Thermal Preference and Habitat Use of Eastern Garter Snakes (*Thamnophis sirtalis sirtalis*) in a Southern Ontario Peatland

By

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Abstract

Garter snakes are the most widespread reptile in North America. Despite occupying vastly different biogeoclimatic zones across their range, evidence suggests that the thermal preference ($T_{sel}$) of garter snakes has not significantly diverged among populations or different *Thamnophis* species. The reason for garter snake’s wide thermal tolerance could lie in their flexible thermoregulatory behaviours and habitat use. I aimed to investigate this relationship by first identifying the $T_{sel}$ of a common garter snake species (*Thamnophis sirtalis sirtalis*) via a thermal gradient in the lab. I used this $T_{sel}$ parameter (27.82 – 32.15 °C) as a benchmark for calculating the thermal quality of open, mixed, and forested habitats all used by the species. I measured the thermal profiles of these habitats by installing a series of operative temperature models that mimicked the thermal properties of living garter snakes and recorded environmental temperatures as living snakes experience them. Lastly, I used coverboards to survey the habitat usage of *T. s. sirtalis*. Of the three habitats, I found that the open habitat offered the highest thermal quality throughout the snake’s active season. In contrast, I recorded the greatest number of snakes using the mixed habitat which had considerably lower thermal quality. Although the open habitat offered the greatest thermal quality on average, environmental temperatures regularly exceeded the upper limits of the animals’ thermal tolerance, greatly restricting the activity window for Eastern garter snakes. Therefore, the open habitat may be less thermally attractive to *T. s. sirtalis*. My data show that not all habitat types used by Eastern garter snakes share the same quality and the relationship is not as simple as warm, open habitats are better. Rather, these animals may preferentially seek out areas that offer a mix of both open and closed-canopy spaces to suit their thermoregulatory needs. In conclusion, *T. s. sirtalis* may select
more thermally stable habitats over those that present them with thermal extremes and
greater constraints to their surface activity.
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Conclusions.......................................................................................................................... 56

Chapter 3: .......................................................................................................................... 57

Evaluating the role of thermal quality on the habitat use of Eastern garter snakes
(Thamnophis sirtalis sirtalis) .............................................................................................. 57

3.1 Chapter Rationale ......................................................................................................... 58

3.2 Methods ........................................................................................................................ 59

Thermal Quality (D_e) – Experimental Design ................................................................. 59

Physical Snake Analogues (PSAs) ..................................................................................... 60

PSA Field Placement ........................................................................................................... 62

Habitat Characterization .................................................................................................... 64

Measuring Operative Environmental Temperature (T_e) ..................................................... 67

Data Analysis of Thermal Quality ...................................................................................... 68

Eastern Garter Snake Habitat Use – Experimental Design .................................................. 69

Coverboard Surveys ............................................................................................................. 73

Incidental Observations of Eastern Garter Snakes .............................................................. 75

Skin-Surface Temperatures of Eastern Garter Snakes in the Field (T_{b\text{field}}) ................. 75

Data Analysis ....................................................................................................................... 76

3.3 Results ........................................................................................................................... 77

Operative Environmental Temperature (T_e) ...................................................................... 77

Thermal Quality (D_e) ......................................................................................................... 82

Habitat Use .......................................................................................................................... 86

Thermal Quality Effects on Habitat Use ............................................................................ 90

Eastern Garter Snake Skin Surface Temperature in the Field ............................................. 92

Anecdotal Observations ...................................................................................................... 92

3.4 Discussion ..................................................................................................................... 92

The Thermal Environment ................................................................................................. 93

Habitat Use .......................................................................................................................... 94

Conclusions ........................................................................................................................ 97

Chapter 4: .......................................................................................................................... 99

General Discussion .............................................................................................................. 99

Thermal Preference (T_{sel}) .............................................................................................. 100

Habitat Use – Study Limitations ....................................................................................... 104

Thermal Quality & Habitat Use ......................................................................................... 105
Thermal Stability ................................................................................................................................. 109
Thermoregulatory Opportunities ........................................................................................................ 110
Activity Time Restrictions .................................................................................................................. 112
General Conclusions & Future Work .................................................................................................. 113
References ........................................................................................................................................... 117
Appendix ............................................................................................................................................. 131
List of Figures

Figure 1. 1: Photo of a typical juvenile Eastern garter snake (*Thamnophis sirtalis sirtalis*) observed in the Niagara region of Southern Ontario. .................................................................27

Figure 2. 1: Housing unit for *T. s. sirtalis* while in captivity at Brock University ..........33

Figure 2. 2: Thermal gradient setup for calculating thermal preference (*T*<sub>sel</sub>) of individual *T. s. sirtalis* .................................................................................................................35

Figure 2. 3: Thermal image of the thermal gradient used in this study .......................36

Figure 2. 4: Sample image from an active *T*<sub>sel</sub> trial showing the snake’s position within the thermal gradient stage (2.23m in circumference) .................................................................39

Figure 2. 5: Locations of an individual Eastern garter snake superimposed on a thermal image of the thermal gradient with the lid and barrier walls removed .......................41

Figure 2. 6: Cumulative activity curves for all 21 garter snakes used for the breakpoint regression analysis performed in R .................................................................44

Figure 3. 1: Sample photo of the dimensions of physical snake analogues (PSAs) used to record *T*<sub>e</sub>. All parts of the instrument were copper.................................................................62

Figure 3. 2: Example placements of PSAs fitted with temperature loggers. .................63

Figure 3. 3: Example canopy cover photos from the forest (A), mixed shrub (B), and open peat (C) habitats to help illustrate how canopy coverage was calculated..........................66

Figure 3. 4: Map of study locations created in Google Earth (2018) .........................70

Figure 3. 5: Example placement of a coverboard in-situ ............................................71

Figure 3. 6: Example diagram of a coverboard aggregate ........................................72

Figure 3. 7: Example photo illustrating how and where individual Eastern garter snakes were marked to estimate recapture rates .................................................................74

Figure 3. 8: Mean operative environmental temperature (*T*<sub>e</sub> ± SE) in degrees Celsius provided by each habitat in each season .................................................................79
Figure 3. 9: Mean daily variation in operative environmental temperature ($T_e$) according to each habitat and hour of day.................................................................81

Figure 3. 10: Mean thermal quality values ($D_e \pm SE$) in degrees Celsius provided by each habitat in each season..................................................................................83

Figure 3. 11: Probability densities representing the distribution of thermal quality values for each habitat during the entire active season...............................................................85

Figure 3. 12: Total number of Eastern garter snake encounters in each habitat according to season..........................................................................................................................87

Figure A. 1: Wide-angle portrait of forest habitat .................................................................134

Figure A. 2: Wide-angle portrait of mixed-shrub habitat ...................................................135

Figure A. 3: Wide-angle portrait of open peat habitat .........................................................136

Figure A. 4: Individual frequency density plots illustrating the distribution of temperatures selected by Eastern garter snakes during their $T_{sel}$ trials.................................................................137

Figure A. 5: GAM smoothing curve illustrating the relationship between signed thermal quality ($D_{es}$) and ACO encounters of Eastern garter snakes.................................................................138

Figure A. 6: GAM smoothing curve illustrating the relationship between signed thermal quality ($D_{es}$) and incidental encounters of Eastern garter snakes.................................................................139
List of Tables

Table 1. 1: A list of indices used to calculate thermal quality and thermoregulatory precision in ectotherms.................................................................16

Table 2. 1: Thermal preference parameters (±SD) for juvenile, fasted Eastern garter snakes (*Thamnophis sirtalis sirtalis*) sampled from a population in Southern Ontario, Canada.................................................................47

Table 2. 2: Results of linear model (LM) performed on Eastern garter snake (n=21) thermal preference where median selected temperature (response variable) was chosen as the proxy for thermal preference........................................................................................................49

Table 2. 3: Results of linear model (LM) performed on the breadth of Eastern garter snake (n=21) T<sub>sel</sub> ranges (response variable)........................................................................................................50

Table 3. 1: Mean values of four abiotic variables measured in each of the three habitats 65

Table 3. 2: Type III analysis of variance results from the LMM performed on operative environmental temperature (response variable).........................................................................................80

Table 3. 3: Type III analysis of variance results from the LMM performed on thermal quality (response variable) measured in absolute value .................................................................84

Table 3. 4: Type III Wald’s analysis of variance results from zero-inflated GLMM performed on coverboard encounters of Eastern garter snakes (response variable), assuming a Poisson distribution .........................................................................................................................88

Table 3. 5: Type II analysis of deviance results from GLM performed on incidental encounters of Eastern garter snakes (response variable), assuming a Poisson distribution. .................................................................................................................................89

Table 3. 6: Results of GAMs performed on thermal quality and Eastern garter snake encounters, assuming a Poisson distribution .................................................................91

Table A. 1: Post-hoc pairwise comparisons of mean operative environmental temperature (T<sub>e</sub>) across all habitats and seasons.................................................................131

Table A. 2: Post-hoc pairwise comparisons of mean thermal quality (D<sub>e</sub>) across all habitats and seasons .................................................................132
Table A. 3: Post-hoc pairwise comparisons of incidental encounters of Eastern garter snakes across all habitats and seasons. ................................................................. 133
Chapter 1:
General Introduction
**Background**

Different strategies have evolved within the animal kingdom to allow species to regulate their internal body temperature (Black *et al.* 2018). Ectotherms rely primarily on their immediate environment and the thermal opportunities therein to regulate their body temperature (Cowles 1957). Maintaining a stable body temperature is important for the optimal functioning and survival of ectothermic species, and is often achieved behaviourally (Cadena & Tattersall 2009; Black *et al.* 2018). In reptiles, temperature influences not only the physiological processes within an individual, but also their behaviour and ecology, as well as their distribution (Blouin-Demers & Weatherhead 2001; Row & Blouin-Demers 2006; Cadena & Tattersall 2009; Black *et al.* 2018; Thompson *et al.* 2018).

In the reptilian body, temperature-sensitive processes exist at the cellular, system, and organismal levels (Tattersall *et al.* 2012). Various biochemical reactions are mediated by temperature, which in turn influences the functioning of systemic processes, such as digestion or locomotion (Seebacher 2005; Cadena & Tattersall 2009; Black *et al.* 2018). The relationship between temperature and physiology is especially strong because temperature affects the stability and structure of the proteins which are the building blocks of a long chain of closely linked reactions (Tattersall *et al.* 2012). Changes in temperature can influence how these macromolecules function, meaning that cellular metabolism and other systemic processes are thermally sensitive (Tattersall *et al.* 2012). Compromised function due to suboptimal temperature, especially over prolonged periods, has been argued to lead to a decrease in individual fitness (Kingsolver & Huey 2008; Angilleta *et al.* 2009). Many biochemical processes and other physical performance proxies (e.g. sprint
speed) reach peak performance within a narrow window of temperatures, and many reptiles (especially those in temperate zones) will invest substantial amounts of energy into regulating their body temperature within this optimal range (Brown & Weatherhead 2000; Herczeg et al. 2006; Cadena & Tattersall 2009; Caldwell et al. 2017; Huey & Pianka 2017). What remains unclear for many reptiles is how wide or narrow these optimal temperature ranges are, and how precisely a given species can live within this range in the wild. There is also uncertainty in how temperature may influence reptilian habitat use, especially of reptiles that live in challenging temperate environments. These uncertainties were topics I aimed to address in my study using a wide-ranging, temperate snake species, the Eastern garter snake.

Thermoregulation

How a reptile regulates its internal body temperature can vary among species. In fact, some reptile species do not employ any forms of thermoregulation and are known as thermoconformers (Tattersall et al. 2012). Thermoconforming species closely match the temperature of their surrounding environment, for example nocturnally active geckos (Huey & Slatkin 1976). Because subtropical and tropical regions typically offer more thermally benign environments than temperate regions, they can support a greater diversity of thermoconforming species (Huey & Slatkin 1976). Temperate regions are characterized by large fluctuations in temperature and limited active seasons which are less conducive to a thermoconforming animal (Huey & Slatkin 1976). Although thermoconforming and active thermoregulating animals are dispersed across many latitudes and climates, I will focus primarily on temperate-zone reptiles that actively thermoregulate to illustrate
different strategies of thermoregulation and some of the costs associated with employing these strategies in the wild.

In temperate regions, most reptiles are considered active thermoregulators, and their internal body temperature can be regulated by physiological mechanisms and/or through behaviour (Tattersall et al. 2012). Behavioural thermoregulation alone can encompass several different strategies which vary in energy cost. Temperate-zone lizards, for example, may utilize shuttling behaviour to physically exploit the thermal environments in their habitat, meaning they actively move between cool and warm microclimates (Cadena & Tattersall 2009; Black et al. 2018). Shuttling behaviour not only requires the animal to move between these different thermal environments, requiring energy expenditure, but it also means the animal may have to temporarily bask in warmer, open environments that may expose them to greater predation risk (Huey & Slatkin 1976; Webb et al. 2005; Cadena & Tattersall 2009; Thompson et al. 2018). Reptiles occupying cooler climes must devote longer periods of time to basking (either in direct sunlight or absorbing radiant heat from their substrate) at the expense of other beneficial activities such as mate searching, foraging, and defending territory (Huey & Slatkin 1976; Row & Blouin-Demers 2006; Cadena & Tattersall 2009; Thompson et al. 2018). Because clear trade-offs exist between regulating body temperature and acquiring resources, reptiles must constantly decide how to allocate their time and energy budgets, and this relationship can be influenced by many factors such as species, individual physiological state, habitat quality, season, etc. (Huey & Slatkin 1976; Blouin-Demers & Weatherhead 2001; Row & Blouin-Demers 2006; Cadena & Tattersall 2009; Caldwell et al. 2017; Sprague & Bateman 2018).
Although shuttling behaviour is a costly thermoregulatory strategy, it is not the only one (Black et al. 2018). Reptiles may also employ subtler behaviours to regulate their body temperature such as adjusting activity times to capitalize on optimal environmental temperature availability (Black et al. 2018). These animals can also make postural changes (i.e. orientation of their bodies) to maximize their exposure to radiant heat, but this strategy can also be used as a heat dissipation mechanism to allow the animal to cool itself (Black et al. 2018). Another strategy used not to gain heat, but rather to lose it, is a gaping behaviour exhibited by some reptile species such as lizards and crocodilians (Black et al. 2018). Heat dissipation strategies are important for reptiles that may be exposed to thermal extremes in their environment and these strategies can permit the animal to dissipate excess heat without the high energetic costs of shuttling (Black et al. 2018). However, gaping also comes with the cost of heightened evaporative water loss and this may not be beneficial in water-limited habitats or seasons (Black et al. 2018). Reptiles in temperate regions may employ a variety of different mechanisms and behaviours to thermoregulate. However, it is important to note that there are inherent energetic and ecological costs associated with all thermoregulatory strategies, and these costs can become even more pronounced at more extreme latitudes where thermal environments change dramatically both temporally and spatially (Row & Blouin-Demers 2006; Tattersall et al. 2012).

**Thermal Preference**

All ectotherms are thought to exhibit an innate preference for a certain range of temperatures (Huey & Slatkin 1976). This preferred range of temperatures is, presumably, what the individual strives to maintain in the wild when environmental conditions permit, and is argued to reflect the optimal temperatures required for maximized physiological
processes such as growth, development, maintenance, digestion, and physical performance (Huey & Slatkin 1976; Hertz \textit{et al.} 1993; Seebacher 2005; Row & Blouin-Demers 2006; Martin & Huey 2008; Angilleta 2009; Cadena & Tattersall 2009; Lelievre \textit{et al.} 2011; Weatherhead \textit{et al.} 2012; Clusella-Trullas & Chown 2014; Buckley \textit{et al.} 2015; Wu \textit{et al.} 2018). Some authors posit that thermal preference is a conserved trait and that different populations of the same species, or even closely related species, have not diverged from a characteristic thermal preference despite occupation of different habitats or latitudes (Rosen 1991; Brown & Weatherhead 2000; Weatherhead \textit{et al.} 2012; Clusella-Trullas & Chown 2014). Although thermal preference has been shown to have a strong phylogenetic affiliation, some studies have suggested that varying levels of plasticity exist among reptile species, allowing them to have rather flexible thermal preferences which may even be acclimatable to different thermal environments (Christian & Bedford 1995; Diaz \textit{et al.} 2005; Seebacher 2005; Clusella-Trullas & Chown 2014; Stellatelli \textit{et al.} 2018; Wu \textit{et al.} 2018). For example, diurnally-active reptiles in tropical Australia have demonstrated (in the lab) a preference for lower body temperatures during the dry season and this has been speculated to be a thermoregulatory strategy allowing for moisture and energy conservation during a period of food and water scarcity (Christian & Bedford 1995; Bedford & Christian 1998). Tocher (1992) reported a strong seasonal effect with geckos in New Zealand preferring colder temperatures in the winter and warmer temperatures in the summer. These observations of thermal preference plasticity have also been demonstrated along latitudinal (Clusella-Trullas & Chown 2014) and altitudinal gradients (Caldwell \textit{et al.} 2017). Despite the appearance of these biological patterns, not all studies are easily comparable given that methods for quantifying thermal preference vary, as well as the indices used to explain
certain thermal preference traits (Gregory 1983; Tocher 1992; Thompson et al. 2018). Before further exploring the ecological and physiological significance of thermal preference, I will define key terms pertaining to thermal preference and highlight how certain thermal indices have changed over the past few decades.

A reptile’s body temperature \( T_b \) can be measured in several ways. Historically \( T_b \) was measured via instantaneous temperature measurements gathered by probing the cloaca of an immobilized individual in the field or lab with a thermometer (see Gregory 1983). With the development of new technologies, \( T_b \) can now be recorded continuously using data loggers implanted into the body of a reptile and later removed and data downloaded (Brown & Weatherhead 2000). Another modern method involves using thermal imagery to capture the body surface temperature of an individual without the need for invasive surgery (Tattersall 2016).

In order to control for some of the costs associated with thermoregulation in the wild that may influence \( T_b \) measurements (e.g. predation risk, access to food or water, etc.), researchers typically use thermal gradients (Hertz et al. 1993). Thermal gradient design may vary between studies but usually consist of closed chambers with a large linear or circular stage that is temperature-controlled and spans several degrees Celsius ranging from hot to cool (Black et al. 2018). Thermal gradients offer experimental animals the equal opportunity to freely choose from a range of temperatures, and the freedom to do so within a low-cost environment (Huey & Slatkin 1976; Hertz et al. 1993; Black et al. 2018). Once a sufficient number of \( T_b \) measurements have been recorded, one can then calculate the species’ setpoint temperature (or thermal preference range), denoted as \( T_{set} \), which refers to the central 50% of body temperatures recorded during a specific timeframe (Huey &
Slatkin 1976; Hertz et al. 1993; Row & Blouin-Demers 2006; Halliday & Blouin-Demers 2006). T_{set} is calculated by separating the T_b dataset into quartiles, with the 25th percentile indicating the lower T_{set} bound and the 75th percentile indicating the upper T_{set} bound (Huey & Slatkin 1976; Hertz et al. 1993; Row & Blouin-Demers 2006).

