

Anthropogenic effects on chronic stress and
nest predation patterns in freshwater turtles.

by

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Abstract

Human altered landscapes contribute to direct and indirect threats on wildlife populations. Chronically high stress, an indirect threat, has been associated with decreased fitness. I examined chronic stress in Midland Painted Turtles (*Chrysemys picta marginata*), Snapping Turtles (*Chelydra serpentina*), and Blanding's Turtles (*Emydoidea blandingii*) in areas with varying densities of anthropogenic structures in Muskoka, Ontario. Enzyme immunoassays were run, in partnership with Dr. Gabriela Mastromonaco (Toronto Zoo), on turtle claws to obtain concentrations of corticosterone (CORT). Most Snapping Turtles had above-minimum CORT, and male Midland Painted Turtles and Blanding's Turtles had relatively high CORT levels. Anthropogenic density did not seem to affect CORT levels, but more research needs to be done to understand the complex relationships. I also determined the rate and pattern of nest depredation, a direct threat, throughout egg incubation in areas of varying anthropogenic densities. Nest depredation occurred throughout egg incubation and relative nest mesopredator abundance did not differ among sites with varying anthropogenic densities. Contrary to some nest caging protocols, my findings suggest that nest cages should be kept on nests until the turtles hatch and priority should be given to caging nests in low road density areas to help increase population recruitment. Understanding indirect and direct threats to turtles can help inform mitigation strategies to improve their fitness.

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General Introduction

Vertebrates use many different specialized habitats seasonally and throughout their life. Those areas can be close to or within areas of varying anthropogenic densities, or densities of anthropogenic structures, such as roads and buildings. High anthropogenic density can fragment habitats, making dispersal and survival difficult (Sanderson *et al.*, 2002) and negatively affecting wildlife populations (Butchart *et al.*, 2010; Hoffman *et al.*, 2010). Conservation programs need to meet the animals' requirements regardless of the density of anthropogenic structures or economic interests (of an area (Lambeck, 1997; Naughton-Treves, 1998; Hoare and Du Toit, 1999). Conservation planning, therefore, needs to be done on a landscape scale (Noss, 1983).

Turtles are, globally, one of the most at risk taxa (Gibbons *et al.*, 2000). They have a “bet hedging” life history, characterized by high adult survivorship, long lifespans, delayed maturity, iteroparity, and low offspring survivorship (Stearns, 1976; Congdon and Tinkle, 1982). This life history is part of the reason why turtle populations are susceptible to direct and indirect threats that especially impact adult survivorship. Indirect threats affect individual physiology, such as stress, by re-directing resources from production to maintenance, while direct threats cause immediate mortality. Indirect threats include habitat destruction and fragmentation (Klemens, 2000), pollution (Gibbons *et al.*, 2000), and climate change (Gibbons *et al.*, 2000). Direct threats include poaching (Gibbons *et al.*, 2000; Velo-Anton *et al.*, 2010), subsidized mesopredators (Snow, 1982; Oehler and Litvaitis, 1996; Prugh *et al.*, 2009) that depredate nests (Oddie *et al.*, 2014), and road mortality (Ashley and Robinson, 1996; Baxter-Gilbert *et al.*,

2015).

Chronic Stress

Stress, an indirect threat, is a vertebrate's physiological response to a negative stimulus, or stressor, such as noise (Blickley *et al.*, 2012), predation risk (Sapolsky, 1990), or habitat destruction (Reeder and Kramer, 2005; Leshyk *et al.*, 2012). Elevated levels of stress hormones increase a vertebrate's heart rate, breathing rate, and blood pressure (Axelrod and Reisine, 1984; Sapolsky, 1990; Dobson and Smith, 2000; Reeder and Kramer, 2005; Sherrif *et al.*, 2011). This acute response is useful to react to immediate unpredictable stressors by triggering the “fight or flight” response (Sapolsky, 1990), and subsequently quickly returning to homeostasis (Axelrod and Reisine, 1984; Reeder and Kramer, 2005; Sherrif *et al.*, 2011). However, if the stress response is chronic, a higher risk of infection due to reduced immunity and reduced fitness can result (Axelrod and Reisine, 1984; Sapolsky, 1990; Dobson and Smith, 2000; Reeder and Kramer, 2005; Sherrif *et al.*, 2011).

A vertebrate's physiological stress response can be summarized as the activation of the sympathetic nervous system to secrete adrenaline, and the hypothalamic-pituitary-adrenal (HPA) axis in terrestrial vertebrates (Sheriff *et al.*, 2011) or hypothalamic-pituitary-inter-renal (HPI) axis in fish (Bonga, 1997) to secrete glucocorticoids (GCs; Bonga, 1997; Reeder and Kramer, 2005; Ashley *et al.*, 2011; Sheriff *et al.*, 2011). Usually the hippocampus inhibits the HPA or HPI axis through a negative feedback system (Reeder and Kramer, 2005; Ashley *et al.*, 2011; Sheriff *et al.*, 2011). When a vertebrate is

exposed to a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH) to the pituitary (Reeder and Kramer, 2005; Ashley *et al.*, 2011; Sheriff *et al.*, 2011). The pituitary makes and releases adrenocorticotropin hormone (ACTH) to the blood stream, which then stimulates the adrenal cortex to secrete an excess of GCs to the blood stream (Reeder and Kramer, 2005; Sheriff *et al.*, 2011), which in turn, increase a vertebrate's ability to escape a dangerous situation (Sheriff *et al.*, 2011). The negative feedback system then stops the production of more GCs, helping the vertebrate to return to homeostasis (Reeder and Kramer, 2005; Sheriff *et al.*, 2011). The negative feedback signals are weaker for a chronic stress response than an acute stress response such that under chronic stress, the system stays active and disrupts long term functions like growth and reproduction for a longer period of time (Reeder and Kramer, 2005; Sheriff *et al.*, 2011).

A common way to quantify the level of stress a vertebrate is experiencing is by measuring the amount of GCs that are produced (Reeder and Kramer, 2005; Ashley *et al.*, 2011; Dantzer *et al.*, 2011; Sheriff *et al.*, 2011; Leshyk *et al.*, 2012). Because GCs are released into the bloodstream, most studies quantifying the level of GCs have sampled blood from vertebrates (Sheriff *et al.*, 2011). This technique is fairly invasive and the blood needs to be collected within the first couple of minutes after the vertebrate is caught to ensure GC levels measured are baseline (i.e. not elevated as a result of the stress of capture and handling: Sheriff *et al.*, 2011). Moreover, blood samples can be difficult to maintain when working in remote field sites as they need to be kept at -20°C (Sheriff *et al.*, 2011). Saliva is another medium that GCs enter (from the bloodstream)

and is less invasive to collect and easier to store than blood, but still provides a short term measure of stress levels (Sheriff *et al.*, 2011). GC metabolites can be found in feces (Malakoff, 1997) and urine and can be used to measure stress hormones on a scale of hours or days, depending on the species' metabolic rates (Sheriff *et al.*, 2011). Should the feces and urine become mixed, the specific metabolite concentration can become difficult to measure (Sheriff *et al.*, 2011). A species-specific proportion of GCs exits the blood stream and gets deposited in tissues (Rosner, 1990), such as hair (Ashley *et al.*, 2011), feathers (Sheriff *et al.*, 2011), and keratinized structures such as nails and claws (Baxter-Gilbert *et al.*, 2014). GCs in these tissues provide a measure of long-term stress levels (Sheriff *et al.*, 2011). My study will examine chronic stress levels of turtles by sampling and analyzing their claws.

Threats to Freshwater Turtle Populations

Habitat fragmentation can come in many forms, including, draining of wetlands and building of roads, buildings, and trails (Gibbons *et al.*, 2000). Climate change may make currently protected habitat unsuitable for turtles pushing them into surrounding fragmented habitats (Gibbons *et al.*, 2000). Female turtles often lay eggs in the gravel shoulders of roads, putting them at an increased risk of road mortality, thus shortening their reproductive years, and the nests and hatchlings are susceptible to mortality from substrate impaction and vehicle collisions. Climate change could alter the sex ratios of turtles with temperature-dependent sex determination (where the sex of the turtle is based on the temperature of the eggs during incubation; Janzen, 1994), if female turtles do not

evolve to use different nest sites (Gibbons *et al.*, 2000).

Turtle eggs are especially at risk from pollution (Gibbons *et al.*, 2000), which could decrease their already low survival rates to unsustainable levels (Stearns, 1976; Congdon and Tinkle, 1982). For example, turtle eggs are adversely affected by endocrine-disrupting chemicals (Guillette and Crain, 1996) and PCBs (polychlorinated biphenyls; Bergeron *et al.*, 1994; Bishop *et al.*, 1994; Guillette *et al.*, 1995; Cobb and Wood 1997).

