), as these are the most limiting factors. When creating artificial nest habitat, we recommend the use of sandy clay loam soil, due to its favourable thermal and hydrological characteristics.

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## **INTRODUCTION**

### *1.1 Decreasing turtle population*

Reptile species are decreasing throughout the globe, holding the highest proportion of species listed as threatened worldwide (Gibbon et al., 2000; Lesbarrères et al., 2014). This is of even greater concern in Canada, where 77% of reptile species are designated as at-risk (Lesbarrères et al., 2014). Included in this phenomenon of population decline are turtles, as their delay to maturity, low reproductive output, and extensive home ranges make this order of reptile vulnerable to any change and disturbance (Browne & Hecnar, 2007). Even if a population looks healthy due to the presence of adults, lack of recruitment of juveniles may still pose a risk (Browne & Hecnar, 2007). Causes of decline may include habitat loss and degradation, invasive species, environmental pollution, disease and parasitism, unsuitable use, and climate change (Gibbon et al., 2000). Loss and degradation of habitat is especially relevant for freshwater turtle species, as not only can it affect the aquatic areas of these species, but it can have a major impact on the upland areas used as corridors and nesting habitat as they move throughout their home range (Marchand and Litvaitis, 2004).

### *1.2 Turtles in Ontario*

As Ontario is in the southern region of Canada, biodiversity is especially high in this region (Kharouba et al., 2008). The rock barren landscape of Eastern Georgian Bay provides suitable habitat for a variety of wildlife, including many

of these reptile species. Home to seven of Ontario's eight freshwater turtles, this region is a crucial turtle habitat, even more so for those that are considered species at risk. Currently, all seven turtle species within the Georgian Bay region are considered either of special concern, threatened, or endangered. Included in this list are the Blanding's turtle (*Emydoidea blandingii*) and spotted turtle (*Clemmys guttata*) both listed as endangered in their Canadian populations, as well as the midland painted turtle (*Chrysemys picta marginata*) and the snapping turtle (*Chelydra serpentina*), both listed as species of concern in their Canadian populations (COSEWIC, 2008; COSEWIC, 2014; COSEWIC, 2016; COSEWIC, 2018).

#### *1.2.1 Blanding's turtle*

The Blanding's Turtle is a species found primarily concentrated around the Great Lakes, in both Canada and the United States (COSEWIC, 2016). Canada has two separate populations of Blanding's Turtles, with one smaller isolated population located in Nova Scotia and another larger population located in the Great Lakes/St. Lawrence region, extending from Southern Ontario to Quebec (COSEWIC, 2016). The Blanding's Turtle is of particular concern as it is listed as a species at risk in 17 of the 18 jurisdictions it is found within (COSEWIC, 2016). The most likely reasons for population decline for the Blanding's turtle is habitat loss and fragmentation (specifically wetland modification and destruction), road mortality, and increased predation (COSEWIC, 2016; Ernst & Lovich, 2009).

As a semi-aquatic species, both aquatic and terrestrial habitats are used throughout its life. Terrestrial habitat allows for movement, nesting, thermoregulation, and hatchling dispersal from nests, making it an essential component, especially during the nesting season (Congdon et al., 2011). Specifically, turtles traverse long distances throughout their home range to reach suitable nest sites (Kiviat, 1997; Millar & Blouin-Demers, 2011). If there are no suitable sites within a certain range, distances may increase, which has the potential to increase mortality due to increased road crossings and predator sightings (Dowling et al., 2010).

Nests of this species are usually created by gravid females in open areas, near water, in areas with well drained soils, and near herbaceous vegetation (Avery et al., 2000; Dowling et al., 2010; Kiviat, 1997; Ross & Anderson, 1990). Studies have recorded nests located in beaches, meadows, rocky outcrops, forest clearings, and human altered sites, with nests occurring in substrates such as sand, organic soil, gravel, and soil-filled rock crevices (Avery et al., 2000; COSEWIC, 2016; Litzgus & Brooks, 1998; Markle & Chow-Fraser, 2014). Oviposition of eggs generally occurs during June, however, has been recorded from the end of May-beginning of July depending on the region and the weather within that year (Congdon et al., 1983; Gibbons, 1968; Linck et al., 1989; Standing et al., 1999). Blanding's turtles have been recorded to show high fidelity to nesting areas, returning to previous nest sites in multi-year studies (Congdon et al., 1983; Congdon et al., 2011; Ernst & Lovich, 2009; Standing et al., 1999). Fecundity is

low, and studies have shown females nesting less than annually (Standing et al., 1999). Nesting is more common during the evening and during or after a rain event, as rain allows for both softer substrate during nest construction as well as decreased olfactory cues for potential predators (Avery et al., 2000; Congdon et al., 1983; Dowling et al., 2010; Linck et al., 1989). Signs of ‘ground nuzzling’ have been seen within this species during nest construction, a potential method to determine temperature during nest site selection, aiding selection of suitable sites. (Linck et al., 1989, Standing et al., 1999). During nest creation, a flask shape is made, with a depth of 12-18cm (Ernst & Lovich, 2009).

### *1.2.2 Snapping turtle*

The snapping turtle, *Chelydra serpentina*, is Canada’s largest freshwater turtle and is listed as special concern according to COSEWIC (2008). Within Canada, this species is located from Nova Scotia to Saskatchewan, and has the largest latitudinal distribution amongst turtles in North America. (COSEWIC 2008). Nesting of the snapping turtle is recorded to begin between mid-May to late-June, and lasts 13-18 days (Congdon et al., 1987, Congdon et al., 1994, Iverson et al., 1997). Later onset of nesting is recorded in more northern sites, with peak nesting recorded in mid-June in Ontario (Ernst & Lovich, 2009; Haxton, 2000). Before this time, gravid females are generally inactive, increasing their movement greatly during the nesting season (Congdon et al., 1987; Obbard & Brooks, 1980). Peak nesting generally occurs in the morning and evening hours and has been recorded to increase in occurrence during or shortly after rainfall (Congdon et al., 1987;

Iverson et al., 1997). In Ontario, known nesting locations include beaver lodges, small clearings, and soil in bedrock cracks, generally found close to water and open areas receiving direct sunlight (Congdon et al 1987; Ernst & Lovich, 2009). Nests are usually 7-20cm deep however deeper nests are possible if the female in question is large (Ernst & Lovich, 2009). Typical clutch size ranges from around 25-45, and varies based on region (Congdon et al., 1987; Ernst & Lovich, 2009; Iverson et al., 1997).

### *1.2.3 Painted turtle*

The painted turtle, *Chrysemys picta*, is largely distributed throughout North America, located throughout Canada and the United States (Christens & Bider, 1987). Three subspecies exist within Canada, with *Chrysemys picta marginata* (known as the midland painted Turtle) being the subspecies found within Ontario and Quebec (Cagle, 1954). Despite having a large geographic range, the painted turtle is listed as special concern within its Canadian populations (COSEWIC, 2018). Nesting in this species has been recorded to occur from mid-May to mid-July (Christens & Bider, 1987; Iverson & Smith, 1993; Mahmoud 1968) with recorded months of mid-June to mid-July in an Ontario location (Schwarzkopf & Brooks, 1987). Nesting has been noted to occur in the morning and the evening, allowing turtles to decrease heat stress during the day and avoid much colder temperatures later in the night (Christens & Bider, 1987; Frye et al., 2017; Mahmoud, 1968; Rowe et al., 2003) Nests are created in a flask-like shape and are generally less than 12 cm deep (Ernst & Lovich, 2009; Mahmoud, 1968).

Average clutch sizes recorded range from 4-10, typically increasing in more northern locations, where fewer clutches are produced annually (Ernst & Lovich, 2009; Mahmoud 1968, Schwarzkopf & Brooks, 1987). Selection of nests by the midland painted turtle have been found to be biased to sites close to water, sites in open areas with little shading vegetation, and sites with a south facing slope (Christens & Bider, 1987; Mahmoud 1968; Schwarzkopf & Brooks, 1987). Fidelity of the midland painted turtle to previous nest locations has been recorded in previous studies (Christens & Bider, 1987; Rowe et al., 2005).

#### *1.2.4 Spotted Turtle*

*Clemmys guttata*, the spotted turtle, is a species found in eastern North America, with populations ranging from southern Ontario to central Florida (COSEWIC, 2014). Threats to the spotted turtle include road mortality, pet trade, and loss and degradation of habitat (COSEWIC,2014). Nesting typically occurs in mid-late June, and lasts approximately two weeks, however may last longer in more southern populations. (Beaudry et al., 2010; Ernst, 1970; Haxton & Berrill, 1999; Haxton & Berrill, 2001; Litzgus & Brooks, 1998; Litzgus & Mousseau, 2004; Wilson, 1994). Female spotted turtles have increased movement during the nesting season, travelling an average of 148m, with nests typically occurring 2-312m from a wetland (Beaudry et al., 2010; Haxton & Berrill, 1999; Litzgus & Mousseau, 2004; Rasmussen & Litzgus, 2010a). Spotted turtles have been found to nest in both natural and anthropogenic sites, nesting in substrates such as loamy soils, sandy soils, loose organic matter, and gravel (Beaudry et al., 2010 ; Ernst,



1970; Litzgus & Brooks, 1998). In the Georgian Bay region, spotted turtles have been found to nest in soil-filled depressions found in rock outcrops (Litzgus & Brooks, 1998). Nest sites tend to be well-drained, and placed in locations receiving direct sunlight (Litzgus & Brooks, 2000; Rasmussen & Litzgus, 2010b). Nesting typically occurs in the evening to early morning (Ernst, 1970; Ernst & Zug, 1994; Litzgus & Brooks, 1998; Wilson, 1994). Nests are typically flask-shaped, relatively shallow, and range from 2-6 cm in depth (Ernst, 1970; Rasmussen & Litzgus, 2010b). Average clutch size for the spotted turtle varies based on location, with more southern populations having smaller average clutch sizes (Litzgus & Mousseau, 2004). In Ontario, the average clutch size is 5.3, with no more than one clutch per female per year, and many females not ovipositing every year (Litzgus & Brooks, 1998).

### *1.3 Temperature*

Temperature is a critical determining factor in the success of a turtle nest site, impacting both survival and life history traits of hatchlings (Elphick & Shine, 1998). In order for viable offspring to be produced, eggs need to reach a certain temperature during this period, with low temperatures leading to failure of nest sites (Gutzke & Packard, 1987; Hughes et al., 2009). Additionally, traits affecting survival later in life (such as size, shape, color, behavior, and locomotive performance) can all be impacted by thermal conditions during the nesting period (Booth, 2006). With Ontario being at the northern range of survival for many of these turtle species, temperature of the incubation environment may be

particularly important in this region, having the potential to compensate for both decreased temperatures and incubation periods during the nesting season (Brooks et al., 1991a). Due to the strong correlation with successful incubation and temperature, any change in habitat that affects the temperature can have large impacts on these freshwater turtle species (Lesbarrères et al., 2014).

Temperature has additional implications in species who undergo temperature-dependent sex determination (TSD), as temperatures need to fit a range to produce both female and male offspring (Ewert et al., 1994). Generally speaking, warmer temperatures are required to produce female offspring, and cooler/intermediate temperatures are required for male offspring (Ewert & Nelson., 1991). The painted turtle, Blanding's turtle, snapping turtle, and spotted turtle are all species which undergo TSD (Ewert & Nelson, 1991). For painted turtles, warmer temperatures (30-32°C) produce all female offspring, while cooler temperatures (22-26°C) produce all male offspring, and intermediate and cold temperature (20°C, 28 °C) produce both female and males (Schwarzkopf & Brooks, 1985). In the snapping turtle similar patterns are found, with intermediate temperatures producing male offspring (24-26°C), and temperature extremes producing female offspring (20°C, 30°C). Temperatures between these values produced both female and male offspring (O'Steen, 1998; Yntema, 1976). The Blanding's turtle follows a typical pattern, with males produced in moderate temperatures, and females produced in high temperatures (Gutzke & Packard, 1987). Fluctuations in temperature may also impact offspring sex. A greater

amplitude in temperature variation has led to increased percentage of females in the painted turtle, potentially correlated to the number of hours in a day the eggs were exposed to higher temperatures (Paitz et al., 2010; Wilhoft et al., 1983) It has also be found that increased fluctuations around high and low temperature means may reverse sex ratios in the painted turtle (Neuwald and Valenzuela 2011).