Although T_{set} experiments are usually performed in laboratory settings (Huey & Slatkin 1976; Row & Blouin-Demers 2006), some studies have used instantaneous field T_{b} measurements to estimate thermal preference (as in Rosen 1991). However, environmental constraints and other pressures associated with thermoregulating in the field may prevent an individual from achieving its desired body temperatures, and as a result, point-sampling techniques from the field may lead to exaggerated ranges and less precise indicators of true thermal preference (Taylor et al. 2004). Recently, T_{sel} (i.e. selected temperature) has been used to specifically refer to the central 50% of selected temperatures within a laboratory thermal gradient to avoid confusion with field T_{b}’s which may be subject to more noise or constraints (Taylor et al. 2004; Wu et al. 2018). For the remainder of this thesis I will use T_{sel} to indicate laboratory thermal preference unless otherwise specified. Other commonly used thermal indices include T_{opt} denoting the optimal temperature at which a particular performance proxy (e.g. sprint speed) reaches maximum performance (Clusella-Trullas et al. 2007). T_{opt} theoretically lies within an individual’s T_{set} or T_{sel} bounds (Clusella-Trullas et al. 2007). Thermal tolerance thresholds are also commonly reported, denoted as CT_{min} and CT_{max}, and these signify the single temperatures below or above which the animal loses locomotor abilities (Clusella-Trullas & Chown 2014).

The thermal preference (T_{sel}) of many reptile species lies very close to their upper temperature threshold (CT_{max}) and this is also true of temperate-dwelling species that may
occupy environments where optimal environmental temperatures are rarely available (Huey & Slatkin 1976; Martin & Huey 2008; Cadena & Tattersall 2009; Huey & Pianka 2017). However, since it is dangerous to maintain body temperatures so close to lethal temperatures, in the wild some reptiles may maintain their body temperatures at the lower end of their thermal preference range and sacrifice maximum performance to avoid overheating (Martin & Huey 2008; Cadena & Tattersall 2009; Huey & Pianka 2017). This maintenance of a slightly lower $T_b$ has been reported for several lizard species and is referred to as negative skew (Cadena & Tattersall 2009; Huey & Pianka 2017). Although not extensively studied, negative skewness may be common in reptiles because of the fact that not far beyond the upper bounds of their thermal preference range there is a precipitous decline in performance and dramatic energetic losses (Martin & Huey 2008; Angilletta et al. 2009; Huey & Pianka 2017). Reptiles can resume surface activity at a wide range of temperatures below their preferred range, but the window of opportunity is much narrower between the upper $T_{sel}$ bound and the animal’s $CT_{max}$ (Martin & Huey 2008; Huey & Pianka 2017). When animals overheat, macromolecules lose their stability and function, proteins denature, and the animal is at risk of death, and this cascade can begin just a few degrees above the upper $T_{sel}$ bound (Tattersall et al. 2012). Although a reptile may experience reduced performance when exposed to temperatures lower than their preferred range, being too cool is rarely fatal, and therefore it is much safer for the animal to maintain slightly lower body temperatures than to operate in hotter thermal environments (Cadena & Tattersall 2009; Huey & Pianka 2017).

Another unusual finding regarding thermal preference is the discovery of some species in colder climes exhibiting similar, or even slightly warmer thermal preferences
than those sampled from warmer environments (Tocher 1992). This phenomenon is known as counter-gradient selection and refers to genetic influences on a given trait opposing the direction of environmental influences (Conover & Schultz 1995). Counter-gradient selection may ultimately result in phenotypic similarities between populations despite large differences in geography (Conover & Schultz 1995). One possible hypothesis for counter-gradient selection in thermal preference is that it is a method of compensating for a limited active season in the temperate environment (Catenazzi & Kupferberg 2017). This relationship has been demonstrated by the gecko species *Hoplodactylus maculatum* (Tocher 1992). Tocher (1992) found that a population sampled from a cooler climate consistently exhibited higher thermal preferences than a population sampled from a warmer climate, and the effect remained even after varying acclimation conditions.

Regardless if a species exhibits counter-gradient selection or not, the question remains as to why cold-climate reptiles prefer such warm temperatures especially when they can be difficult to maintain or achieve. The counter-gradient hypothesis postulates that in colder climates when the growing season is already limited, the selection of warmer temperatures allows animals to speed up their growth rate and partially offset the growing limitations of a shorter active season (Conover & Schultz 1995; Catenazzi & Kupferberg 2017). For temperate-zone reptiles, selection of warmer temperatures permits not only faster growth and spermatogenesis, but also enhanced performance (Tocher 1992; Angilletta *et al.* 2009; Lelievre *et al.* 2011). The evolution and preservation of high thermal preferences in temperate reptile species may be a reflection of the animal’s instinct to maximize physical and physiological performance, energy acquisition, and growth rate to alleviate some of the detriments of prolonged inactive periods (Tocher 1992; Angilletta *et
In warmer environments with longer active seasons, reptiles face different costs associated with thermoregulation such as elevated metabolic rates, oxygen demand, and water loss which may result in the avoidance of hot temperatures (Angilletta et al. 2009). As such, reptile species from more extreme latitudes may evolve similar thermal preferences to relatives occupying tropical regions, thus representing a possible explanation for how counter-gradient selection may arise. Many of the hypotheses proposed above are ongoing and areas of current inquiry in the study of reptile thermal ecology. What is important to note is that temperature is very important to all ectothermic species, and thermal preferences, although hypothesized to reflect optimal temperatures required for maximum performance, may not always be realized in the wild because of the costs and tradeoffs intrinsically linked to thermoregulation.

If we accept the hypothesis that individual driven thermal preferences exist among ectotherms, then this is something that should be measurable using laboratory studies, and in the last 40 years this hypothesis has received much support (Hertz et al. 1993; Brown & Weatherhead 2000; Blouin-Demers & Weatherhead 2001; Row & Blouin-Demers 2006, Yagi & Litzgus 2013). Tropical lizards have been the most commonly studied (Black et al. 2018), however some temperate species such as Northern water snakes (Blouin-Demers & Weatherhead 2001), Eastern milk snakes (Row & Blouin-Demers 2006) and spotted turtles (Yagi & Litzgus 2013) have had their thermal preferences assessed. It has been established that not all reptiles exhibit a preference for the same range of temperatures so measuring thermal preference must be done at the species level (Hertz et al. 1993; Brown & Weatherhead 2000). Compelling patterns have emerged when thermal preference experiments are combined with field experiments looking at habitat use and behaviour, and
such studies have sparked interest into disentangling the complexities of reptile thermal ecology (Row & Blouin-Demers 2006; Yagi & Litzgus 2013; Halliday & Blouin-Demers 2016; Black et al. 2018). Despite the large focus on reptile species, thermal ecology studies have also demonstrated linkages between habitat use and thermal preferences in fish (Pulgar et al. 2005). Natural thermal gradients exist in water bodies (e.g. in intertidal pools) and consequently, fish communities may stratify in the water column by thermophilic species occupying the warmer water higher up, while more thermophobic species occupy cooler water further down (Pulgar et al. 2005). The example of Pulgar et al. (2005) is one of many illustrating how understanding the thermal preference of a species can be indicative of the animals’ ecology, and provides another lens for investigating their distribution, habitat use, and behaviour (Huey & Slatkin 1976; Hertz et al. 1993; Brown & Weatherhead 2000; Blouin-Demers & Weatherhead 2001; Row & Blouin-Demers 2006; Lelievre et al. 2011; Yagi & Litzgus 2013).

**Thermal Quality**

Every species faces challenges in its environment, and for temperate reptiles, achieving optimal body temperature is one of many (Lelievre et al. 2011). Terrestrial reptiles, however, have many choices. In a heterogenous environment, the animals can choose between different substrates such as above ground (e.g. arboreal species), below ground (e.g. fossorial species), or on the ground itself (Brown & Weatherhead 2000; Blouin-Demers & Weatherhead 2001; Row & Blouin-Demers 2006; Lelievre et al. 2011). Gradients of varying sun exposure may also exist depending on the environment, creating different habitat types with varying amounts of sunlight (Row & Blouin-Demers 2006; Elmore et al. 2017). These various habitat types may be equally accessible to a snake or
lizard but can vary considerably in terms of habitat quality. Habitats with varying levels of
sun exposure, moisture availability, and substrate type can all influence the temperatures
available to reptiles, and this temperature availability can make certain habitats more or
less attractive to prospective species (Blouin-Demers & Weatherhead 2002; Elmore et al.
2017). Thermal preferences likely play a role in an animal’s assessment of habitat quality
and can mediate their decisions of which habitat type(s) they select (Blouin-Demers &
Weatherhead 2002; Row & Blouin-Demers 2006; Yagi & Litzgus 2013; Halliday &
Blouin-Demers 2016). In order to quantify just how close the relationship between thermal
preference and habitat selection is, biologists have proposed a parameter of habitat quality
known as thermal quality (Huey & Slatkin 1976; Hertz et al. 1993; Brown & Weatherhead
2000; Blouin-Demers & Weatherhead 2001; Row & Blouin-Demers 2006; Lelievre et al.
2011; Vickers et al. 2011; Yagi & Litzgus 2013). Thermal quality is defined as the
difference between the environmental temperatures available to a species in the field, and
the species’ thermal preference range, measured under low-cost laboratory conditions
(Huey & Slatkin 1976; Hertz et al. 1993; Row & Blouin-Demers 2006; Wu et al. 2018).
However, in order to understand thermal quality and how it is calculated, we must first
understand the distinction between environmental and ambient temperature, and how
environmental temperatures are measured.

Operative environmental temperature (T_e) is defined as the body temperature of an
organism that is in thermal equilibrium with its immediate environment, subjected to the
prevailing convective, radiative, and conductive heat transfer conditions (Hertz et al. 1993;
Thompson et al. 2018). In other words, ambient temperature and operative environmental
temperature are rarely the same under conditions of solar heating or convective cooling
from wind. By implanting temperature loggers inside reptile-mimicking analogues that are built to match the morphological characteristics and thermal inertia of a living animal sitting stationary in its environment, we can obtain \( T_e \) measurements reflective of the thermal environment to which the target species would naturally be exposed (Hertz et al. 1993; Thompson et al. 2018). Operative temperature models (or analogues) are a reliable way of getting in-situ environmental temperature measurements that record temperatures in real time that the target species would be exposed to in a given microhabitat, accounting for the influence of other abiotic factors such as wind speed, solar radiation, and ground heat conduction (Hertz et al. 1993; Vitt & Sartorious 1999; Shine & Kearney 2001; Dzialowski 2005). Usually the analysis of \( T_e \) is restricted to the times of day and year that the species is active, collectively known as the active season or activity time (Hertz et al. 1993). These artificial temperature-recording analogues can record environmental temperatures throughout the active season of a given species, and these measurements can then be used to calculate thermal quality (Table 1). Thermal quality, denoted as \( D_e \), is defined as the absolute deviation between operative environmental temperature and a species’ thermal preference (Hertz et al. 1993; Vickers et al. 2011). Lower deviation values would indicate that the habitat is of higher thermal quality for the target species and would hypothetically require lower amounts of thermoregulatory effort to achieve preferred body temperature (Hertz et al. 1993). Higher deviation values indicate the opposite – that the temperatures of the living environment are not near the animal’s preferred range and therefore larger investments of time and/or energy would be required to achieve preferred body temperature (Hertz et al. 1993), or simply the animal is unable to live within its preferred body temperature range. Brief descriptions of various thermal indices are found
in Table 1.1, and a more detailed formula of the thermal quality calculation can be found in Chapter 3 (Methods).
Table 1. A list of indices used to calculate thermal quality and thermoregulatory precision in ectotherms. Included are brief definitions of each parameter and the methods of how each are measured or calculated.

<table>
<thead>
<tr>
<th>Index</th>
<th>Definition</th>
<th>Calculation method/formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{b(\text{field})}$</td>
<td>Field body temperature</td>
<td>Continuous sampling of cloacal or body surface temperatures in the field</td>
</tr>
<tr>
<td>$T_{b(\text{lab})}$</td>
<td>Lab body temperature</td>
<td>Continuous sampling of cloacal or body surface temperatures in the lab</td>
</tr>
<tr>
<td>$T_e$</td>
<td>Operative environmental temperature</td>
<td>Continuous sampling in the field via temperature logging devices</td>
</tr>
<tr>
<td>$T_{\text{sel}}$</td>
<td>Thermal preference range</td>
<td>Central 50% of lab $T_{b}$ recorded during thermal gradient trial, calculated using interquartile range to denote upper and lower thermal preference bounds. $T_{\text{sel}} = 75^{\text{th}} - 25^{\text{th}}$ percentiles</td>
</tr>
<tr>
<td>$D_b$</td>
<td>Thermoregulatory precision: deviation of $T_b$ from $T_e$ measured in absolute value</td>
<td>$D_b = T_b - T_{\text{sel}}$</td>
</tr>
<tr>
<td>$D_e$</td>
<td>Thermal quality: deviation of $T_e$ from $T_{\text{sel}}$ in absolute value</td>
<td>$\begin{align*} \text{if } T_{e[i]} &amp; &gt; T_{\text{upper}}: &amp; D_e &amp;= T_e - T_{\text{upper}} \ \text{if } T_{e[i]} &amp; &lt; T_{\text{lower}}: &amp; D_e &amp;= T_{\text{lower}} - T_e \ \text{if } T_{\text{lower}} &amp; \leq T_{e[i]} &amp; \leq T_{\text{upper}}: &amp; D_e &amp;= 0 \end{align*}$</td>
</tr>
<tr>
<td>$D_{es}$</td>
<td>Thermal quality: deviation of $T_e$ from $T_{\text{sel}}$ allowing negative and positive values</td>
<td>$\begin{align*} \text{if } T_{e[i]} &amp; &gt; T_{\text{upper}}: &amp; D_e &amp;= T_e - T_{\text{upper}} \ \text{if } T_{e[i]} &amp; &lt; T_{\text{lower}}: &amp; D_e &amp;= T_e - T_{\text{lower}} \ \text{if } T_{\text{lower}} &amp; \leq T_{e[i]} &amp; \leq T_{\text{upper}}: &amp; D_e &amp;= 0 \end{align*}$</td>
</tr>
</tbody>
</table>
Along with other habitat features like resource availability, thermal quality may be an important indicator of habitat quality because it can provide an idea of the thermoregulatory effort required to achieve preferred body temperatures in the wild. For example, forested environments closer to the northern limits of the Eastern milk snake’s range have been found to be of poor thermal quality leading to higher energy investment into thermoregulation, while the preferred open habitats offered more favourable temperatures and overall greater thermal quality (Row & Blouin-Demers 2006). Thermal quality is directly influenced by the landscape and plant communities within them primarily due to the ability of these features to filter incoming solar radiation (Row & Blouin-Demers 2006; Elmore et al. 2017). At more extreme latitudes, heavily shaded environments are generally cooler and offer lower thermal quality to surface-dwelling reptiles like Eastern milk snakes, and this may be why reptile species that inhabit high latitudes typically occupy more open habitats (Huey & Slatkin 1976; Row & Blouin-Demers 2006). In addition, open basking areas are limiting in forested environments and may also (over time) become predictable locations for predation, so not only is it harder for reptiles to thermoregulate in these environments, it can also be more risky (Row & Blouin-Demers 2006).

Different populations of the same species may adjust habitat preferences and behaviours at various latitudes to compensate for changing thermal quality (Weather et al. 2012). Black rat snakes in Canada are exposed to the lowest thermal quality year-round, but do not exhibit different thermal preferences or field body temperatures from populations in Texas (Weatherhead et al. 2012). Northern populations are forced to select warmer environments and invest more time into thermoregulating, while further south they
select cooler refugia or switch to nocturnal activity when daytime $T_e$s become too hot (Weatherhead et al. 2012). The example by Weatherhead et al. (2012) demonstrates how the same species occupying very different thermal environments can survive by employing different strategies to exploit when and where thermal quality is the highest.

**Habitat Selection & Spatial Learning**

Many factors influence an animal’s selection of habitat and usually these factors are thought of in terms of available resources such as food, water, shelter, appropriate nesting sites, and mating opportunities (Goodenough et al. 2010). There are also pressures associated with acquiring these resources such as the risk of predation, competition from conspecifics, and other energy costs which can influence an animal’s assessment of habitat quality (Goodenough et al. 2010). Field studies with reptiles have demonstrated that habitat quality is influenced by the availability of several resources, but there are tradeoffs. For example, Psammodromus lizards select areas with high prey abundance, but this is contingent on the height of the surrounding shrub type (Diaz & Carrascal 1991). Copperhead snakes prefer areas with low shrub and access to small mammal prey, but avoid areas where common kingsnakes (a snake predator) occur in large densities (Steen et al. 2014).

For ectotherms, access to optimal temperatures is also a highly valued resource. Research suggests that temperature selection is prioritized at several spatial scales and can influence a reptile’s use of a certain habitat type, its selection of a microhabitat feature such as a rock crevice, or even its preference for a particular rock (Huey et al. 1989; Pike et al., 2010; Cox et al. 2018). Availability of retreat sites is vital for small-bodied reptiles, especially those that offer suitable thermal conditions (Huey et al. 1989; Pike et al. 2010;
Small-bodied snakes in particular have demonstrated careful selection of retreat sites within their environment which provides an ideal thermal niche; the removal or destruction of these sites can negatively impact these animals and restrict the usable spaces of their habitat (Pike et al. 2010).

Ecological studies have shown tight linkages between thermal quality and broader-scale habitat use in many reptile species (Lelievre et al. 2011; Lelievre et al. 2013; Yagi & Litzgus 2013), and some have even provided empirical support that these animals will favour environments with greater thermal quality versus environments with higher food abundance (Row & Blouin-Demers 2006). A study on Eastern milk snakes in Ontario showed that even though prey abundance was greater in the forest, Eastern milk snakes consistently selected open habitats (Row & Blouin-Demers 2006). The authors had no evidence to support that the snakes catered their habitat preferences to where food was more abundant, but rather they preferentially sought out areas where it was easiest for them to reach their preferred body temperature (Row & Blouin-Demers 2006). Few studies have evaluated the influence of thermal quality against other measurable habitat features like prey abundance, but the study on milk snakes adds weight to the hypothesis that thermal quality may be a reliable predictor of how northern latitude snake species select their habitat (Row & Blouin-Demers 2006).

A possible critique of habitat use being driven by temperature is that snakes are not always spatially aware of the thermally diverse environments available to them, or that they do not have the capacity to remember areas of high or low thermal quality. Snakes may be highly underrepresented in cognitive learning experiments, but studies have shown that they have the capacity to learn tasks, plan routes, and solve problems. In laboratory
studies, garter snakes were capable of rapidly learning how to subdue and consume novel prey items which required learning new visual and chemosensory cues and use of different tactical skills (Krause & Burghardt 2001). Some individuals were even able to navigate mazes to find a food reward (Krause & Burghardt 2001). In another study, corn snakes proved capable of learning the spatial complexities of their environment to locate escape routes without the aid of visual cues (Holtzman et al. 1999). This study concluded that snakes likely use a combination of environmental cues in their spatial learning, some of which are still a mystery (Holtzman et al. 1999). In a more recent study, cottonmouths exposed to a new environment learned how to navigate that environment and could memorize the location of shelter after just one exposure (Friesen 2017). When presented with different visual and chemosensory cues, the snakes learned to exploit specific scents and visuals to select the best ambush sites (Friesen 2017). These studies illustrate how snakes can utilize a variety of different cues to navigate their environment and can distinguish between beneficial or detrimental cues (Holtzman et al. 1999; Krause & Burghardt 2001; Friesen 2017). Since snakes are capable of spatial learning and interpreting different environmental cues (Holtzman et al. 1999; Krause & Burghardt 2001; Friesen 2017), it is possible that some species are capable of recognizing areas of suitable thermal quality and use them to satisfy their thermoregulatory needs.