Hunting and bycatch are serious threat to turtles because population persistence relies on individuals surviving for long periods of time to continue to reproduce (Stearns, 1976; Congdon and Tinkle, 1982). In North America, Alligator Snapping Turtles (*Macrolemys teminckii*) have been negatively affected by hunting for food (Roman *et al.*, 1999), whereas Box Turtles (*Terrapene carolina*; Lieberman, 1994) and Bog Turtles (*Glyptemys muhlenbergii*; Copeyon 1997) by collection for the pet trade. In Ontario, the Snapping Turtle (*Chelydra serpentina*) is listed as a game species under the *Fish and Wildlife Conservation Act* (FWCA, 1997), while also being listed as a species of special concern under the *Species at Risk Act* (SARA, 2003).

Abundance of mammals that predate turtle eggs, a direct threat, is higher where anthropogenic sources of food (Oehler and Litvaitis, 1996) and decreases in populations of top predators (Prugh, 2009) subsidize an ideal environment for middle-sized mesopredators to flourish. Raccoons (*Procyon lotor*) and red foxes (*Vulpes vulpes*) are both well-known turtle nest predators (Snow, 1982) and have increased population sizes near anthropogenic structures (Oehler and Litvaitis, 1996; Prugh *et al.*, 2009). A number of studies have found that the highest predation risk to turtle nests is within the first week

after the eggs are laid (Tinkle *et al.*, 1981; Congdon *et al.*, 1983; Christens and Bider, 1987; Congdon *et al.*, 1987; Robinson and Bider, 1988; Holcomb and Carr 2013), although this is not necessarily a consistent pattern, as other studies have found that depredation of turtle nests occurs throughout the incubation period (Burger, 1977; Snow, 1982; Brooks *et al.*, 1992; Gillingwater, 2002; Riley and Litzgus, 2014). The inconsistencies seen in turtle nest predation patterns may be attributed to the large variety of species that depredate turtle nests and cues available to potential predators.

There are a number of nest cues predators could use to find turtle nests throughout the incubation period. Predators may be able to smell oviposition fluid early in the season (Legler 1954; Congdon *et al.*, 1983; Spencer 2002), rotten eggs throughout incubation, and embryonic fluids when the hatchlings hatch (Riley and Litzgus, 2014). Predators may see turtles laying eggs (Congdon *et al.*, 1987; Eckrich and Owens 1995) or the soil disturbed (Strickland *et al.*, 2010; Spencer 2002) during nesting season, and emerged hatchlings (Riley and Litzgus, 2014) during hatching season. Oddie *et al.* (2015) found that predators were more likely to depredate nests that had tactile cues, such as soft soil resulting from maternal digging and covering. Predators may also hear hatchlings inside their nest cavity after they hatch (Ferrara *et al.*, 2013). Canines and other mammals seem to be the most common nest predators later during incubation (Burger, 1977; Brooks *et al.*, 1992; Spotila, 2011; Riley and Litzgus, 2014).

Study Area, Species, and Objectives

The objective of my study is to investigate the indirect and direct impacts of

human development on freshwater turtles by examining chronic stress levels (Chapter 1) and rate and patterns of nest predation (Chapter 2) in areas of varying anthropogenic densities.

My thesis focuses on the effects of habitat fragmentation, in the form of road and building densities, on chronic turtle stress and nest predation patterns in central Ontario. The study area (roughly 44N 40' to 45N 20' latitude and -79W 50' to -79W 10' longitude; see Figure 1) covers southern portions of the Parry Sound and Muskoka districts and borders the southern boundary of the Boreal Shield Ecozone and the northern boundary of the Mixedwood Plains Ecozone, within the Great Lakes Watershed (Crins *et al.*, 2009). There are many wetlands and lakes among rock barren outcrops and mixed forests (Crins *et al.*, 2009), that provide ideal habitat for the diverse herpetofauna that live in this area (Hecnar *et al.*, 2002). This region of Ontario experiences a humid continental temperate climate, with four distinct seasons (Baldwin, *et al.*, 2000).

Southern Muskoka is an ideal study area for my project because there is a wide range of anthropogenic densities, from very low anthropogenic density, in conservation reserves and Crown land, to high anthropogenic density, in small town centers (Gravenhurst, Bala, etc). Moderately impacted areas include locations with few gravel roads and low building density. Southern Muskoka also simplifies a calculation of anthropogenic density because there are few agriculture and logging practices present (personal observation). In addition, the Muskoka area is home to a diversity of turtle species at risk (SAR; *Species at Risk Act*; SARA, 2003; Committee on the Status of Endangered Wildlife in Canada; COSEWIC, 2011).

My study focuses on three species of turtles: Midland Painted Turtles (*Chrysemys picta marginata*), Snapping Turtles, and Blanding's Turtles (*Emydoidea blandingii*). Midland Painted Turtles and Snapping Turtles occupy different habitat niches (Ernst and Lovich, 2009), which allowed me to compare and make predictions about the effects of anthropogenic density among species. Midland Painted Turtles are small-bodied habitat generalists that spend a significant portion of time basking (Ernst and Lovich, 2009), unlike Snapping Turtles who are large-bodied habitat generalists that spend a significant portion of time underwater (Paterson *et al.*, 2012). Blanding's Turtles, a threatened species in Ontario (COSEWIC, 2005) are more terrestrial than Midland Painted and Snapping Turtles (Ernst and Lovich, 2009). I was able to compare the large sample sizes of data from Midland Painted and Snapping Turtles to small sample sizes of data from Blanding's Turtles.

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Figure

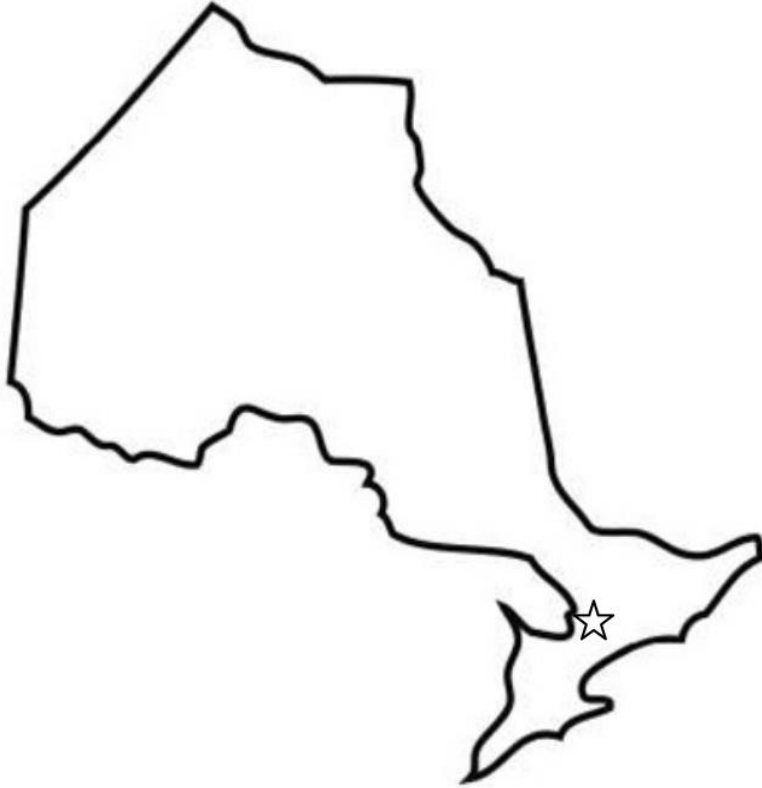


Figure 1: The star represents the study area within Ontario for this thesis. North is toward the top of the page. Due to the potential for illegal collection of turtles, specific location information is not disclosed.

Table

Table 0.1: Turtle species that occur in relative abundance in South Muskoka, Ontario, and their federal and provincial conservation status designations. The federal conservation status is determined by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and *Canada's Species At Risk Act (SARA)*. The provincial conservation status is determined by the Committee on the Status of Species at Risk in Ontario (COSSARO) and *Ontario's Endangered Species Act (ESA)*.

Turtle Species	Federal and Provincial Conservation Statuses
Midland Painted Turtle (<i>Chrysemys picta marginata</i>)	Not Assessed
Snapping Turtle (<i>Chelydra serpentina</i>)	Special Concern
Blanding's Turtle (<i>Emydoidea blandingii</i>)	Threatened

Chapter 1

Do Anthropogenic Structure Densities Cause Chronic Stress in Freshwater Turtles?