A highly influential factor determining temperature during incubation is canopy cover above the nest site (Cotter & Sheil, 2014; Litzgus & Brooks, 2000). Sites with more vegetation or canopy cover allow less heat to reach the surface, leading to a decrease in temperature of the nest that has the potential to affect both hatchling success and phenotype (Hughes and Brooks, 2006; Refsnider et al., 2013a; Weisrock & Janzen, 1999). Vegetation cover has been seen to be correlated with incubation temperature, as well as sex-ratio of hatchlings (Janzen, 1994; Weisrock & Janzen, 1999). This is particularly important at the northern limit of the species range, as increased canopy cover is likely to decrease temperatures which may result in nest failure (Avery et al., 2000; Janzen, 1994).

Nest depth is another important feature in incubation temperature, as it is correlated with variability in temperature throughout the incubation period (Booth & Astill, 2001). Generally, deeper nests have been found to be cooler than shallower nests, having the potential to affect the sex of the eggs within that nest and having a greater impact than shading of nests (Booth & Astill, 2001).

Different nest regions may experience small temperature differences throughout

the incubation period, a possible mechanism for producing more equal sex ratios (Booth & Astill, 2001). With an increase in nest depth, there is a decrease in temperature, and the amplitude of temperature cycles decrease, creating a more stable temperature (Booth & Astill, 2001; Wilson, 1998). Therefore, it is expected that deeper nests have lower temperatures, decreased temperature variation, and an increased developmental period for the egg (Burger, 1976).

#### *1.4 Moisture*

Moisture conditions within the incubation environment also have a determining factor in the success and life history traits of the embryo (Packard et al., 1988). Typically, studies have shown greater success in wetter substrates (Cagle et al., 1993; Packard et al., 1987; Wilson, 1998) and well drained soils (Dowling et al., 2010; Hughes et al., 2009). For example, a study by Wilson (1998) demonstrated that sites selected by gravid females had greater water content than those at nearby randomly sampled sites, potentially illustrating an importance of sites with greater saturation. A study by Morjan (2003) also found gravid females nested closer to water in order to increase moisture within the incubation environment, even prioritizing hydric conditions over thermal conditions (such as canopy cover) in their nest site choices. Other studies have also reported greater influence of water content on the development of embryos within the incubation period in comparison to temperature, indicating a strong importance of the hydric environment (Cagle et al., 1993). While thermal characteristics may have a greater influence in

early stages of incubation, hydric characteristics may have a greater importance in later stages (Sifuentes-Romero et al., 2018).

In general, survival is typically higher in wetter soils, resulting in decreased mortality compared to drier substrates (Cagle et al., 1993; Packard et al., 1987; Packard et al., 1991; Packard 1987). Moisture content can also influence survival of a turtle beyond hatching. Increased water potential typically leads to increased egg mass, length of incubation, yolk consumption, and size and mass of hatchlings (Cagle et al., 1993; Packard et al., 1987; Packard et al., 1991). Wetter substrates are also correlated with increased performance, producing faster hatchlings than drier incubation environments (Miller et al., 1987; Miller, 1993). Additionally, hatchlings incubated in wetter substrates are found to survive longer without dehydration impacting their performance, indicating further benefits of wetter substrate environments (Finkler, 1999).

As moisture is an important mechanism relating to temperature, its importance during incubation may also be related to its effect on the thermal regime of the nesting environment (Morjan, 2003). Wetter substrates were found to decrease temperatures, and consequently produce more males than drier substrates (Sifuentes-Romero et al., 2018). However, other studies have found that moisture content did not have an impact on sex ratios (Packard et al., 1987; Packard et al., 1991)

### *1.5 Nest Site Selection*

Microhabitat characteristics may be used by gravid females as an indicator of optimal site conditions, and cues may contribute to nest site success, helping ensure suitable habitat is chosen for incubation. Cues may exist either in a sequence of thresholds, or as integrated information (Wood & Bjorndal, 2000). Studies have shown greater survival in sites selected by females than randomly selected sites, demonstrating that nest site selection can impact hatchling survival, and therefore nest site success (Hughes & Brooks, 2006). Nest site selection by gravid females can have a critical impact on the thermal conditions of developing embryos during incubation (Janzen, 1994; Kolbe & Janzen, 2002a). Specifically, sites that were selected by females have been shown to have greater temperature than random sites (Hughes et al., 2009; Schwarzkopf & Brooks, 1987). This may be particularly important at the north the species range, as high-quality thermal sites are especially important, and can act as a limiting factor in these environments (Francis et al., 2019; Hughes et al., 2009).

Nest site selection may be determined by vegetation cues, as female turtles are more likely to select sites with decreased vegetation and canopy cover (Hughes & Brooks, 2006; Janzen, 1994; Weisrock & Janzen, 1999; Wilson, 1998). Impacts of vegetation cover due to selection have been shown to directly impact temperature at incubation and increase survival of hatchlings (Wilson, 1998). Nests that are selected by females are also more likely to be found on slopes than randomly

selected sites, a characteristic also linked to increased temperatures (Burger, 1976; Hughes & Brooks, 2006; Schwarzkopf & Brooks, 1987).

Hydrological conditions have also shown to impact nest site selection. For example, one study showed females nested closer to standing water, directly impacting hydric conditions that lead to increased net success (Morjan, 2003).

Another study revealed that selected sites had an overall greater water content than randomly chosen sites, indicating again that this is a characteristic that may be selected for (Wilson, 1998).

Predation may be another factor impacting nest site selection. Female turtles may favour less optimal sites to decrease predation, particularly when more predators are abundant (Spencer, 2002). Although characteristics such as cover and temperature impact nest site selection, a female turtle may tradeoff the benefits of these characteristics to decrease predation, typically nesting closer to the shore (Spencer & Thompson, 2003).

### *1.6 Substrate*

Some studies have found little impact of the type of substrate on the incubation environment (Packard et al., 1987; Paukstis et al., 1984) whereas other studies have found that selection and fidelity was greater for nest substrates than thermal properties (Rasmussen & Litzgus, 2010b). Nesting substrates have the potential to influence temperature, moisture, incubation period, and offspring sex ratios (Mitchell & Janzen, 2019; Ratterman & Ackerman, 1989). Grain size has been

found to directly impact hatchling survival, increasing the likelihood of nest site success or mortality depending on the properties at nest sites (Mortimer, 1990; Mui et al., 2015; Saito et al., 2019). Finer grained soils may lead to cooler and wetter incubation conditions, whereas coarser grain sizes produce warmer and drier environments (Mitchell & Janzen, 2019; Rasmussen & Litzgus, 2010b; Ratterman & Ackerman, 1989; Saito et al., 2019; Tornabene et al., 2018).

Organic matter is a characteristic that influences nearly all soil properties (Brady & Weil, 2008). In general, organic matter is positively correlated with water holding capacity, and consequently, conductivity, particularly in sandy soils (Hudson, 1994; Minasny & McBratney, 2018). Bulk density, influenced largely by clay and organic content, is inversely correlated with organic matter content (Kimble & Follet, 2000; Envo, 1970; Thomasson, 1978).

### *1.7 Road and development impacts*

Increased cottage country and road development within the Eastern Georgian Bay region pose as a threat to this area's reptile species, as these disturbances are likely to have an impact on the already suffering populations. For populations that require extensive corridors between terrestrial and aquatic habitat, such as these freshwater turtles, roads cause a greater problem, increasing mortality within these species (Hamer et al., 2016; Choquette & Valliant, 2016). Areas with a greater road density generally tend toward a male bias in the population compared to less developed areas (DeCatanzaro & Chow-Fraser, 2010). However, it is possible higher temperatures associated with these roads can lead to an increase in



females in species with TSD (Bowne et al., 2018; Vanek & Glowacki, 2019). Due to the life history traits of longevity, slow growth, and late sexual maturity, high adult mortality is of great concern as it decreases the population's ability to respond to disturbance (Brooks et al., 1991b; Congdon et al., 1994). This is of concern during the nesting season, where large distances traversed increases road mortality in females (Haxton, 2000; Ernst & Lovich, 2009). Particularly, an increase in adult mortality of 10% would decrease the number of adults in the population by 50% in less than 20 years, since compensation for adult mortality has not been shown within this species (Brooks et al., 1991b; Congdon et al., 1994).

### *1.8 Thesis objectives*

Moss and lichen covered rock depressions in the rock barrens of eastern Georgian Bay are known nesting habitats of turtles within this region (Markle & Chow-Fraser, 2014). These depressions are microenvironments unique to the rock barren landscape of Eastern Georgian Bay and vary from the sand environment of southern freshwater turtle nests. Continued development within the Eastern Georgian Bay Region amplifies the already dire need for proper conservation of these reptile species. However, for this to be done, a thorough understanding of each species' critical habitats is necessary. In terms of turtle species within Georgian Bay, there is little known about the nesting environment. The rock filled depressions that turtles have been cited to nest in are different from traditional sand environments of most turtle nests, and are poorly understood, quantified, or

qualified within the literature. To properly address conservation of turtle species within this region, an understanding of all critical life stages is necessary, including the nesting habitat. The overall objective of this study is to complete a characterization of the nesting environment in the Eastern Georgian Bay region. By completing a characterization of nesting habitats within this region, we will have a greater insight of what is currently available to turtles in this region, as well as the distribution of these properties. This will give much needed insight for conservation, and will help guide construction and development of artificial nests in the future.

The first objective of this study is to determine current suitability for turtles on the landscape and determine the range of different environmental conditions available to them. We addressed this objective by completing a landscape level survey using linear transects across eight different wetlands, examining micro-environmental characteristics that have already known effects on the incubation environment, including depth, canopy cover, slope, aspect, and vegetation within a quadrat. This allowed us to determine what is available for turtles nesting in the region, what is the variability of these conditions around different wetlands, and distribution of suitable habitat.

The second objective of our study will be to determine the soil properties of available turtle nesting habitat to characterize conditions used for nesting within this environment. Since soil properties have a considerable effect on the thermal and hydrological regime throughout the soil, it is likely that they will also have a

relative effect on the suitability of the substrate for nesting. Our goal of this part of the study was to determine a range of soil conditions that are available in this region, the change of soil properties with depth, as well as the change of soil properties with cover. We addressed this objective by collecting soil samples around the surveyed wetlands, and processing these samples for bulk density, organic matter, texture, colour, and rock percentage. This allowed us to understand what soil turtles are using within this region (based on what is available to them) as well as how these properties are affected by cover and depth.

## **METHODS**

### *2.1 Study area*

Our study took place in the Eastern Georgian Bay region. Specifically, the area of study is the Parry Sound ecodistrict, an area characterized by a mosaic of open rock barrens, wetlands, and forested regions (Catling and Brownell 1999; Wester et al 2018; Figure 1). Shallow rock depressions present throughout the landscape are a known nesting habitat for turtles within this region (e.g; Litzgus & Brooks, 1999; Markle & Chow-Fraser, 2014). Wetlands in this landscape are also used as habitat by these turtles during different critical life stages. During the course of another study, wetlands within the study area were surveyed for turtles during spring emergence to confirm overwintering habitat. We classified wetlands where turtles were found during the survey as ‘confirmed’, to indicate confirmed use as turtle habitat, and wetlands where turtles were not sighted during the survey period as ‘unconfirmed’ (Markle et al., 2020b). Turtles in our study area have been

confirmed to use habitat surrounding these wetlands as nest sites (Markle et al., 2020b)

## *2.2 Transect survey*

We surveyed eight wetlands during the course of this study, covering approximately 5.22 km<sup>2</sup> of area. In ArcGIS 10.7.1 (ESRI, Redlands, California, USA), we randomly selected 6 points around each wetland boundary as the starting point for 300m transects which were surveyed perpendicular to the indicated starting point, using a compass and yard tape to ensure direction and point of measurement. We chose a 300m long transect based on common distances turtles will travel from their base wetland to their nest site (e.g., Baldwin et al., 2004; Avery et al., 2000; Joyal et al., 2001; Ratterman & Ackerman, 1989). Although turtles such as the Blanding's turtle are able to move a greater distance, the mean distance from wetland to nest site has been measured at 242m (Joyal et al., 2001). Every 1m along this transect, we identified land cover within a 1x1m quadrat, according to characteristics representing the majority of that quadrat (Table 1).