For reptiles, factors such as scale coloration, body size, and latitude can influence the animal’s thermoregulatory behaviours and the relationship between its thermal preferences and habitat use (Lysenko & Gillis 1980; Trullas et al. 2007; Weatherhead et al. 2012). Some species may rely on thermal cues more heavily during certain times of year or only during certain life stages (e.g. gestation) and this relationship may be absent in
other situations. This is precisely why variables like thermal quality must be investigated on a species-specific basis. In my case, I aimed to investigate the relationship between temperature and habitat use in a subspecies of one of the widest-ranging and abundant reptile species in North America: *Thamnophis sirtalis*.

**Garter Snakes**

The common garter snake (*Thamnophis sirtalis*) is the most wide-ranging and most abundant reptile species in North America (Larsen 1987; Larsen *et al.* 1993; Shine *et al.* 2001; Krause *et al.* 2003). The average body size of common garter snakes can vary widely between sexes but also between localities (King 1989; Larsen *et al.* 1993; Krause *et al.* 2003). Evidence suggests that island populations may grow slightly larger than mainland populations (King 1989), populations in open habitats may grow larger than those in forested habitats (Halliday & Blouin-Demers 2018), and populations at the species’ northern range limit may exhibit larger body sizes than populations further south (Larsen *et al.* 1993). Despite these spatial differences, adults typically range between 44-60 cm snout-vent length (SVL) for males, and between 55-91 cm for females, with adults being identified as individuals with SVLs of greater than 35 cm (King 1989, Larsen *et al.* 1993, Krause *et al.* 2003). Although many subspecies have been described across the garter snake’s range and may exhibit morphological and ecological differences, *T. sirtalis* has ventured further north than any other terrestrial reptile species on the continent (Larsen *et al.* 1993). However, living at extreme latitudes comes with many costs, one being the incredible fluctuations in temperature both daily and seasonally that the animals must cope with at these high latitudes (Aleksiuk 1975; Larsen 1987; Larsen *et al.* 1993). Despite the struggles of being ectothermic in a cold-climate, common garter snakes have become one
of the most successful reptile species on the planet and have colonized environments that are inhospitable to many other ectotherms (Larsen 1987).

Published observations of garter snake natural history may be biased to specific populations that have been heavily studied such as the inter-lake region of southern Manitoba (Shine et al. 2001). However, various studies investigating the habitat use of common garters have corroborated each other in showing the species’ affinity for open habitats (Charland & Gregory 1995; Engelstoft & Ovaska 2000; Kjoss & Litvaitis 2001; Halliday & Blouin-Demers 2015; Diaz & Blouin-Demers 2017). There may be a variety of reasons for why garter snakes might prefer to use open habitats, but the thermal benefit of an open-canopy habitat has been the most discussed (Charland & Gregory 1995; Engelstoft & Ovaska 2000; Halliday & Blouin-Demers 2015; Diaz & Blouin-Demers 2017). In temperate regions, forested environments are buffered from intense solar radiation and although this might allow less extreme temperature fluctuations, it may limit access to suitable temperatures for garter snakes (Charland & Gregory 1995; Halliday & Blouin-Demers 2015; Diaz & Blouin-Demers 2017).

Several publications exist regarding garter snake thermal biology (Gibson & Falls 1979; Lysenko & Gillis 1980; Gregory 1983; Peterson 1987; Rosen 1991). Although some of these studies report thermal preference traits of *Thamnophis sirtalis*, many methodological refinements have been made since these studies were published and therefore the results may be inconsistent with modern thermal preference experiments. For instance, historical studies of garter snake thermal biology have used instantaneous cloacal temperature measurements taken in the field to estimate preferred body temperature or PBT (Gibson & Falls 1979; Gregory 1983; Rosen 1991). Not surprisingly, the results of these
studies have been highly variable, probably due to the inherent issues of taking body temperatures in the field such as the influences of time of day, thermal environment, and predation risk on individual T_b measurements (Taylor et al. 2004). Because various abiotic and biotic constraints may limit an animal’s ability to achieve its desired body temperature in the field, PBT may not be a reliable indication of the animal’s thermal preference and this is why laboratory thermal gradients that can control for thermoregulatory costs are necessary to study thermal preference (Huey & Slatkin 1976; Hertz et al. 1993; Row & Blouin-Demers 2006; Angilletta et al. 2009; Vickers et al. 2011). Lysenko & Gillis (1980) have tested thermoregulatory behaviour and thermal preference of multiple common garter snakes in a laboratory thermal gradient but used a very small sample size (n=4), limiting interpretation of individual thermoregulatory behaviour. Peterson (1987) has used a thermal gradient for investigating thermal preferences of a close cousin of T. sirtalis (T. elegans), but T_sel bounds are not reported for laboratory thermal preference. To my knowledge, only one study has clearly demonstrated the thermal preference range of Thamnophis sirtalis to be between 25.5°C and 27.4°C (Halliday & Blouin-Demers 2016), and although this study was completed over 400 km from my study site, it can serve as an interesting comparison. Ultimately, most historical data on garter snake thermal preference have rendered variable results and only one population within a massive range has had its thermal preference range clearly defined. Therefore, the laboratory thermal preference of T. sirtalis remains a valuable area for future scientific exploration.

Study Purpose

As previously mentioned, historical thermal preference studies of Thamnophis species (Gibson & Falls 1979; Lysenko & Gillis 1980; Gregory 1983; Peterson 1987;
Rosen 1991) have been inconsistent with recent thermal gradient studies (Hertz et al. 1993; Wu et al. 2018). \(T_{sel}\) ranges have been reported for a few North American snake species, however mean selected temperatures are more often reported with an overall mean being close to 30°C (Weatherhead et al. 2012). Halliday & Blouin-Demers (2016) recognized that their calculated thermal preference range for Eastern garter snakes was considerably lower than those calculated for other snake species (mean range between 26.8°C and 30.7°C), and this may be due to differences in experimental design. For example, the authors utilized a linear gradient which can be problematic for some reptile species because experimental animals may be attracted to the corners and become “stuck” in cold ends of a thermal gradient for extended periods (as observed by Cadena & Tattersall 2009). In these circumstances, animals may not be actively selecting temperatures within the gradient, and as a result thermal preference may be biased towards cooler temperatures (Cadena & Tattersall 2009). Despite methodological differences in my approach to assessing thermal preference, I hypothesized that my sample population of Eastern garter snakes would exhibit a preference for a certain range of temperatures near 30 ºC. Given that thermal preference is likely preserved phylogenetically (Clusella-Trullas & Chown 2014), I predicted that the thermal preference range for Eastern garter snakes from my study population would be similar to the values reported by Halliday & Blouin-Demers (2016), with the caveat that using a circular thermal gradient might render slightly warmer thermal preference results.

In addition to exhibiting a preference for certain temperatures, I also hypothesized that Eastern garter snakes use temperature cues to assess habitat quality, and that this would be evident by their disproportionate use of different habitat types. To address this question,
I sampled a population of Eastern garter snakes (*T. s. sirtalis*) from a large wetland complex in the Niagara region of Southern Ontario (more in Methods: *Study site*). This area boasts a diversity of habitat types from open peatlands, mixed successional zones of shrubs and young hardwood trees, to mature deciduous forest stands. Because plant communities, canopy cover, and substrate greatly differ among these habitats and can affect the ground-level thermal environment (Row & Blouin-Demers 2006; Lelievre *et al.* 2011; Elmore *et al.* 2017), I anticipated that each habitat would have distinct operative environmental temperature availability and thermal qualities for Eastern garter snakes. I define thermal quality as being the deviation of environmental temperature from the $T_{sel}$ bounds of Eastern garter snakes, with lower deviations being indicative of higher thermal quality (formulae in Chapter 3: Methods). Open habitats have been described previously as being areas of higher thermal quality than closed-canopy environments (Charland & Gregory 1995; Halliday & Blouin-Demers 2015; Halliday & Blouin-Demers 2016; Diaz & Blouin-Demers 2017; Elmore *et al.* 2017), and I predicted that I would corroborate this observation by recording the highest thermal quality in the open habitat, with moderate and low thermal quality being recorded in the mixed and forested areas respectively.

The lack of ecological studies of Eastern garter snakes in this part of their range also presents a current knowledge gap regarding what habitat types are preferred by Carolinian populations of *T. s. sirtalis*. Few studies have quantified the abundance of *T. sirtalis* in intermediate (mixed) habitats which represent a matrix of open and closed-canopy spaces. In addition, throughout their wide range garter snakes may explore many different types of open environments, not just fields or meadows which have been more commonly studied. My open habitat comparison is unique in that it is a largely exposed
peatland and may have different thermal characteristics. I hypothesized that thermal attractiveness is a predominant cue used by Eastern garter snakes in their use of habitat, during the active season. I predicted greater abundances of Eastern garter snakes in the habitat(s) that offered the most favourable thermal environment (greatest thermal quality). The overall purpose of my study was to investigate the poorly understood thermal ecology of Eastern garter snakes, and perhaps shed light on a mechanism of habitat use that may have contributed to the species’ success in North America.
Figure 1. 1: Photo of a typical juvenile Eastern garter snake (*Thamnophis sirtalis sirtalis*) observed in the Niagara region of Southern Ontario. This species can be extremely polymorphic so not all species observed were as pictured. *T. s. sirtalis* is one of several recognized subspecies of the common garter snake (*Thamnophis sirtalis*) and is native to Eastern North America.
Chapter 2:
Identifying the thermal preference ($T_{sel}$) of a population of Eastern garter snakes
($Thamnophis sirtalis sirtalis$) in southern Ontario
2.1 Chapter Rationale

Although common garter snakes are among the most heavily studied reptiles, much of what is known about their natural history comes from studies on a few select populations from wide-ranging localities (Shine et al. 2001). The thermal biology of *Thamnophis* species has been a topic of much research, but historical studies have either not clearly defined the thermal preferences of *Thamnophis sirtalis*, or the methods used to quantify thermal preferences were not consistent with agreed upon $T_{sel}$ studies (Gibson & Falls 1979; Lysenko & Gillis 1980; Gregory 1983; Peterson 1987; Rosen 1991). I aimed to clearly define the upper and lower $T_{sel}$ bounds of Eastern garter snakes (*T. s. sirtalis*) at my study location in southern Ontario and not borrow values available in literature. Clearly defining the $T_{sel}$ parameters was necessary so that these values could be used as benchmarks for quantifying the thermal qualities of different habitat types used by my study species (Chapter 3). Without evidence that garter snakes do in fact select a common range of temperatures in laboratory setting, which has been demonstrated to correlate well with reptilian thermal selection in the field (Clusella-Trullas & Chown 2014), it would not be possible to make conclusions regarding thermally-influenced habitat use which I discuss in the next chapter.

I recognize that some reptiles can exhibit plasticity in their thermal preferences. For example, larger animals (on average) may select warmer temperatures than smaller-bodied animals (Seebacher 2005), and gravid females typically select higher temperatures than nongravid counterparts (Blouin-Demers & Weatherhead 2001; Lorioux et al. 2013). Seasonality may also provoke shifts in thermal preferences (Christian & Bedford 1995; Diaz et al. 2005; Stellatelli et al. 2018), exhibited by some species selecting lower
temperatures during hotter and drier seasons to prevent excessive evaporative water loss (Christian & Bedford 1995; Ladyman & Bradshaw 2003) or to conserve energy (Bedford & Christian 1998). The thermal environment of selected habitats may also lead to directional selection of thermal preferences, as Tocher (1992) demonstrated with two gecko populations from different thermal environments expressing distinct thermal preferenda. Acknowledging the potential for these various effects to influence individual T_{sel}, I focused my study on fasted, juvenile animals (<1-year-old). By doing so, I could limit some of the variability of thermal preferences such as thermophilic responses of recently fed animals (Wang et al. 2003; Sievert et al. 2013) or gravid females (Brown & Weatherhead 2000; Lorioux et al. 2013). I incorporated the effects of body condition, capture location, and capture season on Eastern garter snake T_{sel} into my statistical model since these relationships have not yet been demonstrated for the species. In addition, analyzing the effects of habitat and season on garter snake thermal preference may help inform observations of habitat use discussed in Chapter 3.

2.2 Methods

Study Design

I sourced my study animals from a wild population in the Niagara region. In total, I sampled 22 individuals (mean SVL = 27.3 ± 4.4 cm, mean weight = 13.3 ± 4.3 g) from various habitat types and gave each animal time to adjust to the laboratory setting prior to experimentation. For every individual, I calculated several thermal preference parameters including mean and median selected temperatures and T_{sel} ranges, and then averaged these values to obtain thermal preference parameters characteristic of my study population.
**Study Site**

My study site is a large wetland complex spanning a total of 1460 ha of the Niagara region in Southern Ontario (Yagi & Litzgus 2012). The wetland area consists of a high diversity of wetland habitat types including peatland, bog, deciduous swamp, and other successional areas (Yagi & Litzgus 2012). This wetland complex lies within the historical floodplains of Lake Erie and is the largest of its kind in the region (Yagi & Litzgus 2012). The wetland’s unique composition of *Sphagnum* moss and peat deposits has led to large-scale peat extraction throughout the 20th century, greatly disturbing the local ecology (Yagi & Litzgus 2012). Despite this large-scale disturbance, eight snake species can still be found at this site, the most common being the Eastern garter snake. Because of the sensitivity of this study site, its exact location is not disclosed for the protection of the rare biological communities that are still in recovery there.

**Acquiring Animals**

Prior to collecting individuals, approval was sought from Brock University’s Animal Care Committee (AUP#17-01-01). Eastern garter snakes are a common species found in this study site and were captured opportunistically during routine surveys. When an individual was captured, its snout-vent length was measured with a tape measure and its weight was recorded using a Pesola™ scale (to the nearest 0.1 g). The time of day, habitat type, and coordinates of the capture location were also recorded for every animal captured. Animals were caught by hand, and after biological and environmental measurements were taken, the animal was placed into a breathable pillow case for transfer back to the laboratory at Brock University. No more than two individuals were ever captured on the same day. A total of 22 Eastern garter snakes were captured between May and September of 2017. Only
juvenile animals (<1-year-old) were used and were identified as individuals with SVLs of less than 35 cm.

**Snake Husbandry**

Snakes were held individually in a housing unit in the laboratory at Brock University for a maximum of seven days (Figure 2.1) and were kept under a 12-hour light: 12-hour dark photoperiod. Real photoperiod can vary between 14.5-hour light in mid-May to 11.7-hour light in late September, however I ran all T\textsubscript{sel} trials between 0900-1900 which is within daylight hours for the entire study period. The housing unit was partially heated so that a gradient of temperatures was available to the snakes during housing, along with other necessary environmental enrichment as agreed upon by Brock University’s Animal Care Committee (AUP#17-01-01). The housing unit was misted with water every morning to maintain humidity, and water dishes were cleaned and filled with fresh water daily. Upon arrival to the lab, every snake was offered an earthworm cut to 5cm in length. After eating, the snake was left undisturbed in the housing unit for a minimum of 48 hours prior to experimentation. This 48-hour period was an adjustment period for the animals to become accustomed to the laboratory environment and have time to digest any food items. After initial feeding, the snakes were not offered any other food and only fresh water was provided. The 48-hour period allowed us to ensure that every animal used for experimentation was in a similar physiological state to minimize this as a variable that could influence thermal preference results. If the snake did not take the food, either because it had just recently fed or was preparing to shed its skin, the animal was treated as any other. However, if it was obvious that the snake was preparing to shed within a matter of days (evident by dull scale coloration, lifting scales, and/or opaque eye color) then the
snake was held for a longer period until it shed in captivity and it was fed immediately after shedding. After this animal was fed, then its 48-hour fasting period began. Since shedding can influence the thermoregulatory behaviour of snakes (Sievert et al. 2005), I considered it necessary to hold these animals for longer so that I could ensure normal behaviours prior to experimentation. After the snakes completed their adjustment period, they could then proceed to the thermal preference experiment.

Figure 2.1: Housing unit for T. s. sirtalis while in captivity at Brock University. No more than two individuals were kept in the lab simultaneously. The housing unit included a divider to separate the snakes, and both halves of the unit had shelter, a water dish, and enough bedding (coconut husk) so that the snakes could bury beneath it. A heat pad (maximum temperature ~35°C) was placed beneath the far end of the housing unit so that a temperature range of approximately 23-33°C was available to the snakes to support their thermoregulatory needs.
Thermal Gradient Setup

Thermal preference ($T_{sel}$) of Eastern garter snakes was determined by placing individual snakes into a thermal gradient (Figure 2.2) for a total of 10 hours and documenting their movements within the gradient. The thermal gradient I used was specially designed by Brock University’s machine shop. Copper pipes fixed beneath the floor of the gradient were connected to a hot-water bath and cold-water bath on either side and transferred heated or chilled water along the base of the apparatus to create a stable gradient of temperatures. The floor of the thermal gradient ranged in temperatures from 15ºC in the cold end to 40ºC in the hot end (Figure 2.3). Temperatures along the gradient were measured with a thermocouple meter (Sable Systems, model TC-1000) to the nearest 0.01ºC before the experimentation began, midway through the study, and after data collection concluded to make sure there was no drift of temperatures or malfunctioning of the thermal gradient. The gradient chamber was also fitted with a weighted lid to prevent animals escaping and so that internal temperatures of the chamber could be maintained. The thermal gradient’s stage was homogenously lit by a light source fixed above the apparatus, while the setup was enclosed in black-out curtains which eliminated extraneous ambient lighting from polluting study images (Figure 2.2). A high-resolution webcam was situated approximately 1.5 m above the stage of the thermal gradient so that images of the snake’s position could be taken (Figure 2.2). Images were captured every 30 s using an image capture for a total of 10 hours and were later processed in ImageJ so that temperatures could be associated with every position of the snake. The inside of the thermal gradient’s stage was misted before every trial so that the snakes had access to water during experimentation. After the experimental trial finished, the snake was removed, and the
stage of the thermal gradient was disinfected with Lyso™ wipes. Lastly, the thermal gradient was propped up on 15cm of insulation and foam padding to serve as shock absorbers so inadvertent building vibrations would not be disturbing to the snakes during experimentation.

Figure 2. 2: Thermal gradient setup for calculating thermal preference ($T_{sel}$) of individual *T. s. sirtalis*. The setup was equipped with an auto-focusing web camera which captured images of the snake every 30 seconds for 10 hours. A light source was enclosed over top of the thermal gradient and ambient lighting was eliminated via the use of blackout curtains so that the gradient’s stage could be homogenously lit. The gradient was propped up on 15cm of insulation and foam padding to serve as shock-absorbers to minimize disturbance. Not pictured is the hot-water bath (HAAKE, model DC-10) or cold-water bath (HAAKE, model SC-100).
Figure 2. 3: Thermal image of the thermal gradient used in this study. For my study, temperatures ranged from 15°C in the cold end (blue/purple colour) and 40°C in the hot end (yellow/white colour). The track of the thermal gradient was the only part of the gradient which was accessible to the snakes during experimentation and is defined as the area between the black dashed lines. The thermal image was taken with the lid and inner bumper removed.
Calculating Thermal Preference

A total of 1204 images of snakes were captured over the course of each 10-hour experiment. The entire image sequence was downloaded from each $T_{seq}$ trial and imported into ImageJ for image analysis. With ImageJ software I manually tracked the location of the snake in the gradient (using ImageJ’s manual tracking plug-in) for every image in the sequence and obtained a set of cartesian $(x,y)$ coordinates with $x,y = 0,0$ as the lower left of the image. I used the following series of calculations to convert the snake’s head position to a selected temperature (°C) with Figure 2.4 providing a visual reference and sample coordinates.