Abstract

Wildlife near human altered landscapes can exhibit high levels of stress hormones. However, vertebrates can also adapt to chronic high stress situations such that their stress hormone levels readjust to new baseline levels. Chronically high stress hormone levels have been associated with decreased fitness in some reptile and amphibian species. Yet, very little research has been completed on stress hormone levels in reptiles and especially on turtles. Because turtles are long-lived, a measure of chronic stress will help inform long-term conservation efforts in ways that measures of acute stress cannot. I examined chronic stress hormone levels in Midland Painted Turtles (*Chrysemys picta marginata*), Snapping Turtles (*Chelydra serpentina*), and Blanding's Turtles (*Emydoidea blandingii*) in areas with varying densities of anthropogenic structures. Enzyme immunoassays were run on claws sampled from turtles to obtain a concentration of corticosterone (CORT). Male Snapping Turtles were most likely to have above-minimum CORT and male Midland Painted Turtles and male Blanding's Turtles were more likely than females. However, for turtles that had above-minimum CORT, Midland Painted Turtles had the highest relative above-minimum CORT and male Snapping Turtles and male Blanding's Turtles had relatively higher above-minimum CORT levels than females. Surprisingly, anthropogenic density did not seem to affect CORT levels, although variation in the date turtles were captured and the date enzyme immunoassays were run complicated my interpretations. CORT is a byproduct of more than a stress response, and more research needs to be done to understand the complex relationships between vertebrates, anthropogenic influences, and stress. Globally, turtles

are among the most at-risk species and understanding turtle stress physiology can help inform mitigation strategies to improve their fitness.

Introduction

Globally, wildlife populations are in decline and habitat alteration due to human activities is largely the cause (Butchart *et al.*, 2010; Hoffmann *et al.*, 2010). Road mortality is a large contributing factor in global population declines of various animals (Butchart *et al.*, 2010; Hoffmann *et al.*, 2010), but roads and other anthropogenic structures may also be indirectly affecting wildlife populations via stress on individuals. Roads can interrupt migration routes of certain species by acting as barriers to their movement, which could decrease genetic diversity and isolate populations, such as was observed in Timber Rattlesnakes (*Crotalus horridus*; Clark *et al.*, 2010). Gaddy and Kohlsaatt (1987) recorded that the presence of man-made trails and roads decreased reptile and amphibian species richness and density. Wildlife near human altered landscapes can exhibit high levels of stress hormones (Brearley *et al.*, 2012), but vertebrates can also adapt to chronic high stress situations such that stress hormone levels readjust to new baseline levels in a process termed adrenocortical modulation (Wingfield and Ramenofsky, 1999). Chronically high stress hormone levels, over a few days or more, have been associated with decreased fitness in some reptile and amphibian species (Moore and Jessop, 2002). For example, male American Alligators (*Alligator mississippiensis*) secreted less testosterone (Lance and Elsey, 1986), and a species of skink (*Mabuya carinata*) showed impaired spermatogenesis (Yajurvedi and Menon, 2005), when stressed over the course of a couple days. Studying potential stressors, such

as density of anthropogenic structures, which could lead to chronic stress can help inform long-term conservation efforts, such as road mitigation, for species at risk (Busch and Hayward, 2009).

The major glucocorticoid (GC) stress hormone measured in reptiles is corticosterone (CORT; Sandor, 1972). Studies have measured CORT in the blood of tree lizards (*Urosaurus ornatus*; French *et al.*, 2008) and in fecal samples from Green Iguanas (*Iguana iguana*; Kalliokoski *et al.*, 2012), which both represent measures of acute stress. In turtles, urine and feces are mixed, making GC analysis of both samples difficult, and infrequent (Sheriff *et al.*, 2011). Because turtles are long-lived, a measure of chronic stress hormone levels will help inform long term conservation efforts, such as effectiveness of road mitigation. CORT responses are species-specific, so conservation efforts can be more effective if they can target benefits for specific species. CORT, accumulated over a series of weeks to months (chronic CORT), has been measured in keratinized structures, such as hair or fur of mammals (Ashley *et al.*, 2011), feathers of birds (Sheriff *et al.*, 2011), and shed snake skins (Berkvens *et al.*, 2013). A successful pilot study was completed on a newly developed non-invasive technique for measuring CORT from turtle claw samples using enzyme immunoassays (Baxter-Gilbert *et al.*, 2014). Claws can be collected easily from wild-caught turtles with minimal handling, and may be able to indicate a record of long-term stress hormone levels (Baxter-Gilbert *et al.*, 2014). My research expands on this pilot study to increase my understanding of physiological stress in turtles. Studies have quantified the impact of land-use on turtle population structure (Marchand and Litvaitis 2004) and ecological features (Failey *et al.*,

2007). Few studies have quantified the impacts of several anthropogenic factors simultaneously (Gaddy and Kohlsaas, 1987; Crowley, 2006; Dorland *et al.*, 2014), such as road and building densities, especially in low anthropogenic density areas.

Little research has been completed on stress physiology in reptiles (Sheriff *et al.*, 2011). By acquiring most of their energy from the environment, reptiles have different physiologies than mammals. My research furthers our understanding of chronic CORT in a type of reptile. I examined chronic CORT in Midland Painted Turtles (*Chrysemys picta marginata*), Snapping Turtles (*Chelydra serpentina*), and Blanding's Turtles (*Emydoidea blandingii*) in areas varying in anthropogenic density to determine what characteristics influence chronic CORT variability. If turtles living near human structures have acclimated to human presence, and have thus undergone adrenocortical modulation to new baseline levels (Wingfield and Ramenofsky, 1999), then I predicted that turtles from low anthropogenic density sites (more pristine) with infrequent human presence will have higher CORT concentrations in their claws than turtles from higher anthropogenic density sites with frequent human presence and vehicles. Alternatively, if turtles have not acclimated to human presence, then turtles from low anthropogenic density sites will have lower CORT concentrations in their claws than turtles from higher anthropogenic density sites.

Methods

Study Area

The study area covers southern portions of the Parry Sound and Muskoka districts, in Central Ontario. The study area (roughly 44N 40' to 45N 20' latitude and -

79W 50' to -79W 10' longitude) borders the southern boundary of the Boreal Shield Ecozone and the northern boundary of the Mixedwood Plains Ecozone, within the Great Lakes Watershed (Crins *et al.*, 2009). It extends across the Ecodistricts 551 and 552 in the Lake Simcoe-Rideau Ecoregion (6E) and Ecodistrict 413 in the Georgian Bay Ecoregion (5E; Crins *et al.*, 2009). There are many wetlands and lakes among rock barren outcrops and mixed forests (Crins *et al.*, 2009), which provide ideal habitat for the diverse herpetofauna that live in this area (Hecnar *et al.*, 2002). This region of Ontario experiences a humid continental temperate climate, with four distinct seasons (Baldwin, *et al.*, 2000).

Anthropogenic Density

I quantified anthropogenic density using QuantumGIS (QGIS) to calculate the density of buildings, roads, and railways in my study area. Muskoka is an ideal study area because there is a wide range of anthropogenic density, from low anthropogenic density, in conservation reserves and crown land, to high anthropogenic density, in small town centers (e.g Gravenhurst and Bala). Moderately impacted areas include locations with few gravel roads and low building density. This region also simplifies a calculation of anthropogenic density because there are few agricultural and logging practices present (personal observation).

Some studies have focused on vertebrate responses to one measure of density or human influence (Gaddy and Kohlsaas, 1987; Crowley, 2006; Dorland *et al.*, 2014). In contrast, I calculated a number of anthropogenic density measures within 1 km of each

turtle (Table 1.1) at the time it was captured and its claws were clipped. I based this 1 km radius around the capture location on known movements of turtles in central Ontario. Snapping and Blanding's Turtle home range sizes in central Ontario were ~45 ha (0.45 km²) and dispersal distance (square root of an animal's home range size; Bissonette and Adair, 2008) was ~700 m (Baxter-Gilbert, 2015). Roads were classified based on the National Road Network road layer categories (and used as a rough approximation of road quality): Gravel Road, Recreation/Resource Road, Local Streets, Arterial Roads, and Highway. I calculated the length (m) of roads within 1 km of turtles using the 'Sum Line Lengths' function in QGIS. I used the building layer from the Muskoka Web Map and manually added points for buildings in the north part of my study area using the Bing Aerial Layer plugin for QGIS. The types of buildings in the Muskoka Web Map layer was not available and I could not determine types from the Bing Aerial Layer images. I calculated the number of buildings within 1 km of turtles using the 'Points within Polygon' function in QGIS. I examined which combination of anthropogenic density variables best described the variation in chronic CORT of turtles.