Since it is known that turtles nest in lichen and moss filled depressions (Litzgus & Brooks, 1999; Markle & Chow-Fraser, 2014), we classified any quadrat that had lichen or moss present on the surface as 'depression' and recorded soil depth, canopy openness, ground cover type, moss or lichen depth, slope, and aspect at this quadrat. All other land cover types were recorded, but no further data was collected at these locations.

We measured soil depth by inserting a flag into the soil and measuring the length of the flag that had been within the soil, a method that limits destruction of potential habitat on the landscape. We inserted the flag at each corner in the quadrat as well as in the center of the quadrat to roughly estimate the deepest point, recording the cover above this point and measuring the height of the cover if it was identified as moss (species identified) or lichen (*Cladonia spp*). We then recorded the type and percentage of ground cover on the surface, identifying lichen, moss, and vegetation to species level. Any further identification was deemed unnecessary for the purpose of determining nest suitability. Additional measurements, such as the slope and aspect of the point, were taken using a ruler, a compass, and a cellphone with a levelling application.

To determine nest site suitability we measured canopy openness, as temperature is a key component of nest site success, and canopy cover is a key metric in determining the nest temperature. Canopy cover is also a known factor influencing nest site selection (Litzgus & Brooks, 2000; Cotter & Sheil, 2014). To capture canopy openness, we used a camera with a Sunex 185° SuperFisheye 5.6 mm F/5.6 lens, allowing hemispherical photographs to be taken. We marked the lens to indicate the north direction and placed the camera in a level spot on the ground in this orientation. We then processed canopy photos in Matlab, by determining the number of pixels in the photo containing vegetation based on parameters specified for each day and location of the photo. We inputted

distortion parameters of the fisheye lens, allowing the application to adjust for hemispherical photos taken.

### *2.3 Soil survey*

To assess the soil characteristics across the landscape, we took soil samples around the eight surveyed wetlands. Samples were chosen to fit under three different depth categories (shallow, medium, and deep) and three different ground cover types (lichen, moss, and litter). We classified shallow samples as 3-6cm in depth, medium samples as 6.5-10cm in depth, and deep samples as any depth greater than 10cm. For each depth category, we sampled 9 locations in each of the 3 ground cover types, sampling a total of 81 samples across the landscape. We classified ground cover based on the sample having a majority (>80%) cover of that type. We chose sample sites based on previous knowledge of depth, canopy cover, land type, and cover type taken from the transect surveys. We chose sample sites classified as ‘depression’, that had a canopy openness of 60%, as turtle nests require a relatively open canopy for successful incubation (Brown, 2016; Hughes & Brookes, 2006). We chose sample sites that had a height of moss or lichen less than 6cm, as based on observations earlier in the season, turtles did not nest in areas with a height of moss or lichen greater than 5.5cm.

We collected samples in October 2018. Samples were taken in October to reduce potential of disturbing in-use nests. To excavate soil samples, we cut a 10cm x 10cm square in the ground using a knife, and measured 2cm depth increments. We chose a 10cm x 10cm square to allow for a greater volume, accounting for

possible errors in removing the exact volume of samples in-field. Taking samples in this way ensured we knew the volume measurements for further analysis. We measured the height of the overlying moss, lichen, or litter, removed this layer, and placed it into a labelled bag. We removed 2cm of the soil with a trowel, placed the soil into a labelled bag, and continued to take samples at 2cm increments for the full depth of the sample. Once we collected samples from all 81 sites (totalling 327 10x10x2cm samples), we processed each individual sample for bulk density, loss on ignition, sieving, texture, and colour.

We measured the bulk density by oven drying each sample at 65°C until the sample reached a constant weight and no more mass was being lost. Since we already knew the volume of all samples, we calculated the bulk density by dividing the dry weight of the sample by the in-field volume. However, this gives the bulk density of the total sample and does not take into consideration density of wood, roots, and rocks within the sample. To compensate for this, and obtain a more accurate density of the soil itself, we processed the samples through woody root and soil sieving methods to ensure the bulk density was just the weight of the soil. After oven drying, we put the samples in a soil sieve shaker for 10 minutes in a 2mm sieve, as we assumed that anything above 2mm is no longer mineral soil. Once samples were shaken, we separated anything that was greater than 2mm into wood and rock components and measured these components respectively. We removed any woody or root components that were in the bottom of the sieve and added these to the larger wood components. We then recorded the weight of rock

and wood in each sample and, using recorded bulk densities, calculated the volume of the wood and rock components. Wood samples were assumed to be an average of Jack Pine (*Pinus banksiana*) and White Pine (*Pinus strobus*) bulk densities, as these are the most common trees in the area, and rock was assumed to be granite. We were then able to calculate the mass of the mineral soil, without its rock and wood components.

Following this procedure, we determined the organic matter content of samples through loss on ignition (LOI) processing. We placed an ~ 5 g portion of each sample previously weighed crucibles, which we then burned in an oven at 550 °C for 4 hours. After the four hour period, we lowered the temperature of the oven to 65 °C, and allowed samples to cool down within the oven overnight (to avoid addition of moisture and therefore weight to the sample). We weighed the burned samples, ensuring the time exposed to air was minimized to increase accuracy of measurements. We calculated LOI by taking the difference in the mass of the sample before and after the procedure, divided by the pre-LOI weight of the sample. This resulted in a percentage of how much organic matter was within the 5g sample, representative of the sample as a whole.

Once the soil underwent bulk density and loss on ignition processing, we determined the texture of the soil using the guidelines for feel (Thien, 1979). In this process, we wetted the soil and determined the texture based on the samples ability to form a ball and a ribbon. We also recorded the colour of the soil at this



time using the Munsell colour chart to determine its value, chroma, and common name.

#### *2.4 Statistical analyses*

Statistical analyses were completed using JMP 13 Statistical Software (© SAS Institute Inc.). Data was tested for normality using the Shapiro-Wilks test for normality. All data sets being compared were found to be normally distributed and statistical tests were completed accordingly.

Confirmed and unconfirmed sites were compared for differences in canopy cover, depth, organic matter, bulk density, and rock percentage using a t-test. Confirmed and unconfirmed sites were also tested for differences in land type classification as well as depth category using the chi squared goodness of fit test.

Analysis of Variance (ANOVA) and Tukey HSD tests were completed to determine differences between cover types (moss, lichen, and litter) and values of cover height, soil depth, bulk density, and organic matter. These tests were also completed to determine any differences between depths of samples and organic matter, bulk density, and rock percentage values, as well as differences between texture categories and bulk density and organic matter.

## **RESULTS**

### *3.1 Landscape characteristics*

#### *3.1.1 Available habitat*

The majority of the landscape was forest habitat (62.2%), dominated by depression (22.1%) and peatlands (7.3%). Other habitat areas such as shrub, juniper, bedrock, grass, river, and meadow were more sparsely covered on the landscape. Five of the surveyed wetlands (9000 surveyed quadrats) were classified as confirmed based on the above criteria, and three of these wetlands (5318 surveyed quadrats) were classified as unconfirmed. Confirmed wetlands were surrounded by 24.2% potential nesting habitat (2178/9000) compared to unconfirmed wetlands which were surrounded by a significantly lower percentage of potential nesting habitat (18.5%; 983/5318;  $X^2 (1, N = 14317) = 133.91, p = <0.0001$ ; Figure 2). For two confirmed wetlands, the percentage of quadrats classified as depressions exceeded 30%. Furthermore, distribution of nesting habitat around wetlands typically occurs within 200m of the wetland boundary. For four of the five confirmed wetlands, over 20% of quadrats were classified as depression within 200m of the wetland boundary, whereas this is only true for one of the three unconfirmed wetlands.

#### *3.1.2 Suitable habitat*

Given species depth and canopy cover requirements, suitable nesting habitat on this landscape is limited compared to available habitat (Figure 3). ‘Available’ habitat on the landscape is considered any point under the ‘depression’ classification, whereas ‘suitable habitat’ is considered any point that meets both

the soil depth requirements (dependent on species) and canopy openness requirements (generalized among species to 60%). Species with greater depth requirements, such as the snapping turtle, have a lower percentage of suitable nest sites compared to species who can nest in shallower nest quadrats, such as the spotted turtle (Table 2). Average soil depths around each wetland ranged from 7.1-8.8 cm, ( $\pm 0.27$ - $0.4$ cm) with an overall average of 8.2cm ( $\pm 0.12$ cm) across the landscape (Table 3). Mean soil depth in confirmed wetlands ( $8.0 \pm 0.14$  cm, 2178/3161) was significantly lower than unconfirmed wetlands ( $8.6 \pm 0.22$ cm, 983/3161);  $t_{3144} = 2.598$ ,  $p = 0.0047$ ,  $d = 0.099$ ), however the overall averages were similar between both classifications from an ecological perspective, as demonstrated by Cohen's (1998) effect size value ( $d = 0.099$ ). The majority of quadrats (73.9%) had a soil depth between 0-10 cm, making quadrats with deep soils less common than shallow or medium soils. Soil depths greater than 15 cm were especially uncommon, recorded in 12.7% of the measured quadrats.

Unconfirmed and confirmed sites did not have a significant difference in the percentage of depths above 10 cm ( $\chi^2 (1, N = 2146) = 1.29$ ,  $p = 2.565^{-1} \chi^2$ ).

Average canopy openness for the 2012 quadrats in which canopy cover photos were taken was  $46 \pm 0.29\%$  (4.4-88%), with only 16.3% of quadrats having a canopy openness greater than 60%. However, if we reduce the canopy openness requirement from 60% to 50%, the number of available sites increases from 16.3% to 55.1%. Unconfirmed wetlands had a significantly greater canopy openness than confirmed wetlands ( $t_{2010} = 5.382$ ,  $p = <0.0001$ ,  $d=0.035$ ), however

the difference between the average in confirmed wetlands ( $45 \pm 0.33\%$ , 1527/2012) and unconfirmed wetlands ( $49 \pm 0.59\%$  485/2012) is small from an ecological perspective, as demonstrated by Cohen's (1998) effect size value ( $d = 0.281$ ). Canopy cover does not have a relationship with soil depth ( $R^2 = 0.004$ ).

As both depth and canopy requirements increase, abundance of suitable nest sites decreases (Figure 4). This decrease is observed equally as canopy cover requirements exceed 60% and depth requirements exceed 10cm (Figure 4C-E; Figure 4 H-J). The greatest abundance of suitable sites occurs when there are no limitations on canopy cover and a soil depth requirement of 2 cm (Figure 4P). As soil depth increases, the abundance of suitable sites decreases, however it is never severely limited (Figure 4Q-T). When there are no soil depth requirements, abundance of suitable sites is relatively high at 40% and 50% canopy cover, however becomes limited at 70% canopy cover (Figure 4K-O).

When we consider the type of cover in addition to soil depth and canopy cover, habitat is limited even further. It is most common for turtles to nest in lichen-dominated habitat. Of the 16.3% of quadrats surrounding the surveyed wetlands that satisfy the canopy cover requirements of 60%, only 12.6% were lichen-dominated, bringing the total percentage of depression quadrants of which canopy cover measurements were taken that have a canopy openness of at least 60% as well as lichen-dominated cover to 2.06%. If we take into consideration soil depth, only 6 of the above quadrats had a depth greater than 10 cm, totaling 0.28% of the measured depression quadrats.

### 3.1.3 Surface cover properties

There was a significant difference found between cover type and cover height when looking at moss and lichen species ( $F_{4,2022} = 9.8212$ ,  $p = <0.0001$ ,  $\eta^2 = 0.019$ ). A post hoc Tukey test showed that *Polytrichum* had significantly greater cover height than lichen ( $p = 0.0018$ ), and *Sphagnum* had a significantly greater height than lichen ( $p = <0.0001$ ), *Polytrichum* ( $p = <0.0001$ ), and *Dicranum* ( $p = 0.0053$ ). All other cover heights (lichen, *Polytrichum*, *Dicranum*, Feather moss, and *Sphagnum*) were not significantly different from each other. Soil depth was also significantly different with moss and lichen cover height ( $F_{4,1993} = 23.3548$ ,  $p = <0.0001$ ,  $\eta^2 = 0.045$ ). Soil depth was greater in *Polytrichum* dominated quadrats than lichen dominated quadrats ( $p = <0.0001$ ), however was not significantly different between any other cover heights. Litter was the most dominant cover in depression quadrats, with 711/3161 quadrats having greater than 60% litter. This was followed by lichen (374/3161), bedrock (316/3161), and *Polytrichum* (184/3161). Of the depression quadrats measured, 334/3161 had northern facing slopes, whereas 353/3161 had southern facing slopes.