Because the center of the image space did not correspond directly to the center of the gradient circle (centroid), I subtracted the cartesian coordinates of the centroid from those of the snake’s position in the thermal gradient.

$$\text{Centering image} = \text{Snake } (x,y) - \text{Centroid } (x,y)$$

I then converted the cartesian coordinates (pixels) of the snake’s centered position to polar coordinates $(r, \theta)$ where $r$ is the hypotenuse of the triangle prescribed by the $x$ and $y$ distance from the centroid center, and $\theta$ was determined using the following formula:

$$\begin{align*}
\text{If } y < 0, \quad \theta &= 180 + (180) \times \frac{\text{ATAN}(y/x)}{\pi} \\
\text{If } y > 0, \quad \theta &= (180) \times \frac{\text{ATAN}(y/x)}{\pi}
\end{align*}$$

In addition, I accounted for the angular deviation of the camera from the true horizontal line corresponding to the hottest and coldest regions of the gradient respectively (0 and 180°), which was determined to be 11°. Lastly, I converted the final corrected $\theta$ position to
a temperature using a regression equation describing the measured temperature changes between known destinations within the thermal gradient at stable temperature. Because the thermal gradient had mirrored temperatures on either half of the gradient, I calculated two separate regression equations (found below), one for angles 0-180º, and one for angles 181-360º. Both halves provided nearly identical gradients (except for the +/- sign), and returned r² values >0.98, so I was confident that the temperature gradient was linear.

\[
\begin{align*}
  & If \ 0 < \theta < 180, \quad y = 0.1320x + 13.6617 \\
  & If \ 0 \geq \theta \geq 180, \quad y = -0.1351x + 62.5640
\end{align*}
\]
Figure 2.4: Sample image from an active $T_{sel}$ trial showing the snake’s position within the thermal gradient stage (2.23m in circumference). This diagram illustrates the centroid (0,0) of the thermal gradient (represented by the red dot) and the location of the garter snake’s head (red circle). The corrected $x,y$ cartesian coordinates of the snake’s current position (233,175), using the equations explained above, correspond to a resting position of 34.7°C. The light grey lines observed within the thermal gradient stage are connection pieces in the gradient lid and do not reflect the gradient’s origin which is more accurately represented by the yellow lines.
Through the series of calculations explained above, I was able to assign a known temperature value for all 360° within the thermal gradient (Figure 2.5) and obtain exact temperature measurements for each position. With this experimental design, I assumed that the $T_b$ of the snake was in equilibrium with the temperature of the gradient floor. Previous research by Cadena & Tattersall (2009) suggests that over long time periods, mean selected ambient temperature in a thermal gradient is very similar to simultaneous measures of body temperature ($<$0.5 °C difference) in lizards. Thermal images were taken of a subset of snakes ($n=3$) during their $T_{sel}$ trials to confirm they were conforming to the gradient’s thermal environment and were not experiencing notable heat gain or loss external to the thermal gradient chamber. Because the snakes were long enough to span an area varying more than 1°C within the gradient, I always manually tracked the position of the head and kept this consistent for all trials. It has been demonstrated in previous experiments that lizards orient the position of their heads to facilitate thermoregulation (e.g. regulating brain temperature) and therefore head orientation is a valid thermoregulatory reference (Black & Tattersall 2017).
Figure 2. 5: Locations of an individual Eastern garter snake superimposed on a thermal image of the thermal gradient with the lid and barrier walls removed. Each blue dot corresponds to the location of the snake’s head located every 30 seconds throughout an active experiment. The light shaded inner circle represents a bumper made from plastic tubing that was installed along the inner ring of the thermal gradient stage to prevent snakes from disappearing into the camera’s blind spot. All image sequences were taken with a high-resolution web camera and coordinate positions as shown above were downloaded from the manual tracking plug-in from ImageJ.
After image analysis, I divided each snake’s dataset into quartiles; the 25th percentile representing the lower T_{sel} bound, the 50th percentile representing the median selected temperature, and the 75th percentile representing the upper T_{sel} bound, thus providing a range of temperatures representative of the central 50% of the snake’s selected temperatures. This was repeated for all 22 snakes sampled in this study. After each experiment, the snake was then placed back into its respective housing unit and was released the following day back to its exact point of capture in the field. Every snake was treated individually and had its own thermal preference calculated. To determine a T_{sel} range characteristic of the local T. s. sirtalis population, I took the mean of all 22 median selected temperatures and upper and lower T_{sel} bounds reported for each individual.

*Estimating Exploration Time*

Because the snakes were not familiar with the thermal gradient, I needed to allow time for each snake to explore the gradient, gather a spatial understanding of the new environment, and detect how the temperatures changed within it. To achieve this, I calculated the distance moved by each snake between every 30-second interval as the hypotenuse of the displacement between time points: \( L_i = \sqrt{\Delta x_i^2 + \Delta y_i^2} \) when an image was captured and took the sum of these movements to determine the cumulative distance moved by the animal during experimentation (formula below). Since locations of the animal were only taken every 30 seconds, my calculations of cumulative distance \( (D_n) \) traversed by each snake provided only estimates (likely underestimates) of total movement. In the formula below, \( n \) corresponds to the number of images, and \( x \) and \( y \) correspond to the cartesian coordinates in m of the snake’s position.
\[ D_n = \sum_{1}^{n-1} \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2} \]

By partitioning my data into two phases, an exploration phase and an experimental phase, I was more confident that in the latter phase the study animals were actively selecting temperatures coinciding with their thermal preferences and not simply exploring the gradient or trying to escape. To estimate a time allotment for the exploration period for all animals, I used a breakpoint regression analysis in R (package: segmented) to determine approximately when the animal’s activity (cumulative distance) decreased steeply with respect to time, which indicated a slowing down of the initial exploration (Figure 2.6). I calculated the breakpoint time for all individuals and took the average as my designated exploration period for my study population. The final cumulative distance also served as a measure of movement or activity.
Figure 2. 6: Cumulative activity curves for all 21 garter snakes used for the breakpoint regression analysis performed in R. The mean breakpoint time for all individuals, represented by the dashed line, was 2.55 hours. Although some animals remained active past this time, the majority of activity for all trials occurred before this point in the experiment. Therefore, the mean exploration period was treated as the first 2.55 hours of the total 10-hour $T_{sel}$ trial, while the remainder was treated as the experimental period or post-exploration phase.
Data Analysis

All data analysis including calculating $T_{sel}$ quartiles was performed in R (R Core Team 2018). The first step in my analyses was converting snake biological measurements (snout-vent length vs. mass) into a body condition parameter. This conversion involved obtaining the residuals of the linear regression of each snake’s mass (dependent variable) as a function of snout-vent length (independent variable). Positive body condition values represent individuals with above-average weight for their length, and negative values represent individuals with below-average weight for their length.

To help facilitate analysis, I systematically divided my study period into three defined seasons (spring, summer, and fall), which are not to be confused with the real timing of established seasons. I defined my spring season as May 10th to June 25th (47 days), summer between June 26th and August 12th (48 days), and fall between August 13th and September 29th (47 days). In order to detect intraspecific variation in garter snake thermal preferences I used linear regression modelling in R (function: lm). In this model, I input the median selected temperature as the response variable (a proxy for thermal preference), and body condition, habitat, and season as fixed effects. Habitat and season represent those during which each individual was caught, and body condition encompasses the biological measurements of each snake taken prior to experimentation. Since snake activity within the thermal gradient can influence the precision of my individual $T_{sel}$ calculations (e.g. snakes that move more within the gradient may have wider $T_{sel}$ ranges), I performed a separate linear regression for the same predictor variables on individual $T_{sel}$ range. I define $T_{sel}$ range as being the number of degrees Celsius between the lower and upper $T_{sel}$ bounds, or the 25th and 75th percentiles, respectively.
2.3 Results

I determined the lower $T_{sel}$ bound (25th percentile) of Eastern garter snakes to be 27.82°C, the upper $T_{sel}$ bound (75th percentile) to be 32.15°C, and a median selected temperature of 30.18°C (Table 2.1). I chose these values to represent the $T_{sel}$ range of Eastern garter snakes because they encompassed only the selected temperatures in the post-exploration period. In addition, one snake was determined to not be actively thermoregulating in the experiment due to persistent escape behaviour where the animal was trying to squeeze through the gradient lid and this resulted in the individual being stuck in the coldest section of the gradient for more than half of the experiment’s duration. Removing this individual from my analyses did not change my statistical conclusions, although it did result in slightly higher mean values for all thermal preference parameters (Table 2.1). Removing this individual also resulted in notable decreases in standard deviation for all thermal preference parameters (excluding mean distance moved, Table 2.1), which affirmed my decision to remove the animal from analyses. As previously explained, the mean cumulative movement of garter snakes in the gradient was considerably lower in the post-exploration phase of the $T_{sel}$ trial. Lastly, the thermal preferences of Eastern garter snakes represented negative skew. A one sample t-test concluded that the negative skewness observed in my study population was significantly different ($t$: -5.48, df: 20, p-value: <0.001) from 0 skewness which would be expected of a normal, symmetrical distribution. Individual $T_{sel}$ distributions are available in the appendix (Figure A.4).
Table 2. 1: Thermal preference parameters (±SD) for juvenile, fasted Eastern garter snakes (*Thamnophis sirtalis sirtalis*) sampled from a population in Southern Ontario, Canada. The first row represents data from the entirety of the 10-hour experiment (including exploration period). The bottom row shaded in grey represents only post-exploration period data with one non-thermoregulating snake removed from analysis. This shaded row represents the more reliable thermal preference parameters for this species from my experiment.

<table>
<thead>
<tr>
<th>Study Period</th>
<th>N</th>
<th>Mean ºC</th>
<th>Median ºC</th>
<th>25th Percentile ºC</th>
<th>75th Percentile ºC</th>
<th>Skewness</th>
<th>Distance Moved (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploration</td>
<td>22</td>
<td>27.87 ± 2.60</td>
<td>28.56 ± 3.94</td>
<td>24.27 ± 3.68</td>
<td>32.22 ± 2.79</td>
<td>-0.47 ± 0.70</td>
<td>32.23 ± 16.57</td>
</tr>
<tr>
<td>Experimental</td>
<td>22</td>
<td>29.39 ± 2.45</td>
<td>29.75 ± 3.18</td>
<td>27.25 ± 3.64</td>
<td>32.01 ± 2.12</td>
<td>-0.80 ± 0.76</td>
<td>23.4 ± 17.39</td>
</tr>
<tr>
<td>Experimental</td>
<td>21</td>
<td>29.74 ± 1.90</td>
<td>30.18 ± 2.53</td>
<td>27.82 ± 2.46</td>
<td>32.15 ± 2.06</td>
<td>-0.85 ± 0.74</td>
<td>23.65 ± 17.78</td>
</tr>
</tbody>
</table>
Despite having some variation in body size, capture location, and capture season, I found no evidence that any of these variables significantly influenced the thermal preferences of Eastern garter snakes (Table 2.2). Capture location also had little effect on the breadth of individual $T_{\text{sel}}$ ranges (the difference between upper and lower $T_{\text{sel}}$ bounds), however, body condition may have weakly influenced $T_{\text{sel}}$ breadth (Table 2.3). The effect of body condition on the breadth of garter snake $T_{\text{sel}}$ range rendered a nearly significant result (p-value: 0.066, Table 2.3), suggesting larger individuals exhibited narrower $T_{\text{sel}}$ ranges. When all other parameters were removed from the linear model summarized in Table 2.3, the effect of body condition was slightly more pronounced ($t$: 1.13, df: 1, p-value: 0.053), but still insignificant.
Table 2.2: Results of linear model (LM) performed on Eastern garter snake (n=21) thermal preference where median selected temperature (response variable) was chosen as the proxy for thermal preference. The default comparisons are the forest habitat and the fall season. Parameter estimates, confidence intervals, t-statistics and p-values for all predictor variables are included.

<table>
<thead>
<tr>
<th>Response Variable:</th>
<th>Median selected temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Predictors:</strong></td>
<td>Estimate</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>31.89</td>
</tr>
<tr>
<td>Body Condition</td>
<td>0.29</td>
</tr>
<tr>
<td>Habitat (Open Peat)</td>
<td>-1.02</td>
</tr>
<tr>
<td>Habitat (Mixed Shrub)</td>
<td>-0.51</td>
</tr>
<tr>
<td>Season (Spring)</td>
<td>-0.48</td>
</tr>
<tr>
<td>Season (Summer)</td>
<td>-2.07</td>
</tr>
</tbody>
</table>
Table 2. 3: Results of linear model (LM) performed on the breadth of Eastern garter snake (n=21) $T_{sel}$ ranges (response variable). The default comparisons are the forest habitat and the fall season. Parameter estimates, confidence intervals, t-statistics and p-values for all predictor variables are included. Significance was determined at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Response Variable: Breadth of $T_{sel}$ Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Predictors:</strong></td>
</tr>
<tr>
<td>(Intercept)</td>
</tr>
<tr>
<td>Body Condition</td>
</tr>
<tr>
<td>Habitat (Open Peat)</td>
</tr>
<tr>
<td>Habitat (Mixed Shrub)</td>
</tr>
<tr>
<td>Season (Spring)</td>
</tr>
<tr>
<td>Season (Summer)</td>
</tr>
<tr>
<td><strong>Estimate</strong></td>
</tr>
<tr>
<td>3.12</td>
</tr>
<tr>
<td>-0.32</td>
</tr>
<tr>
<td>-1.08</td>
</tr>
<tr>
<td>-1.07</td>
</tr>
<tr>
<td>2.33</td>
</tr>
<tr>
<td>3.10</td>
</tr>
<tr>
<td><strong>Conf. Int.</strong></td>
</tr>
<tr>
<td>0.95 – 5.30</td>
</tr>
<tr>
<td>-0.67 – 0.02</td>
</tr>
<tr>
<td>-3.22 – 1.07</td>
</tr>
<tr>
<td>-3.14 – 1.00</td>
</tr>
<tr>
<td>-0.65 – 5.30</td>
</tr>
<tr>
<td>0.27 – 5.94</td>
</tr>
<tr>
<td><strong>t-stat</strong></td>
</tr>
<tr>
<td>3.06</td>
</tr>
<tr>
<td>-1.98</td>
</tr>
<tr>
<td>-1.07</td>
</tr>
<tr>
<td>-1.10</td>
</tr>
<tr>
<td>1.67</td>
</tr>
<tr>
<td>2.33</td>
</tr>
<tr>
<td><strong>p</strong></td>
</tr>
<tr>
<td>0.008*</td>
</tr>
<tr>
<td>0.066</td>
</tr>
<tr>
<td>0.302</td>
</tr>
<tr>
<td>0.288</td>
</tr>
<tr>
<td>0.116</td>
</tr>
<tr>
<td>0.034*</td>
</tr>
</tbody>
</table>
2.4 Discussion

The thermal preference range of my study population of Eastern garter snakes spanned more than 4.3°C, between 27.82°C and 32.15°C. Median selected temperature for the population was 30.18°C, and this appeared to be unaffected by varying body condition, capture location, or season. The span of individual $T_{\text{sel}}$ range was also not significantly influenced by these parameters, although body condition may have some influence in garter snake thermoregulatory behaviour. I found evidence that temperature selection within the thermal gradient was strongly negative skewed.

*Thermal Preference Range ($T_{\text{sel}}$)*

Thermal preference ($T_{\text{sel}}$) as I have measured it represents only the range of temperatures that Eastern garter snakes prefer when they are exposed to a novel, laboratory setting and one that presents them with fewer costs associated with thermoregulation than what they would typically face in a natural setting. Thermal preferences assessed in the lab may not always be achievable in nature, and therefore, lab thermal preferences and field thermal preferences are not expected to be identical. The $T_{\text{sel}}$ range I calculated for my study population of Eastern garter snakes falls within global mean of thermal preference ranges for every snake species that has had its thermal preferences reported. Halliday & Blouin-Demers (2016) calculated this global mean range to be between 26.2 ± 0.7°C and 32.7 ± 0.8°C, and the $T_{\text{sel}}$ range I determined for my study population of Eastern garter snakes was 27.82 ± 2.46°C to 32.15 ± 2.06°C. Although other species of the genus *Thamnophis* have not had their $T_{\text{sel}}$ ranges documented, the mean $T_b$s of giant garter snakes (*T. gigas*, 29.8°C) and Mexican garter snakes (*T. eques megalops*, 29.3°C) have been reported and deviate <0.5°C from the mean selected temperature I determined for *T. s."
“sirtalis” (Wylie et al. 2009; Sprague & Bateman 2018). These results may indicate preservation of a characteristic thermal preference within the *Thamnophis* species, however further experimentation would be required to support this.

**Negative Skew**

My thermal preference experiment determined that the T$_{sel}$ range of Eastern garter snakes was strongly negatively skewed, meaning the distribution of selected temperatures more sharply declines at high temperatures compared to low temperatures (e.g. tails observed on the left side of the distribution rather than the right). Skewness has been demonstrated in many lizard species (Huey & Pianka 2017), but few studies on snakes have reported skewness values. Western tiger snakes in Australia showed strong negative skewness in laboratory thermal gradients (Ladyman & Bradshaw 2003), but this was the only snake species I could find in literature that reported negative skew. Negative skewness may be common in the field due to a variety of environmental limitations (Huey & Pianka 2017). Some proposed reasons include changes in thermal environments (daily and seasonally) allowing sufficiently warm environmental temperatures for only short time periods, the use of cool retreat sites subsequently lowering individual T$_{bs}$, or because animals move slower at cooler temperatures and as a result spend proportionately more time in cooler environments, regardless of preference (Huey & Pianka 2017). However, even in laboratory thermal gradients some species have demonstrated negative skew where these environmental limitations and costs of movement have been minimized (Ladyman & Bradshaw 2003; Cadena & Tattersall 2009; Huey & Pianka 2017). The reason for this is currently unknown and may have many ecological, physiological, and evolutionary considerations. Modeling by Vasseur et al. (2014) demonstrate that individual long-term
performance can be negatively impacted by positive skewed environmental temperatures, suggesting it may be beneficial for ectotherms to avoid hotter than preferred temperatures. *Thamnophis elegans* can resume activity down to 2°C, well below its preferred range, while it avoids activity above 36°C, only slightly above its preferred range (Huey et al. 1989). This observation is important because it indicates that garter snake thermal tolerances are much wider at cooler temperatures (Huey et al. 1989), which might explain why lab thermal preferences may be negatively skewed. However, more work is required in this area to disentangle the costs and benefits of negatively skewed temperature selection and why negative skew is exhibited by some species even in controlled laboratory environments.