Field Data Collection and Study Species

Data were collected over two field seasons; from April to October in both 2014 and 2015 by up to 6 teams of START project technicians. Turtles were captured by hand, with Frabill landing nets, or in 'Memphis Net and Twine' hoop traps. For each capture, we recorded species, sex and age (if possible), body mass, size (carapace height, length and width, and plastron length), and reproductive condition for females. Body masses were

measured with 300 g, 2500 g, and 2000 g Pesola spring scales (Medioline, Schindellegi, Switzerland) with an accuracy of 1 g, 10 g, or 100 g, respectively. Size measurements were taken with 15 cm vernier calipers (± 0.01 cm; Scherr-Tumico, Minnesota, USA) and 40 cm calipers (± 0.1 cm; Haglöf Mantax, Sweden). Turtles were individually marked by filing, with a triangular file, a unique combination of notches into their marginal scutes (Cagle, 1939) to prevent resampling. Turtles were released at their capture sites within five hours of initial capture.

The turtle species and sample sizes analyzed in my study are listed in Table 1.2, and their associated federal and provincial conservation status designations can be found in Table 0.1. Due to the potential for illegal collection of turtles, specific location information is not disclosed.

Turtle claws were analyzed to quantify chronic, or long term accumulation of, CORT hormone concentration levels. I clipped the turtle claws with ConairPRO small dog nail clippers (Figure 1.1). Female turtles use their hind limbs to dig holes to lay their eggs (dulling some claws), so we clipped whatever claws were longest of female turtles of all species. Male Midland Painted Turtles use their foreclaws during courtship displays (Ernst, 1971), thus we did not clip front claws of male Midland Painted Turtles. We clipped 1-2 mm lengths of claws, ensuring we did not clip the quick thus avoiding blood loss from the turtle. We focused on collecting at least six claws from each Midland Painted Turtle and at least two claws from each Snapping Turtle because they are the most abundant turtle species and yielded large sample sizes for robust statistical analyses that could be made applicable to turtle species at risk that are captured in lower numbers

(Riley and Litzgus, 2013).

Midland Painted Turtles and Snapping Turtles represent different habitat niches (Ernst and Lovich, 2009), allowing me to compare and make predictions about the effects of anthropogenic density among species. Midland Painted Turtles are small-bodied habitat generalists that spend a significant portion of time basking (Ernst and Lovich, 2009), whereas Snapping Turtles are large-bodied habitat generalists that spend a significant portion of time underwater (Paterson *et al.*, 2012). We opportunistically caught and clipped at least eight claws from each Blanding's Turtle, a threatened species in Ontario (Committee on the Status of Endangered Wildlife in Canada; COSEWIC, 2005) that is more terrestrial than Midland Painted Turtles and Snapping Turtles (Ernst and Lovich, 2009). All claws clipped from an individual turtle were stored together in a 7 mL glass scintillation vial (VWR, Mississauga, ON, Canada) at room temperature, similar to hair analysis protocols (Mastromonaco *et al.*, 2014), until analysis (2-11 months after collection). All field work involving animals adhered to the Canadian Council on Animal Care guidelines and an approved Laurentian University Animal Care Committee protocol (AUP# 2014-03-01).

Labratory Analyses

Enzyme immunoassays were run on the claws at the Endocrinology Lab at the Toronto Zoo to quantify the concentration of corticosterone (CORT), that had accumulated in the claws as they grew, in each turtle sample (Baxter-Gilbert *et al.*, 2014). Claws from 152 turtles of the three species (Table 1.2) caught in the summer of 2014

were washed and crushed in December 2014 and enzyme immunoassays were run in April 2015. Claws from 216 turtles of the three species (Table 1.2) caught in the summer of 2015 were washed and crushed in October 2015 and enzyme immunoassays were run in November 2015.

To avoid contamination with other biological fluids that could artificially elevate cortisol levels, all claw samples were washed multiple times. Samples were first washed under a fume hood with 1 mL of distilled water and then washed twice in 1 mL of 100% methanol and vortexed in their 7 ml glass scintillation vial (VWR, Mississauga, ON, Canada) for 10 s. Between washings, all the liquid was removed using a pipette. Samples were left to dry under the fume hood for 12 to 24 hours until the vials were completely dry. Midland Painted Turtle and Snapping Turtle claws were crushed using methods from Baxter-Gilbert *et al.* (2014). Claws were put into 2.0 mL cryovials (Corning Inc., Corning, NY, USA) and placed in a liquid nitrogen dry shipper (Taylor-Wharton, Theodore, AL, USA) at -196°C for a minimum of ten minutes. Frozen samples were placed in a steel cylinder and smashed with a steel pestle to homogenize the claw sections. Blanding's Turtle claw samples were smaller, so instead of risking claw pieces getting lost in the smashing process, I cut Blanding's Turtle claws into pieces with a scalpel.

Cutting and crushing claws can be time intensive, especially when it may not be necessary. Claws have a hard keratin unguis and subunguis outer protective sheath that protects the inner portion and quick of the claws from dirt and debris in the environment (Feldhamer *et al.*, 2007; Homberger *et al.*, 2009). This sheath may be more permeable to

methanol, but other studies crush the claws or nails before extracting the stress hormones (Warnock *et al.*, 2010; Baxter-Gilbert *et al.*, 2014). It takes 5-10 minutes per sample to do the freezing and smashing method described above (Method 1) and 15-30 minutes to do the cutting method described above (Method 2), but the freezing method has a greater chance of losing claw material in the smashing process. Ideally, I would like to use the method that saves the most time while providing a reliable amount of CORT from the extraction process. Excess quantities of claws from six Snapping Turtles collected in 2014 were used in a test of tissue preparation methods. Claws from each turtle were split into three groups to ensure that claws from each turtle were used in each preparation method before the extraction process. One group was used as a control group and the claws were left whole before extracting CORT from them; the claws from the second group were frozen and smashed (Method 1); and claws from the third group were cut with a scalpel (Method 2). The concentration of CORT extracted from claws was compared between preparation methods.

The crushed/cut claw pieces were weighed using a Mettler Toledo balance (model AB54-S; ± 0.0001 g; Mettler Toledo International, Inc., Columbus, OH, USA) and transferred to 7 ml glass scintillation vials (VWR, Mississauga, ON, Canada). At a ratio of 0.005 g/ml, 100% methanol was added to extract corticosterone from claws and mixed for 24 hrs on an orbital shaker (MBI Orbital Shaker; Montreal Biotechnologies Inc., Montreal, QC, Canada) at 200 rpm. After 24 hrs, the vials were centrifuged for 10 min at 2300 rpm. The supernatants were pipetted off into clean glass vials and dried down under air in a fume hood. The dried extracts were stored at -20°C until immunoassay analysis.

Samples were removed from the freezer and brought to room temperature on the laboratory bench prior to analysis. Reconstitution of the dried-down extracts was done by adding 150 µl enzyme immunoassay buffer solution (0.1 M sodium phosphate buffer, pH 7.0, containing 9 g of NaCl and 1 g of bovine serum albumin per litre), resulting in a 1.13- to 16.53-fold concentration, and sonicating for 20 s in an Elmasonic waterbath (Elma GmbH & Co. KG, Singen, BW, Germany). Samples were loaded and incubated on microtitre plates based on methods from Terwissen *et al.* (2013).

Claw CORT values were quantified using modifications of an enzyme immunoassay described previously (Metrione and Harder, 2011; Watson *et al.*, 2013). Antisera were diluted as follows: goat anti-rabbit IgG (GARG) polyclonal antibody (Sigma-Aldrich, Mississauga, ON, Canada), 0.25 µg/well; and CORT polyclonal antibody (CJM006; C. Munro, University of California, Davis, CA, USA), 1:200 000. The cross-reactivities of the antisera have been described previously (GARG and CORT; Metrione and Harder, 2011; Watson *et al.*, 2013). Corticosterone–horseradish peroxidase conjugate (C. Munro, University of California, Davis, CA, USA) was diluted in assay buffer at 1:1 000 000. Standard solutions used were created with synthetic CORT (Steraloids Q1550; 39–10 000 pg/ ml). The control consisted of a laboratory stock of pooled fecal extracts obtained from spotted-necked otters (*Hydrictis maculicollis*) that was run at 65% binding. Results are presented as standardized nanograms of corticosterone per gram of claw (ng/g).

Data Handling and Statistical Analyses

CORT concentration levels for the claw preparation methods test were not normally distributed, therefore I performed a non-parametric Friedman Test to determine if there was a difference in CORT among the three methods used to prepare the claws for the enzyme immunoassay, while controlling for the individual turtles. All statistical analyses were performed with R (R version 2.0.3; R Core Team 2013).