## 3.2 Soil properties

### 3.2.1 Bulk density and organic matter

Average bulk density and organic matter content measurements for each integrated depth profile as well as dominant ground cover are summarized (Table 3). Integrated profiles had different sample sizes, as medium and deep profiles

had more samples than shallow profiles (due to having a greater overall depth, given the standard 2cm measurements).

Soil bulk density on the landscape ranged from 0.11 to 1.83 g cm<sup>-3</sup>, averaging 0.76 ± 0.02 g cm<sup>-3</sup> (S.E.). Average bulk density in confirmed sites (0.77 ± 0.02 g cm<sup>-3</sup> n=269) was significantly higher than average soil bulk density in unconfirmed sites (0.68 ± 0.04 g cm<sup>-3</sup>; n=47; Figure 5;  $t_{314} = -1.913$ ,  $p = 0.0283$ ,  $d=0.421$ ). Bulk density measurements differed by the dominant cover of the sample, as soil samples that were extracted from litter-dominated quadrats had a significantly greater bulk density than moss and lichen dominated sites (Figure 5; Table 4,  $F_{2,313} = 4.83$ ,  $p = 0.0086$ ,  $\eta^2 = 0.03$ ). Lichen and moss bulk densities were similar and did not vary significantly. Bulk density was significantly greater in deep sites compared to both shallow and medium depths ( $F_{2,313} = 19.0751$ ,  $p < 0.0001$ ,  $\eta^2 = 0.109$ ), however, bulk density was not significantly different with integrated profile depth ( $F_{2,313} = 0.3569$ ,  $p = 0.7001$ ,  $\eta^2 = 0.003$ ). Bulk density measurements taken from a particular depth in a deeper integrated profile had a lower bulk density than those from a shallower integrated profile.

### 3.2.2 Organic matter

Organic matter content on the landscape varied substantially, ranging from 2.9 to 80.4%. The average organic matter content was 12.1 ± 0.5%, with 85% of soils having less than 20% organic matter, indicating a majority of mineral soil in the sampled locations. Organic matter content did not vary significantly between

confirmed and unconfirmed sites ( $t_{322} = 0.805$ ,  $p = 0.2107$ ,  $d=0.170$ ). Samples from moss dominated sites had the greatest percentage of organic matter ( $14.2\% \pm 1.1\%$ ), differing statistically only from litter, which had the lowest amount of organic matter present ( $10.5 \pm 0.44\%$ ; Table 5; Figure 6;  $F_{2,321} = 3.825$ ,  $p = 0.0228$ ,  $\eta^2 = 0.023$ ). Shallow soil depths had the greatest organic matter percentage ( $14.9 \pm 0.75\%$ ), and were significantly different from medium and deep sites ( $F_{2,321} = 49.97$ ,  $p = <0.001$ ,  $\eta^2 = 0.237$ ; Table 5).

### *3.2.3 Soil texture and other soil properties*

Of the 327 2-cm soil increments measured, the majority (72.9%) were classified as having a sandy loam texture. Sandy clay loam, loamy sand, and loam were less common soil textures. Sandy clay loam was more common in deep profiles (Figure 7). Samples classified as sandy clay loam had a greater bulk density than other textures sampled, with loamy sand having the lowest average bulk density ( $F_{3,312} = 7.0418$ ,  $p = <0.0001$ ,  $\eta^2 = 0.063$ ; Table 4). Organic matter content varied with texture, with sandy clay loam having the lowest percent organic matter, and loam having the greatest percentage ( $F_{3,320} = 15.762$ ,  $p = <0.0001$ ,  $\eta^2 = 0.129$ ; Table 5). Sandy clay loam was more common in sites with moss and lichen dominated covers, whereas litter dominated sites had a greater percentage of sandy loam textures.

Colour varied between soil samples, with the majority of samples classified under a variant of black, brown, or grey, and samples with red or yellow hues all taken from confirmed wetlands. The percent of rock (sieved material greater than 2

mm) within a sample also varied, as confirmed sites had a significantly greater percentage of rocks than unconfirmed sites ( $t_{319} = -2.784$ ,  $p = 0.028$ ,  $d=0.462$ ). However, there was a greater number of confirmed samples ( $n=273$ ) than unconfirmed samples ( $n=49$ ). Deep depths had a significantly greater percentage of rocks than shallow depths ( $F_{2,318} = 7.526$ ,  $p = 0.0006$ ,  $\eta^2 = 0.0482$ ).

## **DISCUSSION**

### *4.1 Habitat availability*

Without taking into consideration further characteristics that make sites a suitable nest location, only 22.1% of the surveyed points were classified as ‘depression’ sites. Although other habitat categories could potentially act as nesting habitat, such as peatland or grass (Kolbe & Janzen, 2002a; Milam & Melvin, 2001), in this region the majority of turtle nests occur in moss and lichen covered bedrock depressions (Litzgus & Brooks, 1999; Markle & Chow-Fraser, 2014). As such, available habitat (any point classified as ‘depression’) is limited in this location. However, other factors must be taken into consideration to determine total availability of suitable nest sites on this landscape. Following the conceptual model of Moore et al. (2019) soil depth, temperature, and moisture conditions are all integral in influencing nest site suitability.

Depth affects availability as turtles require a particular depth for nesting, depending on the species, to produce viable hatchlings (Table 2). If these depths are not present on the landscape, a turtle cannot nest at this location, regardless of



suitability of the thermal and hydrological qualities of the soil. It was found that a majority of soils in the landscape were less than 10 cm and deeper soils were relatively limited, with soil depth averaging 8.2 cm. This average is less than the required average nest depth for Blanding's turtle nests, snapping turtle nests, and (in some cases) painted turtle nests (Table 2). Species such as the spotted turtle, requiring depths below the average, are likely to have the most success finding suitable depths for nesting. With only 12.7% of depression sites greater than 15 cm in depth, snapping turtles may have even less success finding suitable depths for nesting within the landscape than other turtle species. Overall, given the range of depth and turtle nest depth requirements for most species, soil depth is limited across this landscape. It is possible, given the shallow range of depths on the landscape, that turtles may be nesting in shallower soils. However, if this is the case, this may result in soil desiccation due to limited water storage in these shallower soils, especially if nest sites are exposed to warm and dry conditions (Moore et al., 2019). Therefore, although this may increase the abundance of possible nest sites, it has the potential to decrease nest site success, as thermal and hydrological conditions have an impact on the survival of hatchlings.

A majority of the surveyed points (62%) were forested. These surveyed locations are unlikely to be used as turtle nesting habitat due to the high canopy cover associated with the classification of forest sites. Temperature is a key component in nest site success, and canopy cover is a major factor that influences the temperature of a nest site (Cotter & Sheil, 2014; Litzgus & Brooks, 2000). Greater

canopy cover allows less solar radiation to reach the surface of a site, which in turn results in cooler nest temperatures, and has the potential to affect both hatchling success and phenotype (Hughes & Brooks, 2006; Refsnider et al., 2013a; Weisrock & Janzen, 1999). Therefore, measuring canopy cover acts as a metric of a key component in nest site success, temperature. Our results show that from the surveyed depression sites, 16.3% of sites have 60% or greater canopy openness. Previous studies have shown selected nest sites typically have little canopy and vegetation cover (Christens & Bider, 1987; Hughes & Brooks, 2006; Legler, 1954). Specifically, Hughes & Brooks (2006) found canopy cover at selected sites averaged 17%, whereas Riley et al. (2014) reported canopy cover ranging from 0-54% and 0-37% for painted turtle and snapping turtle species, respectively. Litzgus & Brooks (2000) found that nest site selection was limited by the openness of the habitat, as it related to its ability to provide a suitable thermal environment during incubation. Given the proposed canopy cover requirements, our results show suitable nest sites are limited by the openness of the habitat in this landscape as well. If the canopy cover requirements were only 10% lower (50% openness) for turtles on this landscape, sites with suitable cover would increase substantially, from 16.3 to 55.1%. This difference shows disturbances (e.g. wildfire) resulting in a change in canopy cover have the potential to have a large impact on availability of suitable sites for incubation. Although decreasing canopy may increase suitability, it is not recommended to

decrease the canopy cover (ie. cut down trees in the landscape) as this can impact many other factors that could negatively affect the habitat.

However, it is not enough to look at canopy cover and soil depth alone. To determine what habitat is suitable for turtle nesting, both soil depth and canopy openness must meet certain conditions, and therefore must be considered together. 'Suitable' habitat was therefore classified as any point meeting both soil depth and canopy openness requirements of each species. When considering canopy cover and depth together, nesting habitat requirements resulted in 10% or lower suitability for all species, ranging from 10.0 to 2.6% of measured depression quadrats (Figure 3). Snapping turtles, requiring the deepest soils, had the lowest percentage of suitable habitat available at 2.6%. Spotted turtles have the highest suitability as they can nest in shallower soils, however, were still relatively limited on the landscape at 10%. Overall, turtle species on this landscape are limited by habitat in regard to both canopy cover and soil depth.

To determine what feature is a greater limiting factor on this landscape, we can look at suitability at different soil depth and canopy cover requirements (Figure 4). If there are no limitations on canopy cover on the landscape, there are still a substantial amount of suitable sites at 10-15 cm and 15-20 cm, and even a relatively large amount of sites available that are greater than 20 cm in depth. This indicates that all species, regardless of depth requirements, will have suitable soil depths for successful incubation if open sites were freely available. However, if we look at canopy cover alone (with no limitations on soil depth), the

abundance of suitable sites starts to see limitations at 60% canopy openness, with little sites available at 70% and 80% openness. Although turtles may nest at 60% openness, this percentage is likely the lower limit, representing the lowest canopy openness likely to result in successful turtle nest sites. Sites with greater canopy openness are more likely to be successful and are likely preferential for turtle species in this region, as turtles are at the northern limit of their range. Given the low number of sites at 70% and 80% canopy openness without any other restrictions, canopy openness is especially limited on this landscape. When looking at Figures 4 A-J, limitations are similar above 10cm soil depth and greater than 60% openness. However, more sites are suitable at 40% and 50% openness (with 10cm depth requirements) than at 2cm and 5cm depths (with 60% openness requirements), further demonstrating greater limitations on canopy openness than depth on this landscape.

Nest depth is important in another regard as its correlation to temperature can have an impact on the survivorship of eggs. With an increase in nest depth, there is a decrease in temperature, and the amplitude of temperature cycles decrease, creating a more stable temperature (Booth & Astill, 2001; Wilson, 1998). Therefore, it is expected that deeper nests have lower temperatures, decreased temperature variation, and an increased developmental period for the egg (Burger, 1976; Moore et al., 2019). This lower temperature and decreased temperature variation may impact eggs of turtle species, such as the snapping turtle, that nest in deeper soils. For these species, a greater canopy openness may

be needed to achieve viable temperatures for incubation as temperature is decreased with depth. This may indicate that the already limited number of available sites are further limited. Research going forward could investigate canopy cover requirements and their impact on incubation within and between species. The impact of depth on temperature variation also has the ability to influence hatchling speed, incubation length, mortality and sex ratio, and therefore may also play a vital role in the determination of a successful site (Ashmore & Janzen, 2003; Neuwald & Valenzuela, 2011; Paitz et al., 2010; Refsnider et al., 2013b; Schwarzkopf & Brooks, 1985).