**Individual Differences in Thermal Preference**

According to coadaptation principles, the thermal preferences of an animal should match the $T_{opt}$ for physiological performance and the $T_{bs}$ of the animal should be maintained within this preferred range in the field (Angilletta 2009). However, aside from the costs and limitations which may prevent animals from reaching their preferred $T_{bs}$ in the field, there may be other biological variables that prevent this scenario from being realized. For example, during certain life stages, physiological states, or periods of metabolic compromise, animals can temporarily choose to thermoregulate around higher or lower ranges of temperatures different from their normal $T_{sel}$ range (Angilletta 2009; Black et al. 2018). There may also be certain periods in an ectotherm’s life when achieving preferred temperatures may not be beneficial, such as when an individual is trying to conserve water, blood oxygen levels, or energy (Christian & Bedford 1995; Bedford & Christian 1998). Australian frill-neck lizards (Christian & Bedford 1995) and several
python species (Bedford & Christian 1998) have been shown to decrease their thermal preferences during dry seasons in tropical Australia. Because the dry season is typically associated with food and water scarcities, these reptile species lower their metabolic rates, enhance water conservation, and conserve more energy by maintaining lower body temperatures in the field (Christian & Bedford 1995; Bedford & Christian 1998).

Body size can influence thermal preferences of a species since larger individuals have higher thermal inertia and must therefore spend more time absorbing heat than smaller individuals that heat and cool more rapidly (Seebacher 2005). I detected a weak relationship between body condition and the breadth of \( T_{sel} \) range, and the biological explanation for this is likely simple. Because I was only sampling juvenile snakes, differences in thermal inertia due to body size were likely insignificant, but there may be an alternative explanation for how individual body condition might have influenced temperature selection. Larger, heavier snakes were likely well-fed prior to the 48-hour fasting period and could devote more time to maintenance of optimal body temperatures and conserve their energy by reducing activity. These well-fed individuals on average moved less in the thermal gradient, potentially to increase their thermoregulatory precision, and this would result in inherently narrower \( T_{sel} \) ranges. Individuals of poor body condition may decrease their thermoregulatory precision and be more active in the thermal gradient, possibly in search of food or other resources. The cumulative distance an animal traveled within the gradient was weakly correlated with the breadth of its \( T_{sel} \) range (\( r^2=0.07 \)) and therefore wider \( T_{sel} \) ranges may partly be a result of individual activity in the thermal gradient. Additionally, famished snakes may reduce their thermoregulatory precision, so
there may be ecological consequences of poor body condition on wild Eastern garter snakes.

Although counter-intuitive, some reptile species living in hotter habitats prefer lower temperatures in the lab than conspecifics sampled from cooler habitats (Tocher 1992). This observation comes back to the discussion regarding counter-gradient selection of temperatures and whether cooler-climate specialists have evolved warm thermal preferences to help combat active season limitations. This effect, however, has only been demonstrated in geographically isolated populations and may not be relevant to my study population of snakes which can access various habitat types within their range.

Seasonality may have had a weak effect on the breadth of individual $T_{\text{sel}}$ range as the summer season did render a significant p-value (0.035). However, given that the model was (by default) comparing the summer and fall seasons, and because only two snakes were tested in the fall, the result is likely an artefact of small sample sizes. Since juvenile animals would not have fully experienced all the seasons of a temperate climate, it is unlikely that seasonal changes would strongly influence their thermal preferences at this life stage.

My capacity to test for the relationships explained above was low given that I did not equally sample animals from different habitats, seasons, or age classes. Despite these limitations, I recognized that these effects could influence individual thermal preferences and therefore included them in my analyses. In the end, I found that seasonality, habitat, and body condition had no effect on the individual thermal preferences of Eastern garter snakes.
Conclusions

My T_{sel} study demonstrates that providing an adequate exploration time prior to experimentation for diurnally-active snakes can decrease the variation in selected temperatures. I also demonstrated that juvenile Eastern garter snakes prefer temperatures similar to other temperate snake species which have had their thermal preferences documented such as the Eastern milk snake native to North America (T_{sel}: 29-31°C, Row & Blouin-Demers 2006), and the European whipsnake native to Europe (T_{sel}: 27.5-31.5°C, Lelievre et al. 2010). Although using individuals of varying age classes and body sizes might render slightly different responses than what I observed, the influences of reproduction status are minimized by using juveniles and acquired thermoregulatory behaviours (or behavioural tendencies) may be more limited in younger animals. Lastly, I found no evidence for the influence of season or habitat origin on the T_{sel} of Eastern garter snakes. However, future studies should focus on targeting larger sample sizes that can more accurately address these influences on garter snake thermal preference.
Chapter 3:

Evaluating the role of thermal quality on the habitat use of Eastern garter snakes

(Thamnophis sirtalis sirtalis)
3.1 Chapter Rationale

Thermal quality can change dramatically even within small spatial scales due to the many thermal environments that exist in a heterogenous landscape (Blouin-Demers & Weatherhead 2002; Yagi & Litzgus 2013; Corkery et al. 2018). For my study, I needed to accurately assess the thermal profiles of forested, mixed, and open-canopy habitats that make up a landscape where Eastern garter snakes are found to determine a) whether these habitats offered different thermal profiles from one another and b) the relative energy investment required by Eastern garter snakes in each habitat to achieve their desired body temperature which could be inferred from habitat thermal quality. In other words, given the knowledge I have on Eastern garter snake thermal preference discussed in the previous chapter, what is the most thermally suitable habitat and are the snakes most abundant in this habitat? It has been suggested that open habitats in temperate regions like those found in Southern Ontario provide superior thermal quality for North American snake species compared to forested habitats (Blouin-Demers & Weatherhead 2002; Row & Blouin-Demers 2006; Halliday & Blouin-Demers 2016), but little is known about intermediate successional areas which may exhibit mixed characteristics of both open and shaded environments. Therefore, my study was unique in adding this additional element of an intermediate habitat which may be more attractive to Eastern garter snakes. This study design may also provide a more realistic reflection of a habitat matrix used by garter snakes since they are not confined to strictly open and closed-canopy systems.

It has been previously demonstrated in both milk snakes (Row & Blouin-Demers 2006) and common garter snakes (Halliday & Blouin-Demers 2016) that optimal temperatures can be a more limiting resource in a landscape than food resources, and
therefore thermal quality has been argued to be the best predictor of habitat quality for some species of snakes. Assuming temperate snake species make some degree of habitat assessment based on thermal quality, I aimed to explore whether Eastern garter snakes use thermal cues to inform their use of different habitat types found in Southern Ontario. Using my own $T_{set}$ values from Chapter 2, I set out to quantify the thermal quality of three habitat types found within a relatively small geographical area that are all accessible to a population of Eastern garter snakes. I then needed to relate this thermal quality data to snake encounters to determine if an open habitat would still exhibit the greatest thermal quality for the species and whether Eastern garter snakes were most abundant in this open habitat. The open sites from previous studies have typically been open fields or artificially cleared openings in the landscape with little to no canopy coverage (Row & Blouin-Demers 2006; Halliday & Blouin-Demers 2016). The open habitat at my field site was distinct from those previously explored in that it was part of a drained wetland complex with dark, exposed peat soils and is sparsely vegetated by bog species like bog blueberry ($Vaccinium uliginosum$), cottongrass ($Eriophorum angustifolium$) and sundew plants ($Drosera sp.$).

3.2 Methods

Thermal Quality ($D_e$) – Experimental Design

In order to capture the complexity of the thermal environment in which Eastern garter snakes live I needed to view my study site from the perspective of a snake. This means that I needed to consider the ground-level microhabitat diversity that these animals would use during the times of day and year in which they would use them and incorporate this diversity into my dataset. I achieved this by employing a series of physical snake analogs (PSAs) that could record environmental temperatures. In doing so, I could bypass
two major obstacles: 1) I did not have to regularly disturb garter snakes in the wild, and 2) I could measure temperatures of different habitats remotely and simultaneously. In total, I deployed 27 PSAs across my field site, nine in each of my three distinct habitats. All PSAs were fitted with temperature dataloggers to record operative environmental temperature ($T_e$) every 20 minutes for a total of 142 days from May to September 2017. Once my active season ended, I could then assimilate my dataset into characteristic thermal profiles for each of these habitat types. In doing so, I could make inferences about the thermal quality or attractiveness of each habitat for Eastern garter snakes.

*Physical Snake Analogues (PSAs)*

All 27 PSAs were custom made by Brock University’s machine shop in April 2017. The PSAs were made entirely from copper pipe (2.5cm in diameter) with a soldered copper cap on one end, and a threaded, removable copper insert on the other (Figure 3.1). The thermal transfer characteristics of copper are very similar to those of living snakes, and copper has been the material of choice for several different reptile species used for thermal biological studies (Dzialowski 2005). In fact, out of all the materials used to construct reptile analogues, copper was considered the most reliable because its heat-conductance, radiation, and absorptivity are the most similar to living reptiles (Shine & Kearney 2001; Dzialowski 2005). Although environmental variables such as humidity and wind speed may influence the thermal properties of a PSA, these typically result in negligible differences in overall mean temperatures (Shine & Kearney 2001). PSAs are known to work best when they match the coloration and morphological characteristics of their target species (Shine & Kearney 2001; Dzialowski 2005). In essence, the more realistic the model, the better. This is why I aimed to manufacture my PSAs to mimic the scale colour
and morphological characteristics of juvenile Eastern garter snakes. Admittedly, Eastern garter snakes exhibit large phenotypic variation in scale patterns and coloration and therefore I could only approximate the reflectance of Eastern garter snakes found at my study site. I also took known estimates of juvenile garter snakes’ snout-vent length and body mass and used these measurements to determine the dimensions of the PSAs. In the end, each of our PSAs was 15.5 cm long, and filled with 30 mL of water to approximate the weight of a 30 g juvenile snake. Every model was sand-blasted and coated with non-reflective matte brown spray paint. Our PSAs were limited to 2.5 cm in diameter because I needed to insert iButtons (Thermochron model DS1922L, Maxim Integrated™) inside each PSA. Therefore, the overall size and weight of our PSAs over-exaggerate those of living juvenile garter snakes, but reflect a living, basking snake which is made up of approximately 70% water (Bauchot 1996). Before fitting the PSAs with iButtons, I tested every iButton under a series of different conditions to ensure that they were functioning consistently and would accurately model temperatures in the field.
Figure 3. 1: Sample photo of the dimensions of physical snake analogues (PSAs) used to record $T_c$. All parts of the instrument were copper. All 27 PSAs used in this study had identical dimensions and coloration. Each unit was sand-blasted to allow paint to adhere to the copper exterior. Matte brown spray paint was used to approximate the coloration $T. s. sirtalis$ found specifically at our study location.

**PSA Field Placement**

In each of my three selected habitats, I installed nine PSAs. PSAs were carefully placed in microhabitats that could be accessed by living snakes and would mimic realistic site selections of wild Eastern garter snakes. PSAs were placed beneath fallen logs, in woodpiles, in leaf litter, beside and on top of coverboards (see Figure 3.2). An even ratio of shaded versus non-shaded microhabitats were randomly chosen for all three habitats to avoid introducing biased placements into the dataset. For example, PSAs placed on top of the boards were the most conspicuous and were also most likely to record the highest temperatures, so only two of the nine PSAs from each habitat were placed on top of randomly selected coverboards and this was kept standard for all habitats. Each PSA had a unique numerical code and serial number, and each placement location was carefully
marked so that individual PSAs could be returned to their exact location after routine data downloads.

Figure 3. 2: Example placements of PSAs fitted with temperature loggers. From left to right: an artificial snake amongst leaf litter (A), basking on top of a coverboard (B), and resting beneath natural woody debris (C).
Habitat Characterization

All three habitats used in this study were pre-selected and only coarsely defined by their abiotic features (Table 3.1). I was primarily interested in abiotic features that influenced the thermal properties of a habitat, and therefore I focused my habitat characterization on variables like canopy cover (Figure 3.3), solar radiation, soil moisture and temperature. Canopy cover was only measured once per season after foliage had emerged, while solar radiation was measured every other survey. Soil moisture and soil temperature were intended to be collected during every survey, but machine malfunctions resulted in soil moisture only being measured once in the spring, and soil temperature three times in the spring and only once in the summer. The open peat habitat was chosen because of its absence of a contiguous forest canopy and presence of dark hummocky soils. This area was also slowly regenerating after being drained and harvested for peat-mining in the mid to late 20th century. The forest habitat was chosen because of its solid canopy cover and thick understory. The forest was an area with mature deciduous trees and was not historically mined. The mixed shrub site served as an intermediate between the other two habitats, where canopy over and understory were moderate and where the plant communities were in succession from previous disturbances.
Table 3. 1: Mean values of four abiotic variables measured in each of the three habitats. $N$ refers to the number of surveys which measurements were taken. Canopy cover was only measured once per season while solar radiation was measured every other survey. The open peat habitat had the greatest mean solar radiation and the lowest mean canopy coverage while the forest had the lowest mean solar radiation and the greatest canopy coverage. Soil measurements were taken but are incomplete due to machine malfunctions in the field.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Solar Radiation ($W/m^2$) $n=5$</th>
<th>Canopy Cover (% cover) $n=3$</th>
<th>Soil Moisture (% vol.) $n=1$</th>
<th>Soil Surface Temperature (ºC) $n=4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>266.6 ± 122.5</td>
<td>69.8 ± 3.4</td>
<td>58.6 ± 8.9</td>
<td>18.9 ± 0.4</td>
</tr>
<tr>
<td>Mixed Shrub</td>
<td>324.5 ± 231.7</td>
<td>64.1 ± 4.1</td>
<td>41.4 ± 9.2</td>
<td>18.5 ± 0.6</td>
</tr>
<tr>
<td>Open Peat</td>
<td>416.8 ± 131.2</td>
<td>41.6 ± 4.8</td>
<td>42.0 ± 9.1</td>
<td>18.7 ± 0.4</td>
</tr>
</tbody>
</table>
Figure 3.3: Example canopy cover photos from the forest (A), mixed shrub (B), and open peat (C) habitats to help illustrate how canopy coverage was calculated. To their right are the same photos converted to binary coloration in ImageJ so that vegetative cover and open sky could be distinguished. Photo A shows an example of high canopy coverage at 81.9%, photo B an example of moderate coverage with 68.2%, and photo C an example of low coverage with 32.8%. Canopy cover was measured once per season and mean canopy coverage was taken from all nine coverboard locations within each habitat. Images were captured from the center of each coverboard using a GoPro™.
Measuring Operative Environmental Temperature ($T_e$)

All physical snake analogues (PSAs) collected high-resolution temperature measurements every 20 minutes for 142 days throughout the active season. In total, I collected over 200,000 temperatures across the three habitats and seasons. Although PSAs collected temperatures around the clock, I focused my analysis on temperatures that occurred during a typical garter snake’s active period at this latitude and this was done for biologically relevant reasons. Since $T. s. sirtalis$ is not nocturnally active at this latitude, only daytime temperatures (after sunrise and before sunset) were of interest for questions of thermal preference. As a result, I analyzed temperatures that were recorded between 0700 and 1900. As explained in the previous chapter, I recognize that natural photoperiod in Southern Ontario fluctuates throughout the year. However, I tried to keep my sample sizes (total number of temperature observations) as similar as possible throughout the study period and chose 0700 – 1900 as my standard day length given that both hours were within the natural daylight hours for the entirety of my study duration. Although I sampled nine different locations within each habitat, I did not treat each PSA independently and collectively used the data from all nine units to calculate habitat $T_e$.

For all the temperatures recorded throughout the active season, I calculated how much each measurement deviated from the $T_{sel}$ of Eastern garter snakes (presented in Chapter 2). This parameter is formally denoted as $D_e$ and is used to infer the thermal quality of a habitat. The formula used for calculating $D_e$ is demonstrated below.
\[
\begin{align*}
    &\text{if } T_{e[i]} > T_{\text{upper}}: D_e = T_e - T_{\text{upper}} \\
    &\text{if } T_{e[i]} < T_{\text{lower}}: D_e = T_{\text{lower}} - T_e \\
    &\text{if } T_{\text{lower}} \leq T_{e[i]} \leq T_{\text{upper}}: D_e = 0
\end{align*}
\]

In the above formula, \(T_{e[i]}\) represents an individual operative environmental temperature measurement taken from each \(i^{th}\) interval (20 minutes apart). \(T_{\text{upper}}\) and \(T_{\text{lower}}\) represent the upper and lower \(T_{\text{set}}\) bounds (75\(^{th}\) and 25\(^{th}\) percentiles, respectively) for Eastern garter snakes. \(T_e\) values which fall within the \(T_{\text{set}}\) bounds are assigned a value of 0, signifying optimal thermal quality. Mean \(D_e\) values could then be used to score each habitat in terms of thermal quality, although the way \(D_e\) is calculated above, it is constrained to be a positive number. Thus, for visualization purposes I also present an alternative signed \(D_e\) where negative values represent thermal qualities that are colder than optimal and positive values warmer than optimal.

Data Analysis of Thermal Quality

To help facilitate more meaningful analysis of my temperature data, I systematically divided my 2017 active season into three defined seasons (spring, summer, and fall). The spring season was from May 10\(^{th}\) to June 25\(^{th}\) (47 days), summer between June 26\(^{th}\) and August 12\(^{th}\) (48 days), and fall between August 13\(^{th}\) and September 29\(^{th}\) (47 days). For each of these 142 sample days I calculated daily mean \(T_e\) and \(D_e\) values and used these numbers for statistical analyses. Because reptile behaviour, movement patterns, and habitat preferences can change throughout the active season, and because temperature can fluctuate widely during this period, I considered it more useful to divide my dataset into different seasons to highlight temporal variation.
I used linear mixed effects modelling in R (package: lme4; function: lmer) to determine if operative environmental temperatures were significantly different between the three habitats explained above. For this model, $T_e$ served as the response variable, habitat, season, and the interaction between habitat and season as fixed effects, and PSA ID as a random effect. I performed the same model for thermal quality, replacing $T_e$ with $D_e$ as the response variable. Significance was determined at the 95% confidence level ($\alpha = 0.05$).