Because high levels of variation in stress hormone concentration levels occur among individuals (Mastromonaco *et al.*, 2014), I considered turtles in a group/population more important for describing trends in CORT than individual turtle CORT concentration levels. Using QGIS, I created 96 site polygons around varying numbers of turtles in roughly geographically isolated areas based on the landscape and known turtle movements. I tested the null hypothesis that there were no differences in claw CORT concentration levels among turtle populations from varying anthropogenic density sites using the lme4 (Bates *et al.*, 2013) and mgcv (Woods, 2016) packages in R.

Not all captured turtles could be included in all analyses. One Snapping Turtle and one Blanding's Turtle were removed as CORT outliers, and one Midland Painted Turtle and one Blanding's Turtle were removed as number of buildings outliers, determined by an adjusted boxplot (Hubert and Vandervieren, 2008) using the “adjboxplot” function in R for skewed data in the robustbase package (Maechler, 2015). In 2014, one Snapping Turtle and three Blanding's Turtles, and in 2015, one Midland Painted Turtle, had repeated measures, so a mean of their CORT concentration levels was calculated, after the outliers were removed, and used in analyses. Sex was difficult to determine in

Snapping Turtles that did not show their penis or were not laying eggs. The ratio of the length from the end of the plastron to the cloacal (pre cloacal length), to the length from the middle of the plastron to the end of the plastron (post plastral length) was used to estimate the sex of Snapping Turtles with unknown sex. A ratio less than 86% indicated a female Snapping Turtle and a ratio greater than 86% indicated a male Snapping Turtle (Mosimann and Bider, 1960). Three Snapping Turtles had ratios close to 86%, so were excluded from the analyses because they had unknown sex. Two Snapping Turtles, two Blanding's Turtles, and seventeen Midland Painted Turtles did not have body size or mass measurements, so were not included in the analyses. The final sample sizes used in analyses are found in Table 1.2.

Body condition has been related to stress in wildlife (Rogovin *et al.*, 2003; Raouf *et al.*, 2006; Waye and Mason, 2008; Williams *et al.*, 2008), so I calculated a body condition index (BCI), based on size and body mass for each turtle, as a proxy of health. There are a number of methods that can be used to calculate body condition indices, based on body size, body mass, and the strength of different regressions (Peig and Green, 2009). For each turtle, I performed a regression of each morphometric measure (Table 1.3) on its mass. For Midland Painted Turtles and Blanding's Turtles, I ran two principal components analyses (PCA) of three morphometric measurements to create a linear combination as an index of the average body size of each turtle (Table 1.3). We did not measure carapace height in Snapping Turtles because their reduced plastron makes their body too flexible to get an accurate measurement. Thus, for Snapping Turtles, I ran three Principal Components Analyses (PCA) of two or three morphometric measurements to

create a linear combination as an index of the average body size of each turtle (Table 1.3). I regressed the most representative principal component of body size on the mass of each turtle and used the strongest of the four regressions, based on adjusted R^2 values representing the amount of explained variation, in the final calculation of BCI values. The residuals of the strongest regression represented the BCI of each turtle. Turtles with negative BCIs were considered to be in relatively poor body condition and turtles with positive BCIs were in relatively good body condition. Percentiles of BCI were taken per species to standardize the BCI for statistical models.

A large proportion of CORT concentrations were below the minimum detection limit for the enzyme immunoassays (or below the assay sensitivity), so that minimum was used as a reference point to separate turtles with minimum chronic CORT from turtles with above-minimum chronic CORT. CORT concentrations were generally left-skewed, or zero- or minimum-inflated, so a two-part gamma hurdle model (Zuur *et al.*, 2009; Anderson, 2014) was used to test the effects of the independent variables. First, a binomial generalized additive mixed-effect model (similar to a multiple logistic regression) was run to determine the probability of a turtle's CORT being above-minimum, given certain predictive independent variables. CORT values were coded as either 0 (minimum) or 1 (above-minimum) for the binomial generalized linear mixed-effects model. Second, only turtles with CORT values above the minimum 'hurdle' were used in a gamma generalized additive mixed-effect model, to determine what explanatory independent variables affect the magnitude of the CORT value once it passes the minimum 'hurdle'. The above-minimum CORT concentrations were standardized by

subtracting the minimum CORT concentration from the actual CORT concentration for each species and/or year group. I used Akaike's Information Criterion (AIC) to evaluate the support of potential additive and additive mixed effects models, and chose models with high support ($\Delta AIC < 2$; Zuur *et al.*, 2009). The candidate independent fixed variables are shown in Table 1.1. A number of variables did not have a linear relationship with CORT concentration levels, so I fit them to the models as additive variables (Table 1.1). The additive variable, weeks from the time the claws were collected until the CORT was extracted from the claws (capture to time of extraction; CATE), was much longer in 2014 than 2015, so was used as a proxy for year. Due to the large study area and opportunistic turtle capture and claw collection method, there were 96 sites with 1 – 31 turtles per site. Site was included as a random independent factor. The dependent variable was CORT concentration. The 'anova' command in R was used to summarize the significance of each explanatory variable and is considered an approximate estimator for P values for generalized additive models, so significance was set at $\alpha < 0.01$ (Zuur *et al.*, 2009).

Results

The method used to prepare claws for enzyme immunoassay did not affect the CORT concentration extracted; there were no differences in CORT measured among the three methods tested (control, freezing and smashing, cutting; $\chi^2(2) = 1.13, p = 0.57$).

Inter-assay coefficients of variation (CV between plates) were 17.6% and 22.1%, at 25% and 65% binding, respectively. Intra-assay CV (within plates) for the CORT EIA

was 5.6% at 50% binding. Only data from duplicates with <10% CV were used in analysis. Midland Painted Turtles had CORT concentrations ranging from 3.50 ng/g to 26.32 ng/g (mean 7.53 ng/g +/- 0.02 ng/g SE). Blanding's Turtles had CORT concentrations ranging from 2.00 ng/g to 10.58 ng/g (mean 3.53 ng/g +/- 0.03 ng/g SE). Snapping Turtles had CORT concentrations ranging from 1.20 ng/g to 8.26 ng/g (mean 2.75 ng/g +/- 0.01 ng/g SE).

For Midland Painted Turtles, individual scores from the first principal component (PC) of the PCA with plastron max length, carapace max width, and carapace max height were used to calculate BCI values (Table 1.4). For Blanding's Turtles, individual scores from the first PC of the PCA with carapace max length, carapace max width, and carapace max height were used to calculate BCI values (Table 1.5). For Snapping Turtles, individual scores from the first PC of the PCA with carapace max width, and carapace max length were used to calculate BCI values (Table 1.6).

Sex, BCI, species, time from capture to extraction (CATE), Julian date of turtle capture (JDAY), and site were strong predictive and explanatory variables for CORT values in both binomial and gamma models (Tables 1.7, 1.9). Length of paved roads within 1 km of the turtle (pave) and length of unpaved roads within 1 km of the turtle (unp) were also strong predictive variables for minimum vs. above-minimum CORT values in the binomial model (Table 1.7). The interaction between sex and species was also a strong explanatory variable for above-minimum CORT values in the gamma model (Table 1.9).

Male turtles were more likely to have above-minimum CORT than female turtles ($\chi^2(2) = 25.6$, $p = <0.001$; Table 1.8, Figure 1.2). Male Snapping Turtles were most likely to have above-minimum CORT ($\chi^2(2)=24.05$, $p = <0.001$; Table 1.8, Figure 1.2) and most Snapping Turtles caught in 2015 had above-minimum CORT. CATE ($\chi^2(9) = 39.27$, $p = <0.001$; Table 1.8) and site ($\chi^2(16) = 23.15$, $p = 0.03$; Table 1.8) explained a large amount of variation in the probability of a turtle having above-minimum CORT, but showed non-linear relationships with CORT that did not seem to show a pattern (Table 1.8).