This limitation of habitat across the landscape is without further consideration of other characteristics impacting suitability such as the type of cover or height of cover above the soil, which have the capability to further limit suitable habitat. Cover may be a particularly important characteristic to consider, as turtles in this landscape predominantly nest on lichen covered soils (Litzgus & Brooks, 1999; Markle & Chow-Fraser, 2014). Although moss dominated plots had greater soil depth than lichen dominated plots in our survey, the greater cover height may contribute to its lesser suitability. Additionally, the ability of lichen to create more stable temperature conditions, prevent desiccation during drought, increase soil saturation of soil, and insulate the underlying soil may make lichen critical components of turtle nest success, and important to include as an additional requirement for nest site suitability (Moore et al., 2019). Lichen was the second most dominant cover in depression sites, indicating that it is relatively available

on the landscape. However, despite lichen being the second most dominant cover in depression sites, only 6 measured sites fit under all requirements of depth greater than 10 cm, canopy openness of greater than 60%, and lichen dominated cover, totaling 0.28% of the measured depression points. This result stresses the limitation of suitable habitat on this landscape, and the need to develop and implement conservation and management tools to help turtles-at-risk during this key life stage.

#### *4.2 Soil properties*

Depth and canopy cover should be the main factors considered when determining suitable habitat, however they are not the only determinants of successful incubation conditions. The soil surrounding eggs can have an impact on the thermal and hydrological conditions during incubation which can impact survival and characteristics of hatchlings. Following the conceptual model illustrated by Moore et al., 2019, along with sufficient soil depth, sites need thermal stability, intermediate saturation, and decreased desiccation potential.

##### *4.2.1 Thermal stability*

Texture can influence the thermal regime of a nest site and contribute to nest site suitability. A study by Mitchell & Janzen (2019) found that painted turtle nests located in three different substrates (sand, gravel, and loam) demonstrated different thermal regimes. Loam soils had lower maximum and mean temperatures than gravel and sand nest sites and were the only male-producing substrates within the study. Through our analysis of soil textures, it was found

that sandy loam was the most common substrate in the area. Of the four textures identified, loam was the least common texture found. Loamy sand and sandy clay loam were uncommon, however sandy clay loam was observed more often in deeper sites. As a majority of the landscape was identified as sandy loam soil, and turtles are confirmed to nest in these bedrock depressions, it is likely that this substrate is being used by gravid females as nesting sites. In this situation, sandy loam may be a favourable soil choice as it has properties of both loam and sand soils, allowing for higher temperatures (potentially contributing to successful incubation), while still having the potential to produce male hatchlings (in regards to TSD in painted turtles). Lower maximum and mean temperatures may also indicate thermal stability, a favourable characteristic for nest site success (Moore et al., 2019).

#### *4.2.2 Intermediate saturation*

Lower temperatures are potentially caused by the increased water content in loam soils as increased moisture content can indicate increased volumetric heat capacity and thermal conductivity of the soil (Abu-Hamdeh & Reeder, 2000; Alnefaie & Abu-Hamdeh, 2013; Mitchell & Janzen, 2019; Ratterman & Ackerman, 1989; Smits et al., 2010). These moisture conditions within the incubation environment can, in addition to temperature, have a determining factor in the success and life history traits of the embryo, and must therefore be investigated further (Packard et al., 1987). Typically, studies have shown greater success in wetter substrates (Cagle et al., 1993; Packard et al., 1987; Wilson,

1998) and well drained soils (Dowling et al., 2010). Finer texture soils are more likely to be classified as poorly drained, whereas an increase in sand content can indicate a soil with better drainage capacity. (CanSIS, 2013; Zhao et al., 2008). In a previous study, it was shown that turtles selected nest sites with greater sand content (Mui et al., 2015). This is potentially due to the increased drainage ability in coarser textured soils like sand. As there was no soil classified under sand within this landscape, loamy sand and sandy loam would be the substrates containing the greatest sand content. The intermediate drainage characteristic associated with sandy loam soil is further evidence that sandy loam soil is a suitable, and therefore likely used soil for nest habitat.

Organic matter can also have a significant impact on the water regime of the soil, increasing the water holding capacity and hydraulic conductivity (Arvidsson, 1998; Gupta & Larson, 1979; Hudson, 1994; Minasny & McBratney, 2018; Thomasson, 1978). Previous studies have shown decreased survivorship in nest sites with greater organic matter content, potentially due to the decreased drainage of the soil at these locations (Mui et al., 2015; Thomasson, 1978). However, it should be noted that decreased survivorship in organic matter sites may also be due to the indication of increased canopy cover (indicating decreased thermal quality) at these sites (Mui et al., 2015). Our results indicate overall low organic matter on the landscape (with 85% of sites having less than 20% organic matter, and an overall average of 12.11%), particularly in depths suitable for turtle nest sites (with shallow depths having a significantly greater amount of organic matter



than medium and deep sites). Therefore, the organic matter content of the soil does not appear to be a limiting factor on this landscape in terms of its effect on the hydrological characteristics of the soil. Organic matter varied by cover type and depth, with the greatest percentage of organic matter occurring in shallow depths and moss covered plots. As organic matter is linked with decreased nest site success, this may indicate a higher suitability for lichen dominated plots. This may be further evidence that, on top of having greater temperature stability, intermediate saturation, and reduced desiccation, lichen is an essential component of nest site success (Moore et al., 2019).

As bulk density is inversely related to organic matter content, and females generally select nests with lower organic matter content, soils with increased bulk density may be favourable (Arvidsson, 1998; Erviö, 1970; Kimble & Follet, 2000). Bulk density was greatest in confirmed sites, litter-dominated sites, and deeper sites. However, in sandy and sandy loam soils, a higher bulk density can mean decreased drainage (Thomasson, 1978). This may decrease the suitability of a site as a nest habitat. With the above information, it is difficult to tell what the impact of bulk density would be at the turtle nesting level, and what a suitable range of bulk density would be. Further research going forward can investigate the impact of bulk density on temperature, moisture, and drainage at the nest specific level.

#### *4.2.3 Desiccation potential*

Since water availability can influence hatchling success (Cagle et al., 1993; Packard et al., 1987; Wilson, 1998), nests require some degree of saturation to prevent desiccation, and textures that increase moisture content (such as finer textured soil) may be more suitable (CanSIS, 2013; Moore et al., 2019; Zhao et al., 2008). However, since this quality of higher moisture content is contradictory to the desired quality of water drainage, it is possible that nests are created in an intermediate of both qualities. Soil texture, specifically the sand and clay content within a soil, can also affect the water availability of a soil, especially at higher tensions (Arvidsson, 1998; English et al., 2005; Gupta & Larson, 1979;). Along with intermediate drainage, sandy loam (the most common substrate on this landscape) also has intermediate water holding capacity compared to other textures. The greater ability to hold moisture along with a moderate drainage ability may allow sandy loam to have a desirable combination of these characteristics, allowing for successful incubation.

Increased gravel and rock particles in a soil sample can also impact hydrological regimes, with the ability to decrease the water holding capacity of a soil.

(Arvidsson, 1998; Saxton & Rawls, 2006) This has the potential to either decrease suitability of the soil (as it can decrease water content within the soil) or increase the suitability of the soil through increased drainage (as drainage is impacted by the water holding capacity in the soil). The exact impact of gravel and rock particles on the water holding capacity and drainage of the soil must be further

investigated for any decisive conclusions. Analysis of our data shows that confirmed sites have a significantly greater percentage of rock material (4.2%) than unconfirmed sites (2.3%). This could indicate a higher suitability for soils in confirmed sites if the difference in drainage due to rock and gravel percentages is significant or a lower suitability for soils if the same is true for water holding capacity.

#### *4.3 Management and conservation*

With many of the turtle species around the Eastern Georgian Bay Region categorized as species-at risk, it is essential to identify potential management and conservation techniques for the critical life stages of these species. Due to current limitations in depth and canopy cover conditions, a possible management solution is to construct nest sites with suitable depth and canopy cover. Restoring or improving nest locations has the potential to increase population growth rates through increased recruitment of juveniles into the population (Reid et al., 2016). Previous implementation of artificial nesting habitat has shown that this strategy can create nests that are successful, have the potential to have higher success rates than natural nests and, in practice, are selected as nest sites by gravid females (Paterson et al., 2013; Buhlmann & Osborn, 2011). Even though many turtle species demonstrate nest site fidelity, both Blanding's turtles and spotted turtles have been recorded to use recently established artificial nest sites (Beaudry et al., 2010). This indicates that suitable sites are able to be detected and used by gravid females if designed properly and placed correctly in the landscape.

#### *4.3.1 Artificial nest recommendations*

Through our analysis, we were able to determine the limiting factors affecting turtle nest site success in this landscape. As soil depth and canopy cover are key limiting factors for turtle nest success in this area, it is essential that these factors are considered when creating artificial nest sites. It is recommended that artificial nest sites should be at least 10 cm depth, with a greater focus on deeper sites (10-20 cm) to ensure that species requiring greater depths are able to nest within the area. Greater depths than required are not necessary, as they have little nest-site specific advantage (Moore et al., 2019). Canopy cover should have at least 60% openness to ensure greater thermal quality of sites, however it is recommended that sites are as open as possible (reaching closer to 90-100% openness), as these turtles species are at the northern limit of their range and require high thermal quality sites (Brooks et al., 1991a). Overall, when constructing nest sites in the Eastern Georgian Bay Region, it is recommended to use sandy loam soil, due to its intermediate water holding and drainage properties, as well as its favourable thermal characteristics. Additionally, as sandy loam was the most common substrate found, and fidelity to nest substrate has been recorded in turtle species before, it is possible that sandy loam is more likely to be chosen by nesting females in this area (Rasmussen & Litzgus, 2010b). It is also recommended that organic matter content within the soil is low (below 20%). Further research must be undertaken on bulk density and rock and gravel particle impacts for conclusive recommendations.

#### *4.3.2 Distribution of nests*

Distribution across a landscape must also be considered when creating and placing suitable habitat for turtle nest sites. Although Blanding's turtles can travel large distances, they typically nest within 400 m of water, with the majority of observations recorded within 300 m (Congdon et al., 2011; Joyal et al., 2001; Steen et al., 2012). Spotted turtle, snapping turtle, and painted turtle species have been recorded to nest within 300 m of water, with distances to wetlands averaging 36.48 , 51.8, and 77.83 m respectively for Ontario species (Beaudry et al., 2010; Milam & Melvin, 2001; Steen et al., 2012). Overall, it is estimated that 95% of nest sites can be found within 232 m of a wetland (Steen et al., 2012). Other studies have found greater turtle abundance in areas that had suitable nesting habitat within 30 m of ponds as well as increased habitat suitability around wetland areas (Marchand & Litvaitis 2004; Millar & Blouin-Demers, 2012). This is congruent with our findings, as the greatest amount of depression sites were found within the first 200 m of wetlands. Additionally, the percentage of depression sites was significantly greater in confirmed sites than unconfirmed sites. More specifically, four out of five confirmed sites had a total of 20% or greater depression classified sites within 200 m of the wetland, whereas this was only true for one of the three unconfirmed sites. An increased population may be present in these wetlands due to the increased availability of nesting habitat within the first 200 m. Although canopy openness and soil depth were greater around unconfirmed sites, these values were not ecologically meaningful (with a

difference of 3% and 0.2 cm respectively), and therefore are unlikely to contribute to turtle abundance.

Given these statistics, it is recommended that artificial nesting habitat is placed within 300 m of confirmed wetlands. However, nest depredation typically increases near both wetland edges (within 50 m) and forest edges (Kolbe & Janzen, 2002b; Marchand et al., 2002; Reid et al., 2016; Strickland et al., 2010; Thompson et al., 2017). Predation may lead to low recruitment, which has the ability to negatively impact population growth, even in a healthy population (Browne & Hecnar, 2007). Therefore, it is recommended that nest sites are placed within 50-300 m of wetlands (to avoid predation) and placed away from forest edges. Nest density may also influence rates of predation, and artificial nest sites should be dispersed evenly, rather than clumped, around the landscape (Kolbe & Janzen, 2002b; Marchand et al., 2002). Placing artificial nest sites on southern facing slopes may increase thermal conditions and may act as nesting cues for gravid females (Burger, 1976; Schwarzkopf & Brooks, 1987). As both abundance and distribution affect the suitability of nesting habitat, a further look into the distribution of suitable habitat in a more concentrated depression area may provide further insight into the ability of this landscape to act as suitable habitat.