*Eastern Garter Snake Habitat Use – Experimental Design*

For this field experiment, I installed coverboards in the three habitats previously explained and documented the occupation of these cover objects by snakes. I used this methodology to survey for habitat use as it is a reliable method for documenting snake presence/absence and is a safe and non-invasive method of sampling that can be repeated for long study durations (Scheffers *et al.* 2009; Halliday & Blouin-Demers 2015). For my study, I installed nine coverboards per habitat using an aggregate design (Figure 3.4). All coverboards used in this study were identical and consisted of 1.2 m x 2.4 m untreated plywood sheets that were 2 cm thick (Figure 3.5). Three coverboards were placed in an aggregate with each board being an equal distance (20 m) from each other (Figure 3.6). Three aggregates were placed into each habitat, and every aggregate was positioned a minimum of 150 m from the previous. In addition, each aggregate was located at least 150 m from the nearest road allowance.
Figure 3.4: Map of study locations created in Google Earth (2018). The green polygon represents the forest habitat, the turquoise represents the mixed shrub habitat, and the blue represents the open peat habitat. Triangles indicate approximate locations of coverboard aggregates (accuracy ±10m). Aggregates were placed 150-200 m apart. To limit edge effects being present in the forest, each aggregate was placed at least 150 m from the forest edge. Trails (dashed lines) connecting the three aggregates ranged in length from 755 m (one-way distance) in the forest, 770 m in the mixed shrub, and 730 m in the open peat. Trail lengths were measured using a hand-held GPS unit (Garmin™, model 650t).
Figure 3. 5: Example placement of a coverboard in-situ. Each board was 1.2 m wide, 2.4 m long, and 2 cm thick, and specifically coded to correspond with the habitat and aggregate number it belonged to. Coverboards were installed in March and April of 2017 and were undisturbed at least two weeks prior to surveying.
Figure 3. 6: Example diagram of a coverboard aggregate. Three of these aggregates were placed into each habitat, resulting in a total of nine coverboards in the open peat, mixed shrub, and forest habitats. Each aggregate was treated as an independent sample in analyses.
Coverboard Surveys

I conducted a total of ten coverboard surveys throughout the reptilian active season in Ontario. Although Eastern garter snakes may leave hibernation sites as early as March and can continue activity into October, I considered it more meaningful to focus on the species’ habitat use during the times of day and year with the greatest activity. I surveyed every board on average within a two-week interval meaning that every board was visited at least twice a month from May to September 2017. Only one habitat was surveyed per day, and the sequence of habitat visits was randomly chosen. At the beginning of every coverboard survey I recorded the current weather conditions and approximated cloud cover. During a coverboard survey, all three aggregates within the habitat were surveyed in the same day, and the sequence of aggregate visitation was also chosen randomly. At each coverboard I documented the ambient temperature and relative humidity using a handheld hygrometer (Extech Instruments™, model 445580), the start and end time of the coverboard visit, and documented all species and number of individuals beneath the board. I noted where each individual was found, whether it was beside the board (within 1m), on top of the board, or beneath the board. When Eastern garter snakes were encountered, they were immediately hand-caught and had their biological measurements taken. I took the SVL (cm) of every individual, along with its mass (g). After measuring, the animal’s tail was then painted in non-toxic, water-based acrylic paint so that the animal could be identified as a recapture in later surveys (Figure 3.7). This marking technique was temporary and could only last until the animal’s next shed. Dull, pastel paint colors were chosen so that released snakes would not be more visibly conspicuous to potential predators. Once all the animals occupying the coverboard were vacated, I took the rest of
the environmental measurements. I took soil temperature readings with a probe thermometer (Extech Instruments™, model 39240), and soil moisture readings with a soil moisture sensor (Delta-T™, model ML2x ThetaProbe). For each variable I took three spot measurements beneath the board and recorded the mean measurement. Canopy cover photos were taken above each coverboard three times throughout the active season and later processed in ImageJ to calculate average canopy cover for each habitat (see Figure 3.3). Photos were taken with a wide-angle GoPro™ camera pointed directly towards the sky from the middle of the coverboard. I also measured solar radiation using a pyranometer application (Hukseflux Thermal Sensors™) installed on a smartphone to collect incoming solar radiation (W/m²) at each coverboard. This application was calibrated using a local weather station’s estimates of incoming solar radiation for that day.

![Image of an Eastern garter snake](image)

Figure 3. 7: Example photo illustrating how and where individual Eastern garter snakes were marked to estimate recapture rates. The tail of every individual captured during coverboard surveys was temporarily marked in a pastel colour using water-based, non-toxic acrylic paint.
Incidental Observations of Eastern Garter Snakes

During each routine survey I also noted the total number of Eastern garter snakes I encountered while walking from aggregate to aggregate within each habitat. These observations (referred to as incidental encounters) represent individuals that were actively basking or moving through the habitat away from any coverboards. When walking between coverboard aggregates, I used a standardized search approach where I constantly swept through the understory vegetation along the trail edges with a snake hook and documented any snakes that I disturbed and could positively identify. Most snakes were found basking directly on trails or observed moving within a few meters of the trail edge and could be identified by sight. Snakes encountered in this manner were counted separately from those observed occupying coverboards and were not captured or measured. Although the time spent in each habitat varied depending on capture success and other factors, my effort for incidental observations was standardized due to area searched and not by time spent in-situ. Since the same trails were always used, and the trail lengths for the forest (1.51 km), mixed shrub (1.54 km), and open peat (1.46 km) varied minimally (<90 m), I compared incidental sightings of garter snakes between habitats to corroborate the habitat use results obtained from coverboard encounters.

Skin-Surface Temperatures of Eastern Garter Snakes in the Field ($T_{b(field)}$)

I opportunistically measured skin-surface temperatures of active Eastern garter snakes captured in the field. I used a thermal imaging camera (Spot Thermal Camera, model TG165 FLIR™) to acquire $T_{b(field)}$ measurements of 20 individuals across the active season in 2017. Because of the opportunistic nature of these measurements, the timing and
locations of when these measurements were recorded was inconsistent. However, all 20 measurements were captured from diurnally active snakes during the active season.

Data Analyses

Since my coverboard dataset involved many parameters, I first inspected my data to determine if there was evidence of collinearity between parameters and found that time of day and thermal quality were strongly colinear with habitat and season. As a result, I input only habitat and season, and the interaction of habitat and season, into a generalized linear mixed effect model (GLMM), assuming a Poisson distribution for my count data. I then tested this model for zero-inflation given that zero counts were common during my surveys (Harrison et al. 2018) and found that zero inflation was present in my count data. In the end, I used a zero-inflated generalized linear mixed model to gauge the effects of habitat and season on Eastern garter snake encounters (package: glmmTMB; function: glmmTMB). I input ACO encounters as the response variable, habitat, season, and the interaction between habitat and season as fixed effects, and included aggregate number as a random effect. Significance was determined at the 95% confidence level ($\alpha = 0.05$).

To analyze my incidental encounters, I followed a similar methodology explained above but used a generalized linear model (GLM) to analyze incidental encounters of Eastern garter snakes given that zero inflation was not present. Like the ACO encounters, I assumed my incidental observations followed a Poisson distribution. In the GLM, I input incidental encounters of garter snakes as my response variable, habitat, season, and the interaction between habitat and season as fixed effects. Significance was determined at the 95% confidence level ($\alpha = 0.05$).
Since thermal quality (Dₑ) was not included in the previous models due to collinearity, but I was interested in its effect to address my hypotheses, I analyzed the effect of Dₑ on Eastern garter snake encounters using a separate generalized additive model (GAM) approach (package: mgcv; function: gam), given that I did not suspect a linear relationship between Dₑ and snake encounters. In order to determine if cooler temperatures differed in their effect from warmer temperatures, I slightly modified the Dₑ metric to allow for the distinction between negative (too cool), zero (within the T_{sel} range), and positive (too warm) thermal quality values. Both GAMs were performed using the modified metric which I denoted as D_{es}. In these models, D_{es} served as the only predictor variable, and either ACO or incidental encounters of Eastern garter snakes were input as the response variable. All analyses were performed in R (R Core Team 2018).

3.3 Results

*Operative Environmental Temperature (Tₑ)*

Out of the three habitats studied, only the open peat habitat was capable of providing mean seasonal environmental temperatures near the T_{sel} range of Eastern garter snakes (Figure 3.8). On average, the open peat was the warmest habitat in every season, while the forested habitat was the coolest (Figure 3.8). However, throughout the active season, average morning and overnight temperatures were lowest in the open peat habitat and climbed dramatically in the afternoon, whereas the forest provided the most thermally stable environment (Figure 3.9). A linear mixed effect model determined that the effects of habitat, season, and the interaction between habitat and season were all significant in influencing operative environmental temperature (Table 3.2). Post-hoc pairwise comparisons determined that the open peat habitat was warmer than the forest in every
season, but only significantly warmer than the mixed shrub during the summer, and that all habitats experienced higher environmental temperatures in the summer season (Appendix, Table A.1). Despite providing slightly warmer temperatures in all seasons, the mean operative temperatures in the mixed shrub were not significantly warmer than those offered in the forest (Appendix, Table A.1).
Figure 3. 8: Mean operative environmental temperature ($T_e \pm SE$) in degrees Celsius provided by each habitat in each season. Values shown above were calculated only from activity time temperatures (recorded between 0700-1900). The open peat habitat was the warmest in all seasons while the forest was the coolest. In all habitats, $T_e$ was the highest in the summer season. The red line represents the lower $T_{sel}$ bound of Eastern garter snakes (27.82°C).
Table 3. 2: Type III analysis of variance results from the LMM performed on operative environmental temperature (response variable). F values (f-value), degrees of freedom (df), and p-values (p) for all predictor variables are included. Significance was determined at $\alpha=0.05$.

**Response Variable:**  
Operative Environmental Temperature ($T_e$)

<table>
<thead>
<tr>
<th>Predictors</th>
<th>f-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
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<td>2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Season</td>
<td>98.8123</td>
<td>2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Habitat:Season</td>
<td>3.0774</td>
<td>4</td>
<td>0.0153*</td>
</tr>
</tbody>
</table>
Figure 3. 9: Mean daily variation in operative environmental temperature (Tₑ) according to each habitat and hour of day. Each data point represents a mean temperature for a specific hour and is averaged across the entire active season (n=142 days). In general, the morning and overnight temperatures of the open peat habitat were the lowest, while afternoon temperatures were substantially warmer than the mixed shrub or forest habitats. The forest habitat experienced the least variation in temperature throughout a given 24-hour period and was considered the most thermally stable habitat.
**Thermal Quality (D<sub>e</sub>)**

The mean thermal quality (D<sub>e</sub> measured in absolute value) of all three habitats followed a very similar trend as T<sub>e</sub> (Figure 3.8) in that the open peat habitat consistently provided the greatest thermal quality in all three seasons while the forest provided the lowest (Figure 3.10). The effects of habitat and season were considered significant predictors of thermal quality, while the interaction of habitat and season was weaker (Table 3.3). Post-hoc pairwise comparisons determined that the open peat habitat provided considerably greater thermal quality than the forest in every season, and greater thermal quality than the mixed shrub only in the summer and fall seasons (Appendix, Table A.2). All habitats experienced the greatest thermal quality in the summer. Despite providing slightly better thermal quality in all seasons, the mixed shrub habitat did not differ significantly from the forest.

Although D<sub>e</sub> values were more consistently in the range of zero (i.e. T<sub>e</sub> = T<sub>sel</sub>) in the open peat habitat, this habitat also experienced more positive D<sub>es</sub> values indicating that environmental temperatures were hotter than the thermal preference range of Eastern garter snakes (Figure 3.11A). Temperature observations which rose above 36°C, or the equivalent of a D<sub>es</sub> value of 3.85°C, represent periods when environmental temperatures exceeded the voluntary thermal maxima (VT<sub>max</sub>) reported for garter snakes (Huey et al., 1989). In the open peat habitat, the proportion of D<sub>es</sub> observations exceeding this 3.85°C threshold equated to 11.4% of the total active season (Figure 3.11A). The mixed shrub and forest habitats experienced temperatures above the VT<sub>max</sub> of garter snakes only 1.8% and 0.5% of the time respectively.
Figure 3. 10: Mean thermal quality values ($D_e \pm SE$) in degrees Celsius provided by each habitat in each season. Thermal quality is defined as the deviation (in absolute value) of $T_e$ from the $T_{sel}$ bounds of Eastern garter snakes and lower values indicate greater thermal quality. Values represent mean measurements recorded during the activity time of Eastern garter snakes (0700-1900). The open peat habitat provided the greatest thermal quality (i.e. lowest $D_e$) for Eastern garter snakes in all seasons while the forest provided the poorest. In all habitats, $D_e$ was the greatest in the summer season.
Table 3.3: Type III analysis of variance results from the LMM performed on thermal quality (response variable) measured in absolute value. F values (f-value), degrees of freedom (df), and p-values (p) for all predictor variables are included. Significance was determined at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>f-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
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<td>Season</td>
<td>120.0146</td>
<td>2</td>
<td>&lt;0.0001*</td>
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<tr>
<td>Habitat:Season</td>
<td>2.3264</td>
<td>4</td>
<td>0.054</td>
</tr>
</tbody>
</table>
Figure 3.11: Probability densities representing the distribution of thermal quality values for each habitat during the entire active season. Figure A represents signed thermal quality values which distinguish positive and negative values ($D_{es}$) and Figure B represents absolute thermal quality values ($D_{e}$) as they are commonly reported in literature. Only activity time measurements (0700-1900) were included in analysis. The $D_{es}$ distributions for both the forest and mixed shrub habitats were negatively skewed (A), and the thermal quality of these habitats rarely exceeded the $T_{sel}$ range of Eastern garter snakes (where $D_{es} > 0$). The open peat habitat provided ideal thermal quality the most often of the three habitats, evident by the greatest density of observations within the $D_{es} = 0$ (or $D_{e} = 0$) range. The rectangle region (A) designates the proportion of $D_{es}$ observations which exceeded the $VT_{max}$ of garter snakes (36°C; equivalent to $D_{es}$ value of 3.85°C). Thermal quality surpassed this threshold most often in the open peat (11.4%), and less frequently in the mixed shrub (1.8%) and forest habitats (0.5%).
**Habitat Use**

Coverboard encounter rates for all habitats and seasons were low (Figure 3.12A), often equating to less than two individuals per survey, and zero-inflation was present. No significant differences in coverboard occupancy were detected between habitats or seasons (Table 3.4) although the total number of Eastern garter snakes encountered at coverboards was highest in the mixed shrub habitat (Figure 3.12A), and over 50% of coverboard encounters occurred in the spring season. The interaction between habitat and season was also weak (Table 3.4).

Eastern garter snakes were most commonly encountered either moving or basking in a habitat rather than occupying the coverboards (Figure 3.12). In fact, more than twice as many snakes were observed *in-situ* than at a coverboard (n=67 versus n=27 between all habitats). Similar to the ACO encounters, the effect of habitat was weak on the incidental encounters of Eastern garter snakes (Table 3.5), despite that most snakes were observed in the mixed shrub habitat (Figure 3.12B). There was a strong effect of season on incidental encounters (p-value: 0.0017), and there was an interaction between habitat and season present (p-value: 0.0084, Table 3.5), suggesting that habitat preferences may have been present earlier in the active season for Eastern garter snakes. However, the only comparison which yielded a significant difference was the total number of incidental encounters observed in the spring versus the summer in the mixed shrub habitat (Appendix, Table A.3).
Figure 3. 12: Total number of Eastern garter snake encounters in each habitat according to season. Figure A represents the number of Eastern garter snakes observed occupying coverboards, while Figure B represents Eastern garter snakes that were observed incidentally while in each habitat. Snakes that were encountered incidentally were not associated with a coverboard location, and represent individuals that were detected actively moving or basking in the habitat during a survey. In both figures, the total number of encounters were greatest in the mixed shrub habitat during the spring and fall, but greatest in the open peat during the summer season.
Table 3.4: Type III Wald’s analysis of variance results from zero-inflated GLMM performed on coverboard encounters of Eastern garter snakes (response variable), assuming a Poisson distribution. Chi squared values (Chi sq.), degrees of freedom (df), and p-values for all predictor variables are included. Significance was determined at α=0.05.

<table>
<thead>
<tr>
<th>Response Variable:</th>
<th>ACO Encounters</th>
</tr>
</thead>
<tbody>
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Table 3. 5: Type II analysis of deviance results from GLM performed on incidental encounters of Eastern garter snakes (response variable), assuming a Poisson distribution. Chi square values (Chi sq.) degrees of freedom (df) and p-values (p) for all predictor variables are included. Significance was determined at α=0.05.

<table>
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<td>Season</td>
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<tr>
<td>Habitat:Season</td>
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</table>
**Thermal Quality Effects on Garter Snake Encounters**

Although thermal quality ($D_{es}$) was colinear with other effects (namely habitat and season) for incidental and ACO encounters of Eastern garter snakes, when the $D_{es}$ effect was isolated, the response was weak for both types of snake observations (Table 3.6). Coverboard occupancy may have been greater when habitat $D_{es}$ became positive (Appendix, Figure A.5), but due to low point counts and strong zero-inflation, there was no clear association between thermal quality and coverboard use. More garter snakes were observed incidentally when $D_{es}$ was slightly negative (Appendix, Figure A.6), differing slightly from the trend observed with coverboard occupancy, although the relationship was statistically insignificant. Statistical analyses were also performed on the association of $D_{e}$ and habitat use but resulted in similarly weak effects to the analyses performed using $D_{es}$.
Table 3. 6: Results of GAMs performed on thermal quality and Eastern garter snake encounters, assuming a Poisson distribution. Deviance explained \((Deviance\ exp)\), estimated degrees of freedom \((edf)\), chi-squared values \((Chi-sq)\), and p-values are included for all scenarios.

<table>
<thead>
<tr>
<th>Predictor</th>
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<th>Chi-sq.</th>
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Eastern Garter Snake Skin Surface Temperature in the Field

The mean $T_{b(\text{field})}$ of Eastern garter snakes was 27.4 ± 1.4°C during the active season of 2017. Individual snakes were captured opportunistically between May and September, however only active individuals were measured. Individuals observed beneath cover objects were not included in analysis.

Anecdotal Observations

Included in this section is an anecdotal observation made during the 2017 field season regarding a potential relationship between inclement weather and low snake activity. It is included in results because it may help explain trends observed not only in my study, but in other reptilian thermal biology studies. I documented four separate occasions (June 18, June 29, July 16, and August 04) when I observed little to no snake activity in the hours preceding storm fronts with associated drops in barometric pressure. Only one incidental observation of an Eastern garter snake was made during these four days, whereas the average incidental encounter rate throughout the active season was between two and three snakes per survey.

3.4 Discussion

I determined that the open peat, mixed shrub, and forest habitats all provided different thermal environments, and that the operative environmental temperatures ($T_e$) and resulting thermal qualities ($D_e$) were subject to seasonal changes. Despite the effects of changing seasons, the open peat habitat provided the warmest mean temperatures and the most suitable thermal quality for Eastern garter snakes throughout the reptilian active season. However, Eastern garter snakes were not the most abundant in the open peat habitat, but rather, the greatest number of incidental and ACO encounters occurred in the
mixed shrub habitat, primarily during the spring season. In addition, I determined that the effect of thermal quality did not strongly influence the habitat use of Eastern garter snakes in my study.

**The Thermal Environment**

Consistent with the findings of previous studies (Row & Blouin-Demers 2006; Halliday & Blouin-Demers 2016), I determined that the most open habitat type (in terms of canopy cover) experienced the warmest activity-time temperatures and as a result, also provided the greatest thermal quality for Eastern garter snakes throughout the active season. However, given that vegetation cover was sparse in the open peat habitat, this habitat also experienced the greatest fluctuations in temperature throughout a 24-hour period and was less buffered from thermal extremes than the mixed shrub or forested habitats. Overhead canopy structure can strongly influence substrate surface temperature, and the lack of canopy cover can result in extreme temporal variability in environmental temperature (Chen *et al.* 1999). Artificial clearings in canopy have been shown to benefit reptile assemblies because the increase in solar radiation and ground-level temperatures can allow rock crevices and other retreat sites to retain warmer temperatures later into the day, and this relationship can enhance the thermal attractiveness of open areas (Webb & Shine 1998; Pike *et al.* 2011). However, given that the open peat habitat at my study site represented a mined wetland complex that was cleared and drained, few habitat features served as retreat sites or refugia for reptiles. Even the PSAs placed into shaded microhabitats in the open peat still experienced occasional daytime temperatures that were too hot to support an Eastern garter snake, and therefore thermal refugia are likely a limiting resource in the open peat environment.
Habitat thermal quality can change significantly from season to season, but my data also illustrated how environmental temperatures can fluctuate throughout a 24-hour period. Not surprisingly, morning and overnight temperatures were the lowest in each habitat, and greatest in the afternoon. I documented greater surface activity of Eastern garter snakes (e.g. actively traveling or basking animals) when environmental temperatures and thermal quality were lower than optimal (i.e. when $D_{es}$ was slightly negative). Of course, when thermal quality is lower, the incentive to augment $T_b$ through basking is likely stronger (Nelson & Gregory 2000). In addition, for transient snakes that are moving through these habitats, they may prefer to do so when environmental temperatures are slightly cooler rather than hotter so that they minimize their risk of overheating. Increased surface activity during suboptimal environmental temperatures (e.g. in the morning) has been reported for garter snakes with the caveat that aquatic activity may increase on hotter afternoons in some populations (Nelson & Gregory 2000). At my study site, surveys that were conducted during periods when $D_{es}$ was positive may represent periods when the habitat was too hot for Eastern garter snakes to be active. Therefore, snakes may not have been encountered as frequently due to their restriction to retreat sites, and this observation is consistent with what has been documented for garter snakes in other parts of their range (Nelson & Gregory 2000).