Within turtles that had above-minimum CORT, Midland Painted Turtles had higher CORT than Blanding's and Snapping Turtles ($F(2,243) = 12.86$, $p = <0.001$; Table 1.10; Figure 1.3). Male Snapping and Blanding's Turtles had higher above-minimum CORT than female Snapping and Blanding's Turtles ($F(1,243) = 5.02$, $p = 0.03$; Table 1.10; Figure 1.3), but there was an interaction between species and sex ($F(2,243) = 4.92$, $p = 0.008$; Table 1.10; Figure 1.3). There was no difference in above-minimum CORT between male and female Painted Turtles ($F(2,243) = 4.92$, $p = 0.008$; Table 1.10; Figure 1.3). CATE ($F(1,243) = 29.28$, $p = <0.001$; Table 1.10) and JDAY ($F(1,243) = 26.45$, $p = <0.001$; Table 1.10) seemed to complement one another to account for a large amount of variation in above-minimum CORT, while having a weak positive trend with above-minimum CORT (Table 1.10). Site ($F(4,243) = 0.15$, $p = 0.003$; Table 1.10) accounted for a large amount of variation in above-minimum CORT, but showed a non-linear relationship with CORT that did not seem to show a pattern (Table 1.10).

Discussion

Anthropogenic densities did not affect the probability of Midland Painted Turtles and Blanding's Turtles having above-minimum chronic (or long-term) CORT, nor did it help explain the CORT concentrations once they were above-minimum. This suggests that turtles in my study are not experiencing chronic stress due to the factors I tested. These findings support my hypothesis that turtles living near human structures have acclimated to human presence. The turtles may have undergone adrenocortical modulation, adapting to chronic high stress situations and stress hormone levels by readjusting to new baseline levels (Wingfield and Ramenofsky, 1999). Similarly, road density did not affect Red-eared Slider (*Trachemys scripta elegans*) population demographics in Texas (Mali *et al.*, 2013), and Dorland *et al.* (2014) found that relative abundance of Painted Turtle populations did not seem to differ based on proximity to high traffic volume roads. Even though I sampled turtles much farther from roads than Dorland *et al.* (2014), giving us a larger representative sample of low anthropogenic density, my findings seem to be similar. Some studies have found that wildlife near human altered landscapes exhibit high levels of stress hormones (Brearley *et al.*, 2012), but my results indicate that there may not be negative indirect effects of roads on turtles.

Snapping Turtles were most likely to have above-minimum CORT, especially in 2015, but no other variables seemed to linearly explain their variation in CORT concentrations. Snapping Turtles often get fed fish and/or hooks (by accident or intentionally) by fishermen (Borkowski, 1997; Ernst and Lovich, 2009), including in my

study area, and this may be one anthropogenic variable that I did not measure that could increase chronic CORT. There is likely a complex relationship between chronic CORT and anthropogenic density. Snapping Turtles have higher variation in body size, by a magnitude of 10-100 times the BCI of Midland Painted Turtles and Blanding's Turtles, which may complicate interpretation of their BCI relationships with stress. Calculating the home range sizes of turtles would give a more accurate representation of the anthropogenic densities a turtle experiences (Schoener, 1981), which may have a greater effect on their chronic CORT levels than the anthropogenic densities at the capture location. Unfortunately, turtles do die from vehicle collisions and other human impacts, such as fishing nets and boat collisions (Pappas *et al.*, 2001; Ersnt and Lovich, 2009; Paterson *et al.*, 2012), so turtles readjusting their CORT baseline levels may not always be beneficial.

Future studies should investigate other potential sources of stress, such as land use, vehicle encounters, sound, light and chemical pollution, and natural disasters (White, 1979; Hopkins *et al.*, 1997; Longcore and Rich, 2004). Different forms of pollution have been studied in other species (Hopkins *et al.*, 1997; Blickley *et al.*, 2012). Southern Toads (*Anaxyrus terrestris*) had high CORT circulating in their blood in polluted study areas (Hopkins *et al.*, 1997). My study area is in a relatively pristine part of Ontario (Crins *et al.*, 2009), so environmental pollution is unlikely to affect chronic stress in turtles. Fecal CORT metabolites in Greater Sage-Grouse (*Centrocercus urophasianus*) were higher when exposed to prolonged noise playback or noise pollution (Blickley *et al.*, 2012), although birds have more sensitive hearing than reptiles (Fay, 1988). Midland Painted

Turtles bask more than Blanding's Turtles and Snapping Turtles (Ernst and Lovich, 2009), so may react more to prolonged sound pollution near roads by increasing their CORT levels.

Male Midland Painted Turtles and Blanding's Turtles tended to have higher chronic CORT than females. Baxter-Gilbert *et al.* (2014) also found that chronic CORT was higher in male Midland Painted Turtles than females, regardless of whether turtles were captured near roads. Increased CORT in male Marine Iguanas (*Amblyrhynchus cristatus*) helped with beneficial reproductive behaviours, such as orientation, territoriality, and courtship (Berger *et al.*, 2005). Midland Painted Turtle males have elaborate courtship displays (Moldowan, 2014) and Blanding's Turtles have coercive mating tactics (Baker and Gillingham, 1983) that may benefit from increased CORT levels, but no studies have tested this relationship. Little is known about Snapping Turtle mating behaviours, but males may defend territories or home ranges (Galbraith *et al.*, 1987), which could raise CORT levels (Sheriff *et al.*, 2011). Because male Snapping Turtles do not seem to defend strict territories (Galbraith *et al.*, 1987), their CORT may only show a few acute spikes when they encounter and fight another male. The Southern Toads with high CORT circulating in their blood in polluted study areas were male and also had high testosterone circulation in their blood (Hopkins *et al.*, 1997). Paitz *et al.* (2014) found that female Red-Eared Sliders did not seem to have increased CORT circulating in their blood during nesting season, or over any other time in their active season, which is contrary to most mammals (Sheriff *et al.*, 2011). Midland Painted Turtle and Blanding's Turtle females in my study area, which did not show elevated CORT, may

have been similar to Red-Eared Sliders in their response to reproduction. Cash *et al.* (1997) found no difference in CORT between male and female Red-eared Sliders. Some reptiles have shown differences in CORT levels based on sex [Watersnakes (*Nerodia sipedon*), Sykes and Klukowski, 2009; Common Wall Lizards (*Podarcis muralis*), Galeotti *et al.*, 2010], but others have not [Freshwater Crocodiles (*Crocodylus johnstoni*), Jessop *et al.*, 2003; Hawksbill Turtles (*Eretmochelys imbricata*), Jessop *et al.*, 2004; Tree Lizards (*Urosaurus ornatus*), French *et al.*, 2008].

CORT and other glucocorticoids are associated with processes in the body that are not solely due to stress, such as reproductive status, season, and disease (Sheriff *et al.*, 2011; Sapolsky, 1990). Many of these relationships are understood in other taxonomic groups, but not in reptiles (Sheriff *et al.*, 2011). In my study, the JDAY of turtle capture and claw collection was a significant explanatory variable for above-minimum CORT values, but the relationship between Julian date and CORT was non-linear and weakly positive. The effect of Julian date may represent a seasonal effect on CORT level, with CORT increasing slightly as the summer progresses. My study was short term, and logistical challenges prevented me from pursuing some areas of investigation. Research is still required, in the form of adrenocorticotrophic hormone (ACTH) challenges (Mastromonaco *et al.*, 2014), comparing CORT in blood to CORT in claws to determine if a strong relationship exists (Berkvens *et al.*, 2013). ACTH challenges would be difficult to perform on turtles because they have slow metabolic rates compared to mammals on which ACTH challenges have previously been performed before (Sheriff *et al.*, 2011). Claw growth rates should be measured for individual claws of each species to

determine the amount of time represented in the claw samples (Baxter-Gilbert *et al.*, 2014). For example, human hair grows approximately 1 cm/month (Wennig, 2000). CATE was a strong explanatory variable in both models in my study, but the relationship between CATE and CORT was non-linear and weakly positive. This could suggest that the CORT may be degrading in the claws over the length of time that they are stored. It could also suggest that CORT may degrade over time while the claw is still attached to the animal because claws are not vascularized (Feldhamer *et al.*, 2007; Homberger *et al.*, 2009). Bortolotti *et al.* (2009) found significantly higher CORT levels in Great Horned Owl (*Bubo virginianus*) feathers that were twenty-five years old, than samples less than one year old, but Bortolotti *et al.* (2009) did not find a significant difference between recent and historic CORT levels in feathers of twelve other bird species. Webb *et al.* (2010) successfully extracted cortisol from archaeological hair of humans living 550-1500 AD. No studies have directly tested the effect of storage time on the level of CORT or cortisol on keratin structures (Sheriff *et al.*, 2011), but future studies should extract CORT from samples within a similar time frame after capture. CORT was extracted from turtle claws after many more weeks in 2014 than 2015, which likely contributed to the significance of CATE in both models in my study.