#### *4.4 Road impacts*

Since suitable nest sites across this landscape are limited, it is probable that movement during nest season is increased for gravid females in order to find suitable habitat (Baldwin et al., 2004). This could result in increased road

crossings, leading to high road mortality during the nesting season, as well as male-biased sex ratios (Refsnider & Linck, 2012; Shallow & Morrison, 2000; Steen et al., 2006; Steen & Gibbs, 2004). Due to the life history traits of turtles, decreasing adult survivorship can significantly impact a population, with small adult mortality rates having the capability to decrease population sizes in a relatively short amount of time (Brooks et al., 1991b; Congdon et al., 1994; Refsnider & Linck, 2012). Additionally, anthropogenic sites have been noted to be warmer than natural nest sites, encouraging turtles to nest on locations such as roadsides, causing increased risk to nesting females and potential hatchlings (Beaudry et al., 2010; Francis et al., 2019; Refsnider & Linck, 2012). Although it is possible that male-biased sex ratios are not linked to road density in certain environments and urbanized areas have the potential to increase female proportions in species with TSD due to higher associated temperatures, nesting roadside may also increase predation risks and still poses a risk for road mortality (Bowne et al., 2018; Francis et al., 2019; Vanek & Glowacki, 2019). Providing access to close high-quality nesting habitat within 300m of home wetlands may decrease distance travelled and consequently reduce the risk of road mortality in gravid females, potentially impacting the overall survival of these species (Beaudry et al., 2010; Gunson et al., 2016; Paterson et al., 2013; Zagorski et al., 2019;). This could be critical to turtle species conservation, as anthropogenic adult mortality can have devastating impacts on small turtle populations (Howell & Siegel, 2019).

#### *4.4.1 Road development recommendations*

With the greatest number of roads, vehicles, and species of animals in Canada, Ontario is a particular concern for road-wildlife interactions (Gunson et al., 2009). Although placing artificial habitat can increase recruitment and therefore aid population growth, mortality from vehicles can have large negative effects on reptile populations (Paterson et al., 2019; Rytwinski & Fahrig, 2012). Increasing adult mortality by as low as 2% can greatly impact population growth and increase risk of extinction (Spencer et al., 2017). As well, turtle road mortality coincides with peak nesting season, potentially impacting the number of gravid females that are able to nest (MacKinnon et al., 2005).

This may be an increased concern in the Eastern Georgian Bay area as Highway 69 is currently being expanded from a two-lane highway to a four-lane controlled access freeway (Beebee, 2013; Rogers, 2016). Although steps can be taken to reduce mortality risks, these methods (such as barriers to road access and eco passages) require substantial maintenance and are not always effective, with studies demonstrating exclusion failures and lack of eco passage use, resulting in similar turtle abundance on roads with and without these measures (Baxter-Gilbert, 2014; Baxter-Gilbert et al., 2015; Rogers, 2016).

If roads are to be placed within the area, it is essential that critical habitat components are taken into consideration and protected. Included in these critical habitat components is upland habitat, particularly for females during nesting season (Joyal, et al., 2001; Semlitsch & Jensen, 2001). This is of even greater



importance given the limited nesting habitat within this area. As there are currently limited suitable sites, removing suitable habitat through road development may result in even lower recruitment or increased distance travel, both having harmful effects on species populations. Roads also have the ability to change hydrological regimes, impact erosion, change flow of materials and available resources, and alter spatial patterns, which can have various impacts on species habitat (Coffin, 2007; Forman & Alexander, 1998). It is also important to take into consideration connectivity within the landscape, as roads lead to habitat fragmentation and connectivity loss (Cowie, 2011; Forman, 2012). Loss of connectivity and road development decreases biodiversity and can lead to isolation with the potential of species extinction (Crooks & Sanjayan, 2006; Poschlod et al., 2005). New roads may also result in increased human access, which may lead to species decline (Forman, 2012; Garber & Burger, 1995). Given the species at risk status of turtles within this region, increasing harmful effects such as those mentioned may be detrimental, and should be avoided. Development of roads, if necessary, should avoid proximity to wetlands and nesting sites, especially in areas with open canopy and deeper soils. To ensure the appropriate measures are taken, preliminary surveys should be completed to know what species are present, and what habitat those species are using in order to determine critical habitat and essential connectivity corridors in these areas (Gunson et al., 2016).

#### *4.5 Additional implications*

Openness on the landscape and depth of the soil may fluctuate over time due to events such as fires or processes such as afforestation, having the potential to greatly impact nest site quality on this landscape. Fires have the ability to both increase and decrease the suitability of a site for nest habitat. Depending on the intensity, severity, and location of the fire, openness of a site may be increased by burning of vegetation and canopy cover within that area. If the fire is severe enough to impact canopy cover, this increase in light may be beneficial to some species and has the potential to increase species richness, species diversity, and abundance (Bury, 2004; Greenberg, 2001; Harper et al., 2016; Rochester et al., 2010). With canopy cover acting as a limiting factor on this landscape, an increase in openness may create more availability of suitable nest sites. This has been shown to be true in lizard nest sites, where increased canopy cover from forest clearings significantly impacted the thermal environment, increasing the amount of solar radiation reaching a site (Shine et al., 2002). Similar results were found in a Blanding's turtle population, where forest clearing as a conservation effort resulted in increased reproductive success (Reid et al., 2016). Although forest clearing is not suggested as a conservation method on this landscape, this demonstrates a change in canopy (through means such as wildfires) may impact suitability of nest sites. Although fidelity to sites exists, turtle populations have shown to shift use to these newly open habitats in the summer, potentially related to the nesting season (Roe et al., 2020). This increase in thermal quality of

nesting sites may be related to warmer temperatures in soils, lasting up to 3 years post fires (Hossack et al., 2009; Iverson & Hutchinson, 2002; Smith, 1968).

Increased thermal quality may lead to increased recruitment, which has been shown to increase abundance of reptile populations in areas where high intensity burns after fires resulted in heavy tree kill. (Greenberg et al., 1994; Greenberg & Waldrop, 2008). Given our analysis of canopy cover in our study, it is possible that a fire that decreases the canopy by 10% will have a substantial impact on reproduction and nesting in this population.

Although there is some evidence on fire impacts on herpetofauna, previous research typically focuses on the impact of prescribed fires (Ashton et al., 2008; Howey & Roosenburg, 2013; Melvin, 2017; Platt et al., 2010; Roe et al. 2020), with fewer studying impacts of wildfire, and a gap in research investigating impact on nesting habitat in particular. It should be noted as well that many of these studies are completed in different habitats and climates than our region of study, as well as with different species. How one species reacts and adjusts to these disturbances cannot be assumed of other species (Lindenmayer et al., 2008; Moorman et al., 2011; Roe et al., 2020). Some studies have found no differences in movement patterns, habitat use or fecundity in burned sites when compared to unburned areas (Ford et al., 1999; Lindenmayer et al., 2008; Roe et al., 2017; Sanz-Aguilar et al., 2011). The post-fire recovery may also be related to the habitat availability on the landscape pre-fire as well as resource availability post-fire (Lovich et al., 2011; Roe et al., 2020). Sites with high quality habitat pre-fire

may have increased resilience to these types of disturbances and see fewer negative effects. As a site that is already limited in habitat, a wildfire may have detrimental impacts in our study area, and resources may not have great enough availability to allow recovery. Specifically, in our landscape, turtle nesting habitat is not only limited by canopy cover but by soil depth as well. Although a wildfire can have positive impacts for canopy cover, it is likely that a fire severe enough to burn a large amount of canopy is likely to burn soil material or permit increased erosion as well. A post-fire study completed in the rock barrens of Eastern Georgian Bay confirms this, reporting burned open rock barrens having 71-73% fewer suitable sites for turtle nesting than unburned sites (Markle et al., 2020a). Given the current average of 8.2 cm across the landscape and the depth requirements of most turtle species (Table 2), lowering this already shallow average can be detrimental to the Blanding's, snapping, and painted turtle's availability of suitable nest sites. Considering that depth is a large limiting factor, fires are likely to do more to decrease nest site suitability than increase it.

Fire can also lead to direct mortality, injuries, and poorer conditions of herpetofauna, which, due to removal of individuals, can lead to extirpation and extinction of species (Dodd et al., 2016; Esque et al., 2003; Howey & Roosenburg, 2013; Lovich et al., 2011; Lovich et al., 2017; Melvin, 2017; Oliveira et al., 2019; Platt et al., 2010; Rochester et al., 2010; Roe et al., 2019; Sanz-Aguilar et al., 2011). A long-term post-catastrophe study demonstrated a failure of species recovery despite relevant management and recovery strategies in

a turtle population (Keevil et al., 2018). In general catastrophes, specifically those increasing female mortality in turtles, may have long lasting impacts on populations subject to them (Keevil et al., 2018). Given likelihood of increases in future catastrophes (development in the region leading to increased road mortalities and habitat fragmentation, changes in climate leading to increased weather-related events such as wildfires) this is an increased concern (Keevil et al., 2018). Therefore, it is expected that although fires may increase canopy openness, the possibility of adult mortality, failure to recover post-catastrophe and decrease in death may be detrimental for species within this area.

## **CONCLUSION**

The rock barren landscape of Eastern Georgian Bay provides suitable habitat for a variety of wildlife, including seven of Ontario's eight freshwater turtles, all of which are considered at risk (COSEWIC 2008; COSEWIC 2014; COSEWIC 2016; COSEWIC 2018). Although it is known that shallow soil-filled rock depressions throughout the Eastern Georgian Bay region are used as nesting habitat by these turtle species, little is known about the nesting microenvironment for turtles within this area. For a turtle to nest at a particular site, depth must meet certain species-specific requirements, and for eggs to complete successful incubation, warm temperatures must be reached, correlating with increased canopy openness at the northern limit of their range (Brooks and Bobyn, 1991; Cotter & Sheil, 2014; Hughes & Brooks, 2006; Litzgus & Brooks, 2000). We found that soil depth and canopy openness on the landscape was low, averaging 8.2cm and 46% respectively. When considering soil depth and canopy cover requirements together, there were only 2.6-10% suitable sites on the landscape (dependent on species depth requirements), indicating limited nesting habitat on the landscape. Through our analysis, we determined canopy cover to be the most limiting factor for turtle nest suitability in this landscape. As it is known turtles nest in lichen dominated soils, adding this requirement decreased the number of suitable sites even further, emphasizing the limitation of suitable habitat on this landscape.

As nest sites are limited across this landscape, this may increase distance travelled for gravid females seeking a suitable nest site, potentially leading to increased road crossings, and subsequently, increased road mortality (Baldwin et al., 2004; Refsnider & Linck, 2012; Shallow & Morrison, 2000; Steen et al., 2006; Steen & Gibbs, 2004). Construction and placement of artificial nest sites within 50-300m of a resident wetland is a conservation method that, given limited habitat available in this region, is likely to increase nest site success and subsequently population growth.

Soil properties can also contribute to nest site success, impacting hydrological and thermal regimes within the nest environment, and therefore should also be considered when constructing artificial nest sites. High quality nest sites are characterized by thermal stability, intermediate saturation, and decreased desiccation potential (Moore et al., 2019). To determine the soil properties that best fit these characteristics, soil samples were taken across the landscape at different depths and ground cover types and analyzed to determine bulk density, organic matter percentage, texture, colour, and rock percentage. Our survey determined that a majority of the soil on this landscape was sandy loam, a soil likely to increase soil temperatures, increase thermal stability, and have intermediate drainage and water holding capabilities, making it suitable as turtle nesting habitat (CanSIS, 2013; Mitchell & Janzen, 2019; Zhao et al., 2008). Organic matter, likely to be unfavourable in turtle nest sites due to decreased drainage and indication of canopy, was low on our landscape, with a majority of

samples below 20% (Mui et al., 2015; Thomasson, 1978). We recommend that when creating and placing artificial nest sites, open canopy (90% or greater), deep soils (10cm or greater in depth), sandy loam soils (due to intermediate hydrological qualities and favourable thermal characteristics), as well as low organic matter content (below 20%) are used to ensure the greatest success for turtles within this region.

As future disturbances (such as road development and wildfires) are likely to increase, it is even more important to ensure that these species at risk have suitable habitat at all critical life stages, including nesting. Through characterizing the landscape and analyzing the soil within the Eastern Georgian Bay Region, we are able to better understand the environment in which these turtles-at-risk are nesting, and therefore are better equipped to create successful management and conservation solutions for these species.