**Habitat Use**

Upon initial inspection of my results, I suspected an interaction between season and habitat given that the Eastern garter snakes appeared to be more common in the spring time, especially in the mixed shrub habitat for both ACO and incidental encounters. However, a Fisher’s Exact test corroborated that there was no strong interaction between
habitat and season for coverboard encounters, and this may have been due to the issue of zero-inflation. I did discover that an interaction between habitat and season was present in the incidental encounters, but this interaction may have been strongly influenced by the high snake activity in the spring season. I discuss the potential relevance of seasonal changes in habitat use to help explain why more snakes might have been observed in the spring season and in the mixed shrub habitat, acknowledging that this interaction was only present in my incidental observations of Eastern garter snakes.

Coverboard occupancy was low in my study and could be argued to be a poor representation of habitat use by Eastern garter snakes, but the results from the coverboard surveys are very similar to the results of the incidental encounters. In fact, both datasets corroborate each other in showing the relative abundance of Eastern garter snakes being greatest in the mixed shrub habitat in the spring and fall seasons, but greatest in the open peat during the summer. Although few significant differences were found, the two datasets may still provide compelling results regarding Eastern garter snake habitat use during different periods of their active season. For example, one study on the common garter snake (*Thamnophis sirtalis*) found that gravid females can shift their habitat preferences in favour of hotter, open habitats that facilitate thermoregulation (Charland & Gregory 1995). Since gravid female garter snakes give birth late into the summer, they may prioritize thermal quality more in the summer season to facilitate gestation and the development of their offspring. Anecdotally, I did observe larger snakes in the open peat habitat during the summer which I presumed to be gravid, however because I did not capture incidentally observed individuals, I could not ascertain gravidity in the field. Given that the open peat habitat was the hottest habitat that also provided the greatest thermal quality in the summer
season, it is logical that gravid snakes would temporarily seek out this habitat for gestation purposes, and this may explain the slight increase in encounter rates that appear to be specific to the summer season. Further research into the habitat use of gravid female garter snakes in response to changing seasons is needed to support this hypothesis.

A large proportion (>50%) of all incidental and ACO encounters of Eastern garter snakes occurred in the spring season, primarily in the mixed shrub habitat. This trend is difficult to explain because the thermal quality in the mixed shrub habitat was rather low in the spring time, and without data on other habitat features, I can only speculate as to why garter snakes were most abundant in this habitat at the beginning of the active season. A possible explanation may involve the location of the mixed shrub habitat in my study area which was situated adjacent to a previously mined peatland. Due to the close proximity, peat spoils (mounds of rejected peat from the mining operation) were common throughout the mixed shrub habitat. These peat mounds, although artificially constructed, may mimic suitable overwintering areas that peat hummocks (naturally occurring peat mounds) are known to provide several North American reptile species (Smolarz et al., 2018). Natural peat hummocks are typically less than one meter tall but provide unique subterranean spaces for overwintering reptiles that are situated above the water table, resistant to deep frosts, provide ample moisture, and provide temperature stability throughout the winter (Smolarz et al., 2018). The artificial peat mounds in my mixed shrub habitat were larger than those found naturally but may still offer suitable conditions for overwintering snakes. If these artificial peat mounds were used by snakes in the winter, then their spring emergence could explain why the abundance of Eastern garter snakes was greater earlier in the active season.
Statistical analysis of my incidental encounters supported that there was an interaction present between habitat and season, despite the habitat effect alone being weak. This interaction may have been influenced by the spring time activity in the mixed shrub habitat which, in turn, may have been exceptionally high due to the presence of overwintering sites in this area. However, the fact that more garter snakes were observed in the mixed shrub habitat overall may also indicate a more attractive habitat for Eastern garter snakes. With more sampling, it may be possible to statistically support that nongravid Eastern garter snakes prefer the mixed shrub habitat over the other two available habitat types, at least during the spring and fall seasons. The reasons for why the mixed shrub habitat might be more desirable to Eastern garter snakes may involve many factors relating to thermal heterogeneity, abundance of resources (e.g. food and retreat sites), and predation risk. These relationships are explored in further detail in Chapter 4 (General Discussion).

Conclusions

Due to many limitations my encounter rates of Eastern garter snakes in all habitats were likely underestimated and may poorly reflect the real habitat use and preferences of the species. Nonetheless, the combination of ACO surveys and incidental surveys provide some indication that Eastern garter snakes may prefer an intermediate habitat type that provides a thermal landscape not quite as limited as a closed-canopy system (e.g. forest), but still provides some refuge from thermal extremes. There have been several studies that conclude that open habitat types such as fields, meadows, or artificial clearings are the preferred habitats of garter snakes (Charland & Gregory 1995; Engelstoft & Ovaska 2000; Kjoss & Litvaitis 2001; Halliday & Blouin-Demers 2015; Diaz & Blouin-Demers 2017).
However, my study shows that not all open-canopy habitats are equal in terms of their attractiveness to garter snakes, and that open habitats may only be preferred during particular life stages and/or seasons. Furthermore, my results indicate that within a mosaic of habitat types that vary in canopy coverage, substrate type, and overall thermal quality, a mixed habitat type may be used more often by Eastern garter snakes for some of their active season.
Chapter 4:

General Discussion
My study demonstrates that Eastern garter snakes likely prefer warm temperatures, similar to those selected by other members of the *Thamnophis* genus found at lower latitudes. I found no evidence that this species shifts its thermal preferences due to season or capture location, indicating that my results may be a reliable representation of Eastern garter snake thermal preference. I discovered that different habitat types offer different thermal profiles and subsequent thermal qualities for Eastern garter snakes. Thermal quality, as it is currently presented in literature, can mask the severity of temperature variation within habitats, and may not reliably predict habitat usage of Eastern garter snakes as a result. It is likely that other biotic and abiotic features contribute to habitat quality. I propose several biologically relevant scenarios for why I observed a mismatch between thermal quality and habitat use in Eastern garter snakes and suggest improvements to the current thermal quality calculation.

*Thermal Preference (T_{sel})*

Although thermal preference has been studied extensively in reptiles, changing nomenclature and sampling methodologies have resulted in inconsistent assessments of reptile thermal biology. This issue is not a novel one, as more than two decades ago biologists were explaining the difficulties and inconsistencies in defining preferred body temperature (Tocher 1992). Even studies completed on the same species just two years apart can find different results, as is the case with my study and that of Halliday & Blouin-Demers (2016). The latter study found that their sample population of *T. s. sirtalis* (n=49) exhibited a $T_{sel}$ of 25.5°C to 27.4°C (Halliday & Blouin-Demers 2016), which is a range considerably lower than what I calculated for my study population (n=21) which was between 27.82°C and 32.15°C. I hypothesized that my results would be slightly higher
given that I utilized a circular thermal gradient and Halliday & Blouin-Demers (2016) used a linear thermal gradient, but the 2.3–4.7°C difference was unexpected. The reason for this large difference may be partly due to the differences in thermal gradients used, but there may be a variety of other confounding variables which can explain this discrepancy. Firstly, the $T_{sel}$ trials performed by Halliday & Blouin-Demers (2016) were seven hours in duration with only the first hour allotted as the exploration period. I used an objective approach to determine that the first 2.5 hours was appropriate for the exploration period, and the $T_{sel}$ trials I performed were 10 hours in duration. In my study, the post-exploration period showed higher selected temperatures and less variation in all thermal preference parameters than when the entire experimental period was analyzed, so an overall shorter trial and shorter exploration period may account for some of the difference. Halliday & Blouin-Demers (2016) also only took body temperature measurements once per hour resulting in six sample points per animal. They then calculated the interquartile range of their entire population, ignoring intraindividual variation (i.e. individual differences in $T_{sel}$). Since I recorded 1200 temperatures per animal, I calculated individual $T_{sel}$ ranges and then used mean values for each $T_{sel}$ parameter to indicate the thermal preference range of my study population. By doing so, I incorporated intraindividual variability into my analysis which is important considering that variation of thermal preference may exist within and between populations (Angilleta 2009). Intraindividual trait variation can have many evolutionary and ecological impacts such as the adaptive ability of a species to environmental change (Bolnick et al. 2011). There is some empirical evidence supporting that $T_{sel}$ is heritable and that directional selection of thermal preference occurs in nature (Logan et al., 2014), thus individual $T_{sel}$ expression is relevant to thermal biological
studies. By ignoring intraindividual variability, Halliday & Blouin-Demers (2016) may have underestimated the breadth and the bounds of the $T_{sel}$ range of *T. s. sirtalis*.

I observed some garter snakes select temperatures in the thermal gradient consistent with the values reported by Halliday & Blouin-Demers (2016). However, the overall median selected temperature for my study population was 30.18°C, similar with what is reported in the literature for many snakes (average of ~30°C) despite phylogenetic and ecological differences between species (Weatherhead *et al.* 2012). Further studies of reptile thermal preferences should carefully consider the behaviours of their study animals within a thermal gradient prior to calculating $T_{sel}$. For example, using a null-model approach as explained by Cadena & Tattersall (2009) where individual movements in a gradient are observed in the absence of a thermal gradient (i.e. when the thermal environment is homogenous) prior to an active $T_{sel}$ trial. This approach can help illustrate behavioural tendencies and spatial preferences of an individual which may affect its thermoregulatory behaviours in the gradient. There may be individuals which exhibit a greater propensity to occupy corners of linear thermal gradients and become trapped in these corners for long durations and this can lead to skewed $T_{sel}$ calculations. Given that circular gradients can remove these corner or edge effects, comparative studies may be useful in determining the suitability of different gradient types (e.g. circular versus linear) and can determine whether these different approaches can significantly influence $T_{sel}$ calculations. In addition, it is recommended that future researchers use an objective criterion to determine an adequate exploration period for their animals. Allowing exploration time of novel thermal gradient environments can allow researchers to refine $T_{sel}$ calculations and increase the accuracy of their results by focusing on periods of the experiment where true temperature selection is
most likely. Lastly, although many variations of thermal indices have been reported in literature, T$_{sel}$ range may provide the most accurate representation of thermal preferences given that in the wild and in the laboratory, reptiles exhibit a preference for a range of temperatures rather than a single setpoint temperature. T$_{sel}$ ranges are realistic for any ectothermic species which shuttles between warmer and cooler temperatures to suit their thermoregulatory needs (Huey & Slatkin 1976; Lysenko & Gillis 1980; Hertz et al. 1993; Clusella-Trullas et al. 2007; Black et al. 2018).

Another conclusion that can be made when comparing my T$_{sel}$ study results with those of Halliday and Blouin-Demers (2016) is that Eastern garter snakes exhibit a wide tolerance to different temperatures. I focused my study on juvenile snakes (<1-year-old), so it is possible that replicating this study with adult animals that have had the ability to learn and acquire different thermoregulatory behaviours could render slightly different outcomes. Regardless of age class, when tested in a thermoregulatory low-cost environment, the T$_{sel}$ range of Eastern garter snakes spanned more than 4ºC. It is possible that my study design over estimated this range, but it may also represent a wide breadth of temperature selection exhibited by the species. The T$_{sel}$ range of Eastern garter snakes may be interpreted as a broad range given that most snake T$_{sel}$ ranges reported in literature span 3ºC or less (Weatherhead et al., 2012). Therefore, a broad T$_{sel}$ range may represent a wide thermal tolerance which may have enhanced the garter snake’s ability to occupy various ecological niches and climes. Thermal resilience and more relaxed thermal constraints can contribute to ectotherm success especially in systems undergoing environmental change (Huey & Kingsolver 1993). Therefore, a wide range of thermal preferences and a tolerance
to changing thermal conditions may have facilitated the garter snake’s expansion into new habitat types and latitudes throughout their evolutionary history.

_Habitat Use – Study Limitations_

Before drawing conclusions regarding habitat use from my ACO surveys, I should note the limitations of my experimental design. First, I used new plywood coverboards placed into novel areas that were previously unstudied. ACOs of this nature take time to be discovered by snakes and may require several months of maturation time _in-situ_ for them to become predictable areas for encountering snakes (Engelsoft & Ovaska 2000; Scheffers _et al._ 2009). Unfortunately, because of the constraints of time and site access, I could not allow a lengthy maturation time for my ACOs and this could have influenced coverboard occupancy. Secondly, I recorded greater coverboard occupancy when $D_{es}$ was positive, presumably on hot afternoons. If Eastern garter snakes used the boards most often when temperatures were sufficiently warm, then it is likely I underestimated habitat usage because not all surveys were conducted during these conditions. Lastly, the weather patterns of the 2017 active season were very inconsistent. Frequent weather systems involving significant rainfall events, high winds, thunderstorms, and even tornado warnings occurred in my study region. It may be entirely coincidental, but the low snake activity documented preceding a storm may also present a learning opportunity. Studies have shown that ectothermic species may be able to anticipate impending low-pressure systems and change their usual behaviour and activity patterns in response to these threats (Pellegrino _et al._ 2013). At least one snake species has been shown to respond to incoming storms by decreasing surface activity suggesting an innate ability to sense either drops in barometric pressure, or other early warning signals associated with an impending storm.
front (Liu et al. 2010). Consequently, my low capture rates may be due to a combination of factors, such as the use of novel (immature) ACOs, inconsistent weather patterns influencing snake behaviour, and poorly timed ACO surveys. In turn, these low capture rates made it difficult to detect significant differences regarding habitat usage by Eastern garter snakes. Although this study was not without its limitations, my data may still suggest potential patterns of habitat use that are equally important to discuss.

**Thermal Quality & Habitat Use**

The first major discovery I found in my 2017 field season was that contrary to my hypothesis, *T. s. sirtalis* did not exhibit a preference for the open peat habitat despite it being the habitat of the greatest thermal quality. In fact, the open peat habitat rendered the fewest incidental sightings of Eastern garter snakes of all three habitats studied. In the mixed shrub and forested habitats Eastern garter snakes were easier to spot because of the presence of understory vegetation and dry leaf litter that can quickly give away a snake’s position if it is moving. The vast majority of snakes were heard before they were visibly observed, and this is because leaf litter and small understory plants make noise when they are disturbed by a moving snake. The exposed peat soils of the open peat habitat are soft and characterized by large sections of little vegetation. This made hearing snakes in this habitat much more difficult, and this may have affected my ability to detect snakes in this habitat. Despite this detection bias, in both the case of incidental encounters and coverboard occupancy, the mixed shrub habitat was dominant for most of the active season. In the sections that follow, I propose some biologically relevant reasons for why my hypothesis regarding thermally-informed habitat use may not have been supported, and why an
intermediate habitat like the mixed shrub may be more favourable for Eastern garter snakes.

Originally, I hypothesized that environmental temperature would strongly influence habitat use in Eastern garter snakes because there is a large body of evidence supporting that squamates tend to select habitats that offer the most thermally suitable environments for their preferences (Blouin-Demers & Weatherhead 2002; Row & Blouin-Demers 2006; Elmore et al. 2017; Cox et al. 2018). From an evolutionary perspective this relationship makes sense. For example, if a snake actively selects the habitat that offers $T_c$ consistently within its preferred range, then less of its time needs to be devoted to thermoregulation and this frees up time where the animal can pursue other beneficial activities (Blouin-Demers & Weatherhead 2002). In a thermally superior habitat, if the snake’s body temperature does happen to drop too low, then the effort required to raise its $T_b$ would be minimal compared to other more thermally challenging environments like a forest, for example (Blouin-Demers & Weatherhead 2002). In essence, the life of an ectotherm can be less demanding depending on the thermal suitability of their selected habitat. However, in my study, I did not find any substantial evidence that thermal quality was strongly associated with habitat use (in terms of ACO occupancy or incidental encounters). This leaves me with two possible scenarios regarding Eastern garter snake habitat selection. Either this species does not prioritize thermal quality when assessing habitat and the influence of other habitat features such as prey density or predation pressure are stronger, or Eastern garter snakes do use temperature cues to select their habitat, but my temperature dataset was too variable, and my snake encounters were too few to detect a significant relationship. At present, both scenarios seem equally plausible. Eastern garter
snakes may have found their success in being an extremely tolerant and flexible species that are not strictly confined by temperature in terms of their movements or habitat use. Parlin et al. (2018) have observed a similar trend in Eastern box turtles, concluding that the effects of precipitation and time of day better predicted turtle movements than did temperature, so there may be some degree of thermal independence exhibited by reptiles. Furthermore, garter snakes may be examples of habitat generalists as they explore and exploit many habitat types in response to predation risk, competition pressures, or prey abundance (Angilleta 2009). Although Eastern garter snakes exhibit a preference for a certain range of temperatures in the presence of a low-cost lab environment, they may not consistently use the most thermally optimal habitats due to the many costs and pressures which they are susceptible to in nature. It is possible that not having narrow thermal constraints has allowed the subspecies (T. s. sirtalis) to colonize large portions of Eastern North America. Perhaps garter snake success can be attributed more to their ability to behaviourally thermoregulate in thermally challenging environments, as has been demonstrated for other cold-climate specialists (Besson & Cree 2010), rather than their instinct to select habitats of the highest thermal quality. Further research into the thermoregulatory efficiency of garter snakes in the wild could address this hypothesis and provide a new perspective for understanding their thermal ecology.