Although BCI was an explanatory variable in the top AIC ranked models in my study, it was not a significant predictor of CORT levels. Immunocompetence was measured in Marine Iguanas and was decreased when their CORT levels were high after experimentally stressful events were created (Berger *et al.* (2005). Immune function may be more directly related to CORT than a body condition index and future studies should

further explore the relationships. Stress responses were species-specific in other studies (Sheriff *et al.*, 2011) as well as my own, and an enzyme immunoassay validation has only been run on Midland Painted Turtles (Baxter-Gilbert *et al.*, 2014). Caution must be taken when interpreting chronic CORT levels before more is understood about CORT in turtles.

My study increased understanding of CORT relationships in turtles and provides a basis for future research directions. Conservation management plans could include measuring the indirect effects of anthropogenic disturbance as well as the direct effects. Conservation projects are often limited in time and funding (Wilson *et al.*, 2006; McDonald-Madden *et al.*, 2008). To increase sampling efficiency in future projects, researchers can save up to thirty minutes per claw sample by leaving the claws intact before extracting the CORT, thus reducing analytical expenses. An increased number of samples can be processed in less time, thus improving sample sizes and accuracy of future studies. Turtles do not seem to have higher chronic CORT around roads or buildings, but they are still subject to road mortality. So, mitigating road effects will also benefit the species. Road mitigation projects need to take into account that snapping turtles can climb chain-link fences and hatchling turtles are small enough to pass through many gauges of fencing. There were species-specific differences in chronic CORT response, but Midland Painted Turtles and Blanding's Turtles were similar in that CORT was higher in males. Because I caught more than twice as many Midland Painted Turtles as Blanding's Turtles, my statistical power was much higher for Midland Painted Turtles. Most turtle species in Ontario are species at risk (*Species at Risk Act*; SARA, 2003) and some projects may not be able to catch a large number of rare at risk turtles for strong

statistical analyses. The more common Midland Painted Turtles may have chronic CORT concentrations that can be made applicable to Blanding's Turtles in the same habitat. Understanding different levels of stress hormones, with non-invasive methods, can further inform and help to evaluate mitigation strategies to improve reproduction and survival, rather than impede it (Busch and Hayward, 2009).

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Figures



Figure 1.1: Clipping Painted Turtle claws into 7 mL scintillation vials to store before running enzyme immunoassays to obtain a concentration of corticosterone.

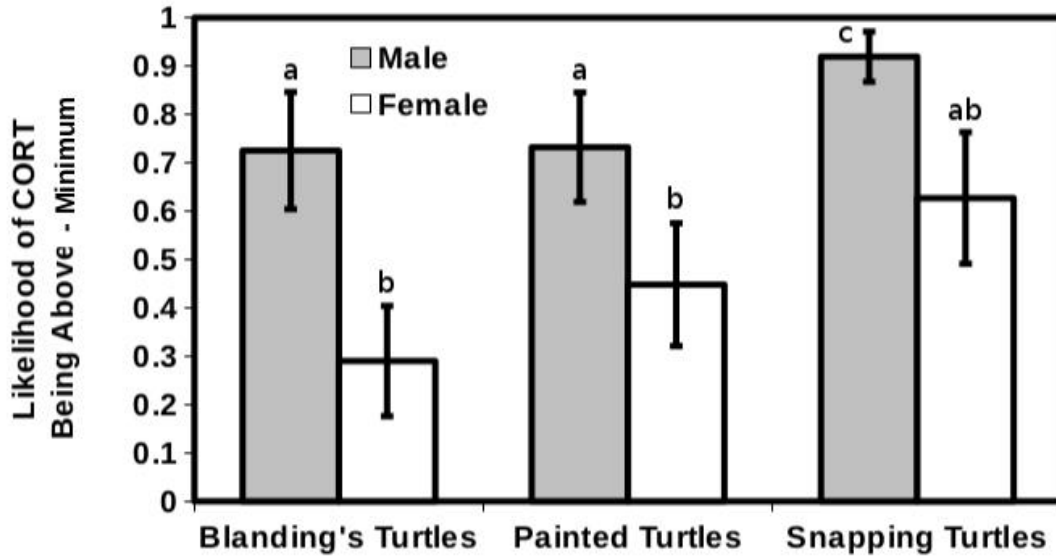


Figure 1.2: Differences between species and sex in probability of chronic CORT being above-minimum. Letters indicate significant differences. Errors bars are +/- SE. Male turtles are more likely to have above-minimum chronic CORT than female turtles. Male Snapping Turtles are most likely to have above-minimum chronic CORT.

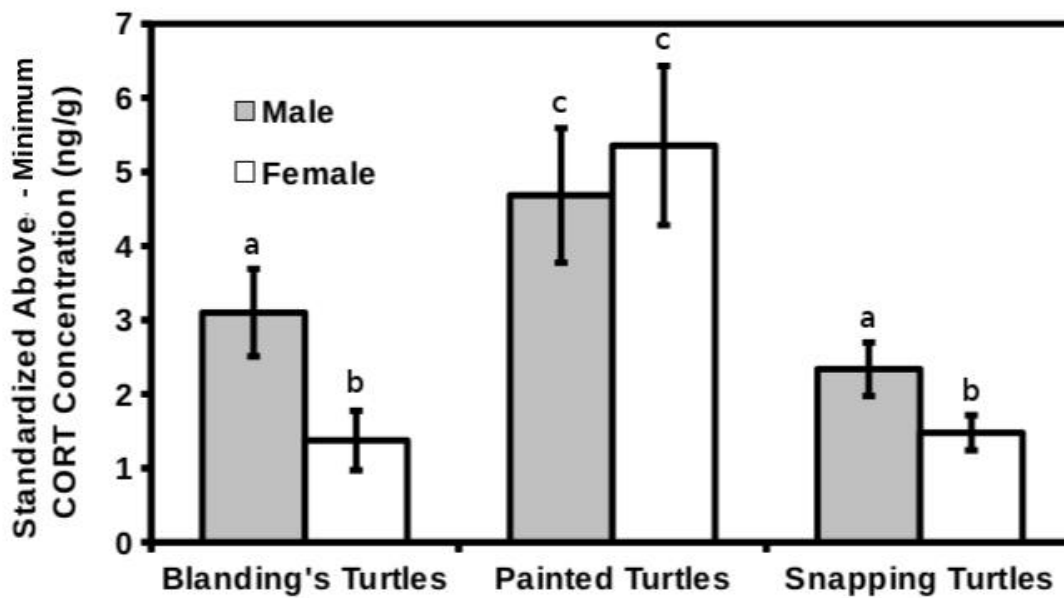


Figure 1.3: Differences between species and sex in level of above-minimum chronic CORT. Letters indicate significant differences. Errors bars are +/- SE. Male Blanding's Turtles and Snapping Turtles have higher relative above-minimum chronic CORT than female Blanding's Turtles and Snapping Turtles. Painted Turtles have the highest relative above-minimum chronic CORT.

Tables

Table 1.1: Independent variables used in two-part gamma hurdle model selection with the codes used in the results. Anthropogenic Structures are the categories used to calculate anthropogenic densities within 1 km of turtles. QGIS was used to calculate the number of buildings and length (m) of roads within 1 km of turtles. ' * ' indicate an additive (non-linear) variable. ' ‘ ' indicates a random variable.

Anthropogenic Structure	Code	Non-Anthropogenic Variables	Code
Buildings	build	Sex	sex
Railway tracks	rt	Species	species
Unpaved roads	unp	Body Condition Index (BCI)	BCI
Paved roads	pave	Weeks from the time the claws were collected until the CORT was extracted from the claws*	CATE
Recreational roads and trails	rec	Julian date of the claw collection*	JDAY
Local roads	local	Site*’’	site
Arterial roads	art		
Highways	high		
All roads	allrd		
All linear features (roads and railway tracks)	rdrt		

Table 1.2: The turtle species that were analyzed for this study and their sample sizes in each year of the study.

Turtle Species	Individuals Sampled in 2014	Individuals Sampled in 2015
Midland Painted Turtle (<i>Chrysemys picta marginata</i>)	55	117
Snapping Turtle (<i>Chelydra serpentina</i>)	65	64
Blanding's Turtle (<i>Emydoidea blandingii</i>)	32	35

Table 1.3: Individual and PCA measurement variables (max=maximum) regressed against body mass to determine the strongest regression. The residuals of the strongest regression represented the body condition index (BCI) of each turtle. A mean of measurements taken more than once throughout the season was used, where applicable.