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**Table 1:** Description of land type classifications for quadrat survey.

<b>Land Type Classification</b>	<b>Description</b>
Bedrock	Area of plain bedrock with no moss or lichen present on the surface
Depression	Bedrock depression filled to a varying degree with inorganic and/or organic material. Moss or lichen is present on the surface.
Forest	Area with a high density of trees, coinciding with high canopy cover.
Grass	Area covered with grass species.
Litter	Area covered by leaves, twigs, or bare soil on the surface.
Loose Rock	Area covered by rock material that was movable on the surface.
Peat	Large deposits of organic material, usually part of a peatland.
River	Area covered by water.
Shrub	Areas completely covered with vegetation.

**Table 2.** Nest depth requirements by turtle species.

	<b>Soil Depth (cm)</b>	<b>Author</b>	<b>Study Location</b>
Blanding's Turtle ( <i>Emydoidea blandingii</i> )	12	Standing et al., 1999	Kejimkujik National Park, Nova Scotia
	15	Dowling et al., 2010	LaGrange, New York
Painted Turtle ( <i>Chrysemys picta</i> )	6-13	Morjan, 2003	Illinois and New Mexico
	10.1 +/- SD 1.2	Schwarzkopf & Brooks, 1987	Algonquin Park, Ontario
Snapping Turtle ( <i>Chelydra serpentina</i> )	12-18	Congdon et al., 1987	Livingston County, Michigan
	14- 21.25 (16.5 average)	Francis et al., 2019	Algonquin Provincial Park, Ontario
Spotted Turtle ( <i>Clemmys guttata</i> )	2-6	Rasmussen & Litzgus, 2010b	Lake Huron, Ontario
	4.5-5.9	Ernst, 1970	Lancaster County, Pennsylvania

**Table 3.** Average characteristics of properties taken around each measured wetland, sorted by habitat confirmation. N refers to the number of soil samples.

	<b>Soil Depth (cm)</b>	<b>Depressions (%)</b>	<b>Organic Matter (%)</b>	<b>Bulk Density (g/cm<sup>2</sup>)</b>	<b>N</b>
Confirmed	7.14 ± 0.32	18.00 (324/1800)	8.55	0.93	12
	8.10 ± 0.27	32.44 (584/1800)	11.97	0.78	27
	7.68 ± 0.36	17.11 (308/1800)	11.51	0.78	3
	7.40 ± 0.30	22.28 (401/1800)	13.71	0.93	5
	8.77 ± 0.30	31.17 (561/1800)	11.86	0.70	21
Unconfirmed	8.60 ± 0.40	13.4 (239/1777)	11.51	0.78	2
	8.66 ± 0.40	18.06 325/1800	14.72	0.65	8
	8.50 ± 0.33	24.07 419/1741	15.38	0.59	2
<b>Total</b>	8.18	22.07	12.11	0.77	80



**Table 4:** Summary of Tukey-Kramer HSD test statistics for bulk density measurements

		Difference	Standard Error Difference	95% Confidence Interval		p-Value
				Lower Bound	Upper Bound	
Cover	Litter, moss	0.1244	0.0411	0.0276	0.2211	0.0075*
	Litter, lichen	0.0881	0.0415	-0.0094	0.1858	0.0867
	Lichen, moss	0.0362	0.0414	-0.0612	0.1337	0.6561
Depth (Full)	Medium, Shallow	0.0390	0.0497	-0.0781	0.1562	0.7126
	Medium, Deep	0.0273	0.0402	-0.0674	0.1220	0.7762
	Deep, Shallow	0.0117	0.0446	-0.0933	0.1168	0.9625
Depth (Integrated)	Shallow, Deep	0.2863	0.0497	0.1692	0.4034	<.0001*
	Medium, Deep	0.1533	0.0565	0.0201	0.2865	0.0193*
	Medium, Shallow	0.1330	0.0392	0.0406	0.2254	0.0023*

Texture	Sandy Clay Loam, Loamy Sand	0.3056	0.0677	0.1307	0.4806	<.0001*
	Sandy Clay Loam, Loam	0.2005	0.1277	- 0.1293	0.5302	0.3971
	Sandy Loam, Loamy Sand	0.1599	0.0565	0.0140	0.3057	0.0253*
	Sandy Clay Loam, Sandy Loam	0.1457	0.04644	0.0258	0.2657	0.0100*
	Loam, Loamy Sand	0.1051	0.1316	- 0.2349	0.4452	0.8550
	Sandy Loam, Loam	0.0547	0.1220	- 0.2605	0.3700	0.9699

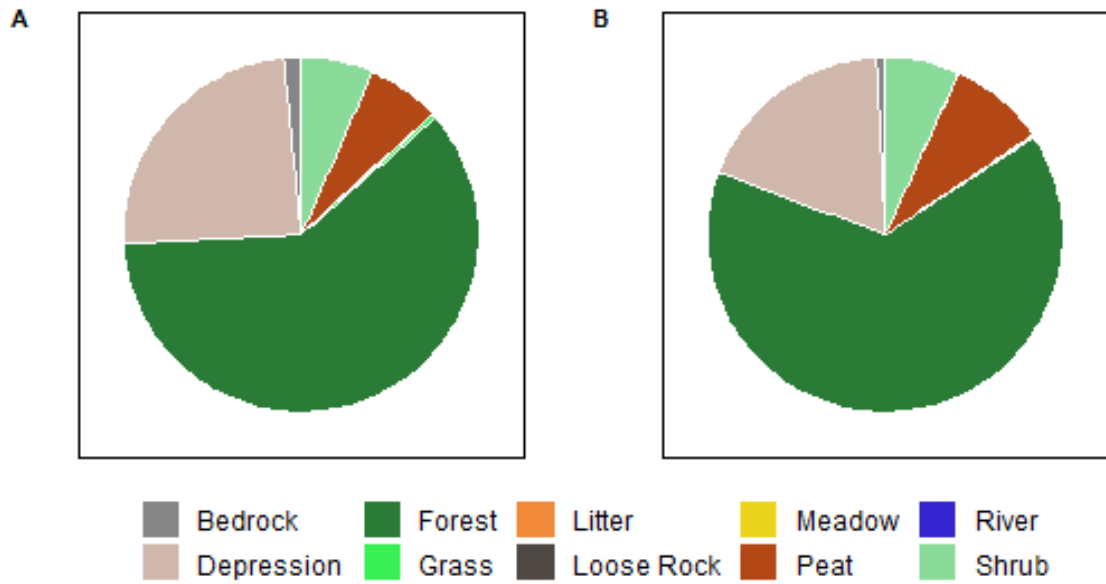
**Table 5:** Summary of Tukey-Kramer HSD test statistics for organic matter measurements

		Difference	Standard Error Difference	95% Confidence Interval		p-Value
				Lower Bound	Upper Bound	
Cover	Litter, moss	0.1711	0.0796	-0.0162	0.3584	0.0815
	Litter, lichen	0.0330	0.0786	-0.1521	0.2182	0.9073
	Lichen, moss	0.2041	0.0788	0.01850	0.3898	0.0271*
Depth (Full)	Medium, Shallow	0.2244	0.0955	-0.0004	0.4492	0.0505
	Medium, Deep	0.0152	0.0762	-0.1641	0.1945	0.9782
	Deep, Shallow	0.2092	0.0851	0.0088	0.4095	0.0384*
Depth (Integrated)	Shallow, Deep	0.6830	0.0823	0.4893	0.8768	<.0001*
	Medium, Deep	0.1710	0.0956	-0.0540	0.3961	0.1747
	Medium, Shallow	0.5120	0.0703	0.3464	0.6776	<.0001*
Texture	Sandy Clay Loam, Loamy Sand	0.7866	0.1260	0.4612	1.112	<.0001*

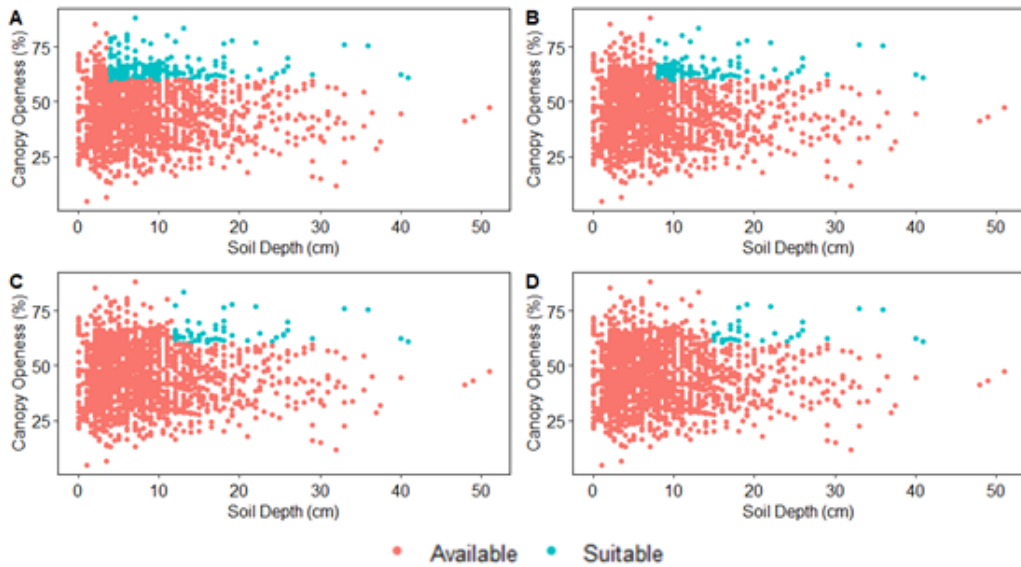
	Sandy Clay Loam, Loam	0.8916	0.2375	0.2781	1.505	0.0012*
	Sandy Loam, Loamy Sand	0.3444	0.1049	0.0736	0.6152	0.0062*
	Sandy Clay Loam, Sandy Loam	0.4423	0.0861	0.2198	0.6647	<.0001*
	Loam, Loamy Sand	0.1049	0.2449	-0.5276	0.7374	0.9736
	Sandy Loam, Loam	0.4493	0.2270	-0.1369	1.035	0.1980



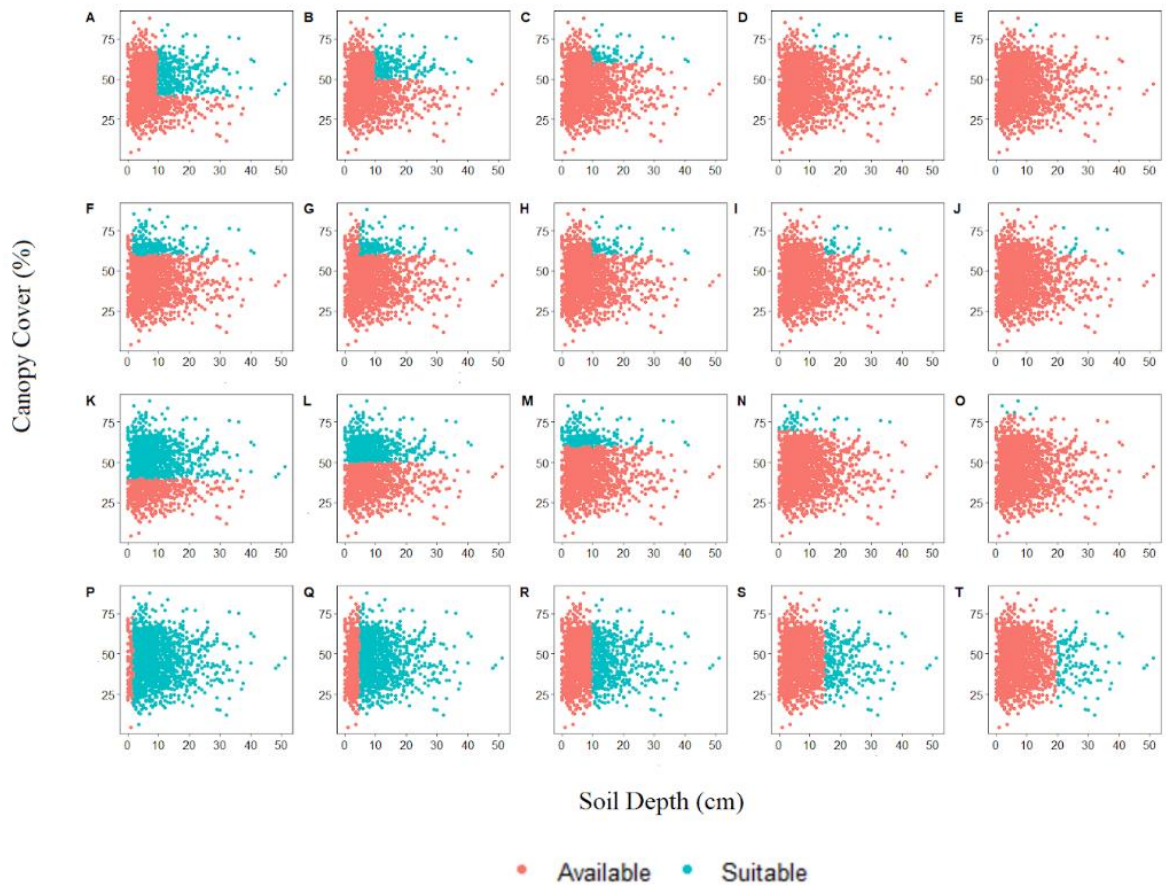
**Figure 1:** Open rock barrens in the Parry Sound Ecodistrict of Eastern Georgian Bay.