On the other hand, because my study was conducted at various times of day and year, my Tc dataset was extremely variable. Even with a larger sample size it would be difficult to support an unequivocal relationship between habitat use and temperature. Nonetheless, my statistical analyses did reveal a relationship between habitat use and seasonality for my incidental encounters. Environmental temperature and season are highly
correlated parameters, so this may indicate a potential relationship with temperature after all. As previously noted, I also documented extreme variability in temperatures in the open peat habitat which may have been less conducive to an ectothermic lifestyle than previously thought. If Eastern garter snakes do prefer thermal stability, then this could explain why they appeared to avoid the open peat habitat in certain seasons. Thermal stability aside, the open peat habitat was very hot, and consistently hotter than the surrounding habitat types. There is some evidence that suggests that snakes may prefer to live in environments with slightly suboptimal temperatures as it is easier for them to maintain their body temperatures within a preferred range when temperatures are cooler rather than hotter than their $T_{sel}$ (Martin & Huey 2008). In fact, when I recorded instantaneous $T_b$ measurements of 20 different Eastern garter snakes, I found that the mean $T_b(\text{field})$ of these snakes was 27.4°C, which fell just below the species’ lower $T_{sel}$ bound (27.8°C) that I calculated in the lab. This difference may provide preliminary support that smaller-bodied snakes may prefer to maintain lower body temperatures in the field because they can heat up quickly and in a hot environment like the open peat, they could reach dangerously high $T_b$s that could negatively impact their performance (Halliday & Blouin-Demers 2015). With these considerations, it is possible that *T. s. sirtalis* does consider thermal quality when selecting habitat. However, from the perspective of a smaller-bodied snake like garter snakes, higher thermal quality may mean slightly sub-optimal temperatures with low variability rather than habitats which offer mean temperature values which fit the confines of the animal’s $T_{sel}$ bounds.
**Thermal Stability**

The high operative temperature variation in the open peat habitat exemplifies an environment that is thermally unstable, and $T_e$ variability may be an overlooked parameter of thermal quality that is important to consider. Vickers *et al.* (2013) noted a fundamental flaw with the thermal quality calculation because it oversimplifies the complex thermal landscapes of hot, highly variable environments and argued that the current thermal quality framework is not enough to adequately explain or predict ectotherm habitat use. A major impetus for improving the thermal quality calculation is acknowledging that reptiles experience a disproportionately large negative impact on their fitness when exposed to hotter temperatures rather than cooler ones (Martin & Huey 2008; Vickers *et al.* 2013). Reptiles exhibit asymmetrical fitness curves where the costs of being too hot are much steeper than those associated with being too cool and this may explain why negative skew is commonly reported for reptiles. Perhaps this is also why certain snake species prefer to maintain slightly cooler temperatures well below their $CT_{max}$ and why they may exhibit a preference for thermally suboptimal habitats (Martin & Huey 2008).

Several studies have shown that reptiles will strategically select thermally stable microhabitats. Some snake species exhibit a clear preference for warm and stable environments such as those beneath coverboards (Hecnar & Hecnar 2011), rocks (Cox *et al.* 2018), and even inside barns (Blouin-Demers & Weatherhead 2002). Smaller ectothermic species like small-bodied snakes spend a considerable portion of their active season in retreat sites, so it is reasonable for these animals to seek out retreats that offer the most suitable microclimate (Huey 1991; Cox *et al.* 2018). This could also be true of habitat selection on a larger scale. Reptile diversity reaches its peak in the most thermally stable
tropical regions of the world (Huey & Slatkin 1976). Some species will take great risks in order to achieve stable body temperatures especially when they are bearing young (Lorioux et al. 2013), so careful selection of safe and stable thermal environments is paramount. Even for snake species preferring open habitats, such as the grass snake (*Natrix natrix*), individuals rarely basked completely in the open where $T_c$ can be the highest but rather seek out more thermally stable areas that are partially concealed conceivably so they can thermoregulate without exposing themselves to predators or dangerously high $T_b$s (Isaac & Gregory 2004). There is ample evidence suggesting that when snakes are actively thermoregulating, they maintain remarkably stable body temperatures. Especially in thermally challenging environments with great variation in $T_c$, snakes will select microhabitats and retreat sites that allow them to reap the benefits of a warmer habitat while not exposing themselves to a more dangerous and thermally chaotic environment. Considering the observations made in my study and others like it, it is likely that snakes select habitats not just on the premise of being able to achieve optimal temperatures, but also those which can provide these temperatures (or slightly suboptimal temperatures) for the longest period of time without exceeding the animal’s thermal tolerance.

*Thermoregulatory Opportunities*

A sympatric species of the Eastern garter snake, the black rat snake, prefers edge habitats, considered the interface where shaded environments meet non-shaded environments and provide a mixed matrix of microhabitats, in a population studied in Eastern Ontario (Blouin-Demers & Weatherhead 2002). This preference is especially strong in gravid females (Blouin-Demers & Weatherhead 2002). They also noted that when in this edge habitat, black rat snakes moved less, suggesting that they were meeting their
feeding and thermal requirements with ease (Blouin-Demers & Weatherhead 2002). A similar study involving rat snakes and racers report that these snakes frequent the edge habitats most often in the spring season when $T_e$ is most variable (Carfagno & Weatherhead 2006). Because thermal heterogeneity is greatest in the edge habitat, the snakes can maintain desired body temperatures by accessing different thermal environments despite high variation in $T_e$ (Carfagno & Weatherhead 2006). A similar effect was reported in an Australian study that found artificially engineered gaps in canopy cover greatly improved the thermal attractiveness of the habitats below by increasing $T_e$ (Pringle et al. 2003). Reptiles benefitted from the increases in canopy openness and colonized areas that were previously unused (Pringle et al. 2003). It was considered most beneficial to the local reptile community to have an intermediate level of canopy coverage, which presumably, would increase the ground-level $T_e$ enough to increase the amount of usable space within that habitat for both diurnal and nocturnal reptile communities (Pringle et al. 2003; Elmore et al. 2017).

In my study, the mixed shrub habitat was analogous to an edge habitat because it was a transitional zone where previously logged areas were gradually being reclaimed by the forest. The mixed shrub habitat was clearly undergoing succession and could be viewed as an extension of forest edge habitat. The mean canopy cover calculated across the active season was still over 60% in this habitat suggesting that shade was plentiful. However, distinct from the forested habitat used in this study, the mixed area was dominated by shrubs and immature hardwood trees and lacked the understory vegetation typical of a climax community or mature forest. Although shade was not limiting in the mixed area, the lack of contiguous canopy cover and presence of shorter, immature trees allowed for
more incoming solar radiation which in turn allowed this region to get rather hot. Environmental temperatures in this habitat did occasionally surpass the VT\(_{\text{max}}\) of garter snakes, and although not as hot as the open peat, the mixed shrub area would certainly get hot enough to force active reptiles into refugia to escape the heat. However, shaded areas and cooler microclimates in the mixed shrub habitat were abundant. Peat spoils, fallen trees, leaf litter and other salient ecological features meant that the mixed shrub was also a structurally diverse habitat. This structural diversity could translate into greater thermoregulatory opportunities for Eastern garter snakes, similar to what was noted by Blouin-Demers and Weatherhead (2002). These opportunities would allow Eastern garter snakes to meet their thermoregulatory needs without heightened exposure to predation or dangerously hot temperatures, and they would also not have to travel as far to seek out warmer or cooler microclimates within their environment (Blouin-Demers & Weatherhead 2002). Ultimately, the combination of a more stable (or rather, less extreme) thermal environment with ample thermoregulatory opportunities may act synergistically to make the mixed shrub a more desirable habitat for Eastern garter snakes.

*Activity Time Restrictions*

Another biological explanation for why Eastern garter snakes were not found using the open peat habitat most frequently was that the open peat imposed serious restrictions to their activity time, and this likely reduced the attractiveness of this habitat to garter snakes. This parameter is referred to as \(Hr\) (hours of restriction) and is argued to be an important parameter for assessing habitat quality and addressing the vulnerability of reptile species to climatic changes (Pontes-da-Silva *et al.*, 2018). Hours of restriction are defined as the number of activity time hours when \(T_e\) surpasses the \(VT_{\text{max}}\) of a particular
ectothermic species, thus indicating periods of the day when surface activity is limited or entirely off limits to a species of interest (Pontes-da-Silva et al., 2018). Voluntary thermal maxima may provide more accurate estimates of activity restriction given that they denote the maximum temperature at which a species can resume surface activities, but beyond which it will seek refuge (Pontes-da-Silva et al., 2018). A voluntary thermal maximum is distinct from CT$_{\text{max}}$ because exposure to the latter induces a loss of locomotor abilities, whereas the voluntary maximum may only elicit an aversive response. I quantified the proportion of activity time which exceeded the VT$_{\text{max}}$ of garter snakes and discovered that the open peat habitat imposed the greatest restrictions to Eastern garter snakes. In fact, the open peat habitat exceeded the VT$_{\text{max}}$ of garter snakes more than six times that of the mixed shrub, which could be substantial in terms of affecting habitat quality. Since other habitat types are accessible to Eastern garter snakes throughout their range, it is likely that they would avoid habitats which expose them to thermal extremes and greater restrictions to their activity time. Reptiles at this latitude must already cope with limited active seasons and exposure to many abiotic pressures, so additional restrictions to their activity time imposed by environmental temperatures are considerable and may decrease long-term habitat suitability.

**General Conclusions & Future Work**

Despite being the most widespread reptile species in North America and occurring further north than any other terrestrial reptile on the continent, the T$_{\text{sel}}$ of Eastern garter snakes is not drastically different from other sympatric snake species with more restricted geographic ranges. The median selected temperature for my study population was 30.18°C, which is considered slightly above average for the snake species which have had their T$_{\text{sel}}$
quantified over the last few decades (Weatherhead *et al.* 2012), but similar to the mean selected temperatures reported for other *Thamnophis* species. The $T_{\text{sel}}$ of Eastern garter snakes may even lend support to the counter-gradient hypothesis which suggests that colder-climate reptiles may have evolved warmer thermal preferences than warmer climate relatives. However, until more snake species from varying latitudes have been studied, the counter-gradient selection of preferred temperatures remains hypothetical.

My study contradicts the hypothesis that snakes seek out habitats that offer the greatest thermal quality. There may be many reasons for why I did not find this relationship in my study, however it is likely that not all open habitats provide the same quality to snakes. Habitats that frequently get too hot subject these animals to restrictions to their surface activity and this can have many negative impacts. I suggest that the current framework for quantifying habitat thermal quality be modified to improve the utility of this parameter and allow it to better predict habitat selection in ectotherms. Perhaps if statistical analyses focused on treating positive and negative thermal quality ($D_{\text{es}}$) values separately, this could help alleviate some uncertainty regarding the disproportionate effects of hot and cool temperatures on snake habitat use. For instance, negative $D_{\text{es}}$ values which indicate cooler temperatures do not impose the same limitations or risks to snakes as positive $D_{\text{es}}$ values, and this should be considered when assessing the thermal quality of hot environments.

More thermally stable and intermediate habitat types that provide ample thermoregulatory opportunities may be the most favourable for Eastern garter snakes. These habitats appear to support the greatest relative abundance of snakes and the thermal benefits of a spatially heterogenous habitat may benefit an entire reptile community
(Pringle et al. 2003), not just garter snakes. In essence, habitat use of Eastern garter snakes may follow a similar pattern to the Goldilocks principle: habitats that are too hot or too cool are used less than habitats that are more thermally neutral. In addition, since edge habitats and mixed successional habitats seem to be preferred by northern snake communities (Blouin-Demers & Weatherhead 2002), protecting a mosaic of forested, mixed, and open areas may prove beneficial for the conservation of terrestrial reptile communities in temperate regions.

The fact that some Eastern garter snakes were still spotted using the open peat habitat during extreme heat events suggests that these animals may have been attracted to this habitat for thermal reasons. Since environmental temperatures were above the voluntary thermal maximum for garter snakes for more than 11% of their active season in the open peat, the usable space of this habitat is greatly reduced for Eastern garter snakes (Elmore et al. 2017). Their realized niche would be limited to cooler microhabitats and refugia that shelter them from thermal extremes (Elmore et al. 2017), and these can be scarce resources in an open environment. To make matters worse, the open peatlands of my study site are subject to stochastic events, and wildfires are becoming increasingly common in this area. Ultimately, the open peat habitat is not an ideal habitat for snakes and could represent a poor choice in habitat selection made by the animals. Gravid females may use this site for basking/gestating purposes, but if they give birth in this habitat, it may become too hot for the young to survive. Beginning life in a harsh environment like the open peat habitat could present a serious barrier to neonatal survival and is likely to result in reduced survivorship. Any snake species being drawn to this habitat via temperature cues and mistaking it for high quality habitat could be subjecting itself and its young to
reduced fitness. In turn, this could eventually result in a potential trap for the local snake community (Battin 2004). Continued monitoring of these open peatlands is encouraged to determine if snakes are actively selecting this habitat or simply passing through them incidentally. Such monitoring would also highlight the influences of large-scale disturbances (such as wetland draining and clearing) on habitat quality and the potential negative consequences for resident reptile communities.
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Appendix

Table A.1: Post-hoc pairwise comparisons of mean operative environmental temperature ($T_e$) across all habitats and seasons. Contrast estimates (*estimate*), z-ratios (*z*), and p-values (*p*) are included for each comparison. Significance was determined at the 95% confidence level ($\alpha=0.05$).

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>estimate</th>
<th>$z$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest,Fall - Mixed Shrub,Fall</td>
<td>-1.3777</td>
<td>-1.3485</td>
<td>0.9166</td>
</tr>
<tr>
<td>Forest,Fall - Open Peat,Fall</td>
<td>-4.5595</td>
<td>-4.8099</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Forest,Spring - Forest,Fall</td>
<td>0.6015</td>
<td>2.278</td>
<td>0.3555</td>
</tr>
<tr>
<td>Forest,Spring - Forest,Summer</td>
<td>-1.6441</td>
<td>-6.5051</td>
<td>$&lt;0.0001^*$</td>
</tr>
<tr>
<td>Forest,Spring - Mixed Shrub,Spring</td>
<td>-1.0355</td>
<td>-1.041</td>
<td>0.9819</td>
</tr>
<tr>
<td>Forest,Spring - Open Peat,Summer</td>
<td>-3.8015</td>
<td>-4.0862</td>
<td>0.0014*</td>
</tr>
<tr>
<td>Forest,Summer - Forest,Fall</td>
<td>2.2456</td>
<td>8.5042</td>
<td>$&lt;0.0001^*$</td>
</tr>
<tr>
<td>Forest,Summer - Mixed Shrub,Summer</td>
<td>-0.8317</td>
<td>-0.8307</td>
<td>0.996</td>
</tr>
<tr>
<td>Forest,Summer - Open Peat,Summer</td>
<td>-4.8012</td>
<td>-5.1391</td>
<td>$&lt;0.0001^*$</td>
</tr>
<tr>
<td>Mixed Shrub,Fall - Open Peat,Fall</td>
<td>-3.1818</td>
<td>-3.0821</td>
<td>0.053</td>
</tr>
<tr>
<td>Mixed Shrub,Spring - Mixed Shrub,Fall</td>
<td>0.2593</td>
<td>0.7172</td>
<td>0.9986</td>
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<td>Mixed Shrub,Summer - Mixed Shrub,Fall</td>
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<td>4.7102</td>
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<tr>
<td>Mixed Shrub,Summer - Open Peat,Summer</td>
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<td>Open Peat,Spring - Open Peat,Fall</td>
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<td>0.9999</td>
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<td>Open Peat,Spring - Open Peat,Summer</td>
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<tr>
<td>Open Peat,Summer - Open Peat,Fall</td>
<td>2.4873</td>
<td>8.2631</td>
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</table>
Table A. 2: Post-hoc pairwise comparisons of mean thermal quality (D<sub>e</sub>) across all habitats and seasons. Contrast estimates (estimate), z-ratios (z), and p-values (p) are included for each comparison. Significance was determined at the 95% confidence level (α=0.05).

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>estimate</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest,Fall - Mixed Shrub,Fall</td>
<td>1.1622</td>
<td>1.8139</td>
<td>0.6730</td>
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<tr>
<td>Forest,Fall - Open Peat,Fall</td>
<td>3.4850</td>
<td>5.8994</td>
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<tr>
<td>Forest,Summer - Forest,Fall</td>
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<tr>
<td>Forest,Summer - Forest,Summer</td>
<td>1.7338</td>
<td>8.1571</td>
<td>&lt;0.0001*</td>
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<td>Forest,Summer - Mixed Shrub,Summer</td>
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<td>1.3932</td>
<td>0.9008</td>
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<td>Mixed Shrub,Summer - Mixed Shrub,Summer</td>
<td>-1.8228</td>
<td>-6.0167</td>
<td>&lt;0.0001*</td>
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<tr>
<td>Mixed Shrub,Summer - Open Peat,Summer</td>
<td>2.1646</td>
<td>3.4809</td>
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<td>Open Peat,Summer - Open Peat,Fall</td>
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<tr>
<td>Open Peat,Summer - Open Peat,Summer</td>
<td>2.1274</td>
<td>9.5027</td>
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<tr>
<td>Open Peat,Summer - Open Peat,Fall</td>
<td>-1.6646</td>
<td>-6.5833</td>
<td>&lt;0.0001*</td>
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Table A. 3: Post-hoc pairwise comparisons of incidental encounters of Eastern garter snakes across all habitats and seasons. Contrast estimates (estimate), z-ratios (z), and p-values (p) are included for each comparison. Significance was determined at the 95% confidence level (α=0.05).

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>estimate</th>
<th>z</th>
<th>p</th>
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</thead>
<tbody>
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<td>Forest,Fall - Forest, Spring</td>
<td>-1.2993</td>
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<td>0.5470</td>
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<td>Forest,Fall - Forest, Summer</td>
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<td>0.9966</td>
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<td>Forest,Fall – Mixed Shrub, Fall</td>
<td>-0.8473</td>
<td>-1.2279</td>
<td>0.9506</td>
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<tr>
<td>Forest,Fall – Open Peat, Fall</td>
<td>0.0000</td>
<td>0.0000</td>
<td>1.0000</td>
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<tr>
<td>Forest, Spring - Forest, Summer</td>
<td>0.7397</td>
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<td>Forest, Spring – Mixed Shrub, Spring</td>
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<td>-1.4426</td>
<td>0.8813</td>
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<td>Forest, Spring – Open Peat, Spring</td>
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<td>1.7326</td>
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<td>1.2279</td>
<td>0.9506</td>
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<td>Mixed Shrub, Fall – Mixed Shrub, Summer</td>
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<td>1.6447</td>
<td>0.7799</td>
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<tr>
<td>Mixed Shrub, Fall – Open Peat, Fall</td>
<td>0.8473</td>
<td>1.2279</td>
<td>0.9506</td>
</tr>
<tr>
<td>Mixed Shrub, Spring – Mixed Shrub, Summer</td>
<td>2.1335</td>
<td>3.4342</td>
<td>0.0173*</td>
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<tr>
<td>Mixed Shrub, Spring – Open Peat, Spring</td>
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<td>Mixed Shrub, Summer – Open Peat, Summer</td>
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<td>Open Peat, Fall – Open Peat, Spring</td>
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<tr>
<td>Open Peat, Fall – Open Peat, Summer</td>
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<td>0.9013</td>
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<tr>
<td>Open Peat, Spring – Open Peat, Summer</td>
<td>-0.6286</td>
<td>-1.0625</td>
<td>0.9794</td>
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</tbody>
</table>
Figure A. 1: Wide-angle portrait of forest habitat
Figure A. 2: Wide-angle portrait of mixed-shrub habitat
Figure A. 3: Wide-angle portrait of open peat habitat
Figure A. 4: Individual frequency density plots illustrating the distribution of temperatures selected by Eastern garter snakes during their $T_{sel}$ trials. Median selected temperature (top value) and skewness of distribution (bottom value) are reported for each participant in the top left of every plot. Negative skewness can be identified by longer tails observed on the left of the distribution, while positive skewness is identified by longer tails on the right of the distribution.
Figure A. 5: GAM smoothing curve illustrating the relationship between signed thermal quality (Des) and ACO encounters of Eastern garter snakes. The probability of encountering an Eastern garter snake at a coverboard increased slightly when thermal quality was positive but the relationship was not significant (chi sq: 2.83, edf: 1, p-value: 0.093).
Figure A. 6: GAM smoothing curve illustrating the relationship between signed thermal quality ($D_{es}$) and incidental encounters of Eastern garter snakes. Incidental encounters were greatest when thermal quality was slightly negative but the relationship was not significant (chi sq: 4.67, edf: 1.82, p-value: 0.118).