**Figure 2:** Distribution of land type across the surveyed area for A) confirmed (n=8998) habitat and B) unconfirmed (n=5317) habitat locations.

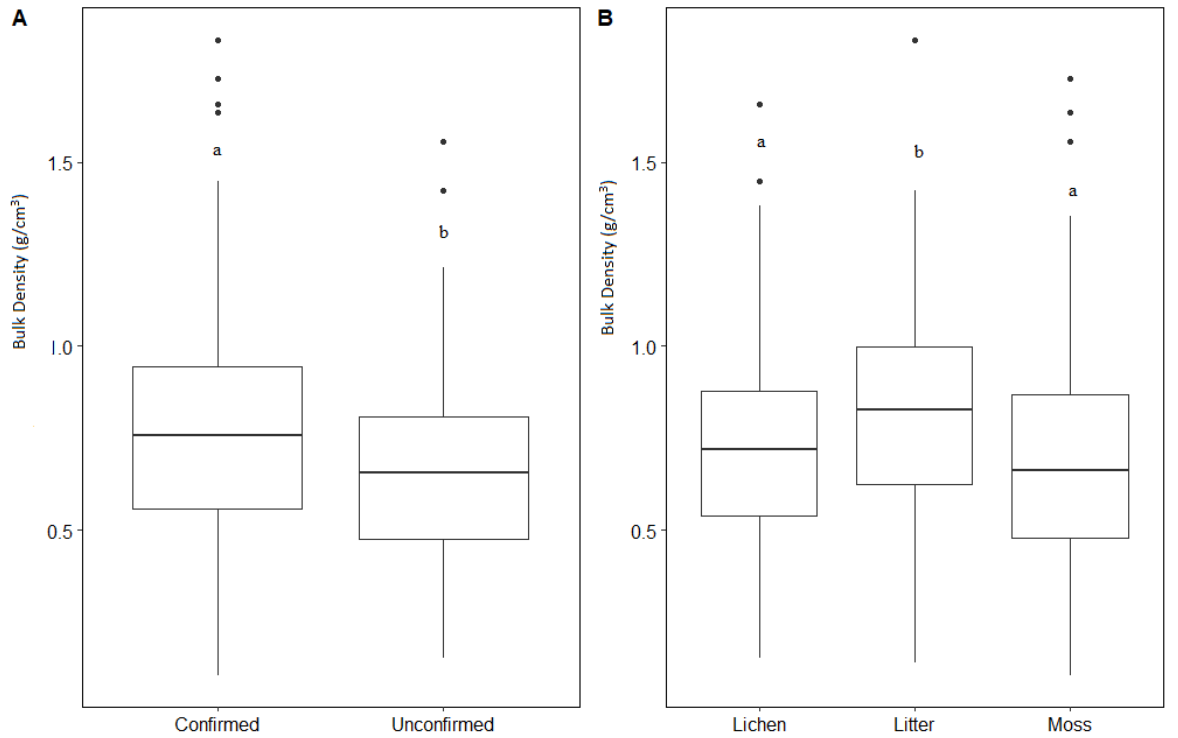


**Figure 3:** Measured soil depth and canopy openness, depicting suitable habitat for A) spotted turtle B) painted turtle C) Blanding’s turtle and D) snapping turtle species. Available habitat is classified as any point labelled as depression, and suitable habitat is dependent on canopy openness and soil depth characteristics. Canopy openness is considered ‘suitable’ if above 60% openness, whereas soil depth is dependent on species (Table 2).

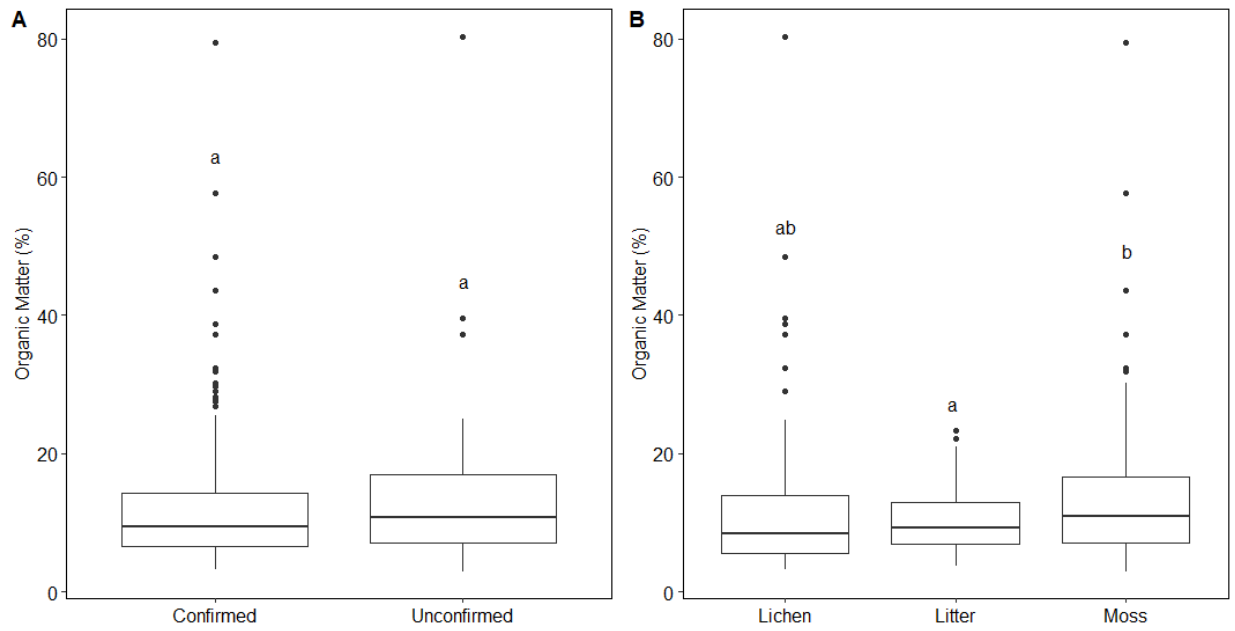


**Figure 4:** Available and suitable habitat given different canopy cover and soil requirements. Available habitat is classified as any point labelled as depression, and suitable habitat is dependent on conditions, whereas A-E have requirements of 10cm or greater soil depth with canopy openness requirements of 40, 50, 60, 70, and 80% respectively; F-J have requirements of 60% or greater canopy openness with soil depth requirements of 2cm, 5cm, 10cm, 15cm, and 20cm respectively; K-O have no soil depth requirements and canopy openness requirements of 40, 50, 60, 70, and 80% respectively; P-T have no canopy cover requirements with soil depth requirements of 2cm, 5cm, 10cm, 15cm, and 20cm respectively.

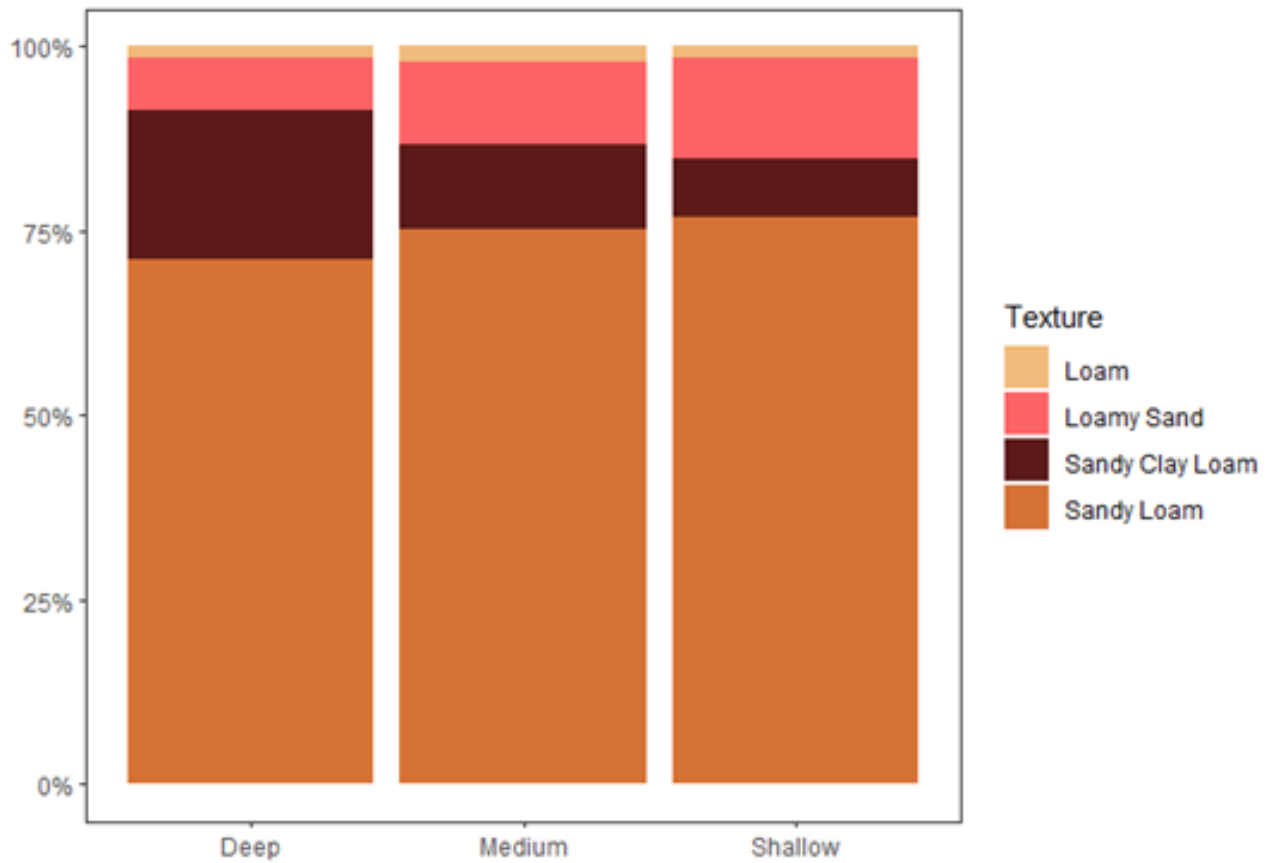




**Figure 5:** Bulk density by A) habitat confirmation of surrounding wetland (n=269 for confirmed sites, n=47 for unconfirmed sites) and B) dominant cover above sample (n=103, 106, 107 for lichen, litter, and moss respectively).



**Figure 6:** Organic matter percentage by A) habitat confirmation of surrounding wetland (n=273 for confirmed sites, n=51 for unconfirmed sites) and B) dominant cover above sample (n=111, 107, 106 for lichen, litter, and moss respectively).



**Figure 7:** Percentage of soil samples of each texture given integrated profile depth (n= 49, 73, 205 for deep, medium, and shallow samples respectively)