Blanding's Turtles (<i>Emydoidea blandingii</i>) and Midland Painted Turtles (<i>Chrysemys picta marginata</i>)	Snapping Turtles (<i>Chelydra serpentina</i>)
Plastron max length	Plastron max length
Carapace max length	Carapace max width
Carapace max width	Carapace max length
Carapace max height	Plastron max length, carapace max width PCA
Plastron max length, carapace max width, carapace max height PCA	Carapace max length, carapace max width PCA
Carapace max length, carapace max width, carapace max height PCA	Plastron max length, carapace max width, carapace max length PCA

Table 1.4: Midland Painted Turtle (*Chrysemys picta marginata*) principle components analyses (PCA) and regressions of morphometric measures with body mass. The morphometric regression model (P values and adjusted R² values reported) that described the most variation in the data (highest adjusted R² value) is in bold and was used to calculate the body condition index (BCI) of each turtle. Loading of each morphometric measurement on the first principal component of the PCA is in brackets after each morphometric measure.

Measurement Variable Regressed Against Mass	Variance Explained by First Principal Component (PC) (%)	P value	Adjusted R ²
Plastron max length	NA	<0.0001	0.861
Carapace max length	NA	<0.0001	0.840
Carapace max width	NA	<0.0001	0.863
Carapace max height	NA	<0.0001	0.836
Plastron max length (0.59), carapace max width (0.58), carapace max height (0.57) PCA	92	<0.0001	0.915
Carapace max length (-0.58), carapace max width (-0.58), carapace max height (-0.57) PCA	92	<0.0001	0.913

Table 1.5: Blanding’s Turtle (*Emydoidea blandingii*) principal components analyses (PCA) and regressions of morphometric measures with body mass. The morphometric regression model (P values and adjusted R² values reported) that described the most variation in the data (highest adjusted R² value) is in bold and was used to calculate the body condition index (BCI) of each turtle. Loading of each morphometric measurement on the first principal component of the PCA is in brackets after each morphometric measure.

Measurement Variable Regressed Against Mass	Variance Explained by First Principal Component (PC) (%)	P value	Adjusted R ²
Plastron max length	NA	<0.0001	0.763
Carapace max length	NA	<0.0001	0.849
Carapace max width	NA	<0.0001	0.834
Carapace max height	NA	<0.0001	0.539
PC1: plastron max length (-0.60), carapace max width (-0.56), carapace max height (-0.57) PCA	84.4	<0.0001	0.825
PC1: carapace max length (-0.60), carapace max width (-0.60), carapace max height (-0.53) PCA	86.1	<0.0001	0.879

Table 1.6: Snapping Turtle (*Chelydra serpentina*) principle components analyses (PCA) and regressions of morphometric measures with body mass. The morphometric regression model (P values and adjusted R² values reported) that described the most variation in the data (highest adjusted R² value) is in bold and was used to calculate the body condition index (BCI) of each turtle. Loading of each morphometric measurement on the first principal component of the PCA is in brackets after each morphometric measure.

Measurement Variable Regressed Against Mass	Variance Explained by First Principal Component (PC) (%)	P value	Adjusted R ²
Plastron max length	NA	<0.0001	0.761
Carapace max width	NA	<0.0001	0.856
Carapace max length	NA	<0.0001	0.887
Plastron max length (0.71), carapace max width (0.71) PCA	93	<0.0001	0.872
Carapace max length (0.71), carapace max width (0.71) PCA	96	<0.0001	0.905
Plastron max length (0.57), carapace max width (0.58), carapace max length (0.58) PCA	93	<0.0001	0.903

Table 1.7: Akaike's Information Criterion (AIC) for selection of best binomial models.

Abbreviations of independent variables are defined in the Methods in Table 1.1. '*'

indicates an additive term. ':' indicates an interaction term.

Model	Number of estimated parameters (K)	AIC	Δ AIC	Akaike weight (w_i)
sex+species+BCI+site*+CATE*+unp	29	384.46	0.00	0.27
sex+species+BCI+site*+CATE*+unp+pave+ JDAY*	26	384.80	0.34	0.22
sex+species+BCI+site*+CATE*	31	384.89	0.43	0.21
sex+species+BCI+site*+CATE*+unp+pave	26	384.91	0.45	0.21
sex+species+BCI+site*+CATE*+unp+ sex:species	30	386.45	1.99	0.10

Table 1.8: Influence of effects of top binomial Akaike's Information Criterion (AIC) model on turtle claw corticosterone concentration levels. 'edf' is the effective degrees of freedom. 'df' is the degrees of freedom. Significant effects are shown as bold P values. Abbreviations of independent variables are defined in the Methods in Table 1.1.

Parameter	Effect	edf	df	χ^2	P values
Claw corticosterone	sex		1	25.6	<0.001
	species		2	24.05	<0.001
	bci		1	0.05	0.83
	unp		1	2.68	0.10
	CATE	8.45		37.55	<0.001
	site	15.83		23.15	0.03

Table 1.9: Akaike's Information Criterion (AIC) for selection of best gamma models. '*' indicates an additive term. ':' indicates an interaction term. Abbreviations of independent variables are defined in the Methods in Table 1.1.

Model	Number of estimated parameters (K)	AIC	Δ AIC	Akaike weight (w_i)
sex+species+BCI+CATE*+JDAY*+sex:species+site*	14	988.66	0.00	0.92
sex+species+BCI+CATE*+JDAY*+sex:species	10	993.48	4.82	0.08

Table 1.10: Influence of top Akaike's Information Criterion (lowest AIC) model on turtle claw corticosterone concentration levels. Significant effects are shown as bold P Values.

'.' indicates an interaction term. Abbreviations of independent variables are defined in the Methods in Table 1.1.

Parameter	Effect	edf	df	F	P Value
Claw corticosterone	sex		1	5.02	0.03
	species		2	12.86	<0.001
	Sex:species		2	4.92	0.008
	BCI		1	1.72	0.19
	JDAY		1	26.45	<0.001
	CATE	1		29.28	<0.001
	site	4		0.15	0.003

Chapter 2

Do Anthropogenic Structure Densities Affect Rates and Patterns of Nest Predation on Freshwater Turtles?

Abstract

Abundances of mammals that predate turtle eggs are higher where anthropogenic sources of food and decreases in populations of top predators allow middle-sized mesopredators to flourish. Many studies have found that the highest predation risk to turtle nests is within the first week after the eggs are laid. However, other studies have found that depredation of turtle nests occurs throughout the incubation period. I hypothesized that if human presence increases mesopredator abundance, then the frequency of depredation events on turtle nests will be greater and will occur over a longer period of time at field sites with higher anthropogenic structures than at more pristine field sites. I monitored three road transects and one remote site in Ontario that connect high road and building density areas to low density areas and one remote location, for three species of nesting turtles in June; Midland Painted Turtles (*Chrysemys picta marginata*), Snapping Turtles (*Chelydra serpentina*), and Blanding's Turtles (*Emydoidea blandingii*). I determined the rate and pattern of nest depredation throughout incubation. The distribution of the percentage of predator interactions with nests that occurred weekly (PRED) was different than the expected PRED based on published studies; nest depredation was not restricted to the early days of egg incubation. Using a non-parametric product-limit survival analysis, I found that the nests of all three turtle species had similar probabilities of survival. Predator exclusion nest cages and higher road densities close to a turtle nest increased the nests' survival. Relative nest mesopredator abundance indices, calculated from trail camera photos, did not differ among sites with varying ADA, but were highest during the nesting season. Of the two

major nest mesopredators in my study area, raccoons were more abundant than foxes. Contrary to some nest caging protocols, nest cages should be kept on nests until the turtles hatch and priority should be given to caging nests in low road density areas to help increase population recruitment.

Introduction

Habitat alteration due to human activities has largely caused global declines in wildlife populations (Butchart *et al.*, 2010; Hoffmann *et al.*, 2010). Road mortality is a direct cause of global population declines of various animals such as turtles (Gibbons *et al.*, 2000; Butchart *et al.*, 2010; Hoffmann *et al.*, 2010), but roads and other anthropogenic structures may also have indirect effects on turtle ecology, such as the predation patterns of turtle nests. The egg life stage is one of the most vulnerable periods of time for turtles (Tinkle *et al.*, 1981; Congdon *et al.*, 1983; Riley and Litzgus, 2013). In some areas, predators depredate all of the turtle nests (Fordham *et al.*, 2008), and if more than half of the turtle nests in a population are depredated each year, turtle populations decline at increasing rates (Reed, 2009). Abundance of mammals that depredate turtle eggs is higher where anthropogenic sources of food (Oehler and Litvaitis, 1996) and decreases in populations of top predators (Prugh, 2009) subsidize an ideal environment for middle-sized mesopredators to flourish. Raccoons (*Procyon lotor*) and red foxes (*Vulpes vulpes*) are both well-known subsidized turtle nest predators (Snow, 1982) that display increased population sizes near human-altered landscapes (Oehler and Litvaitis,

1996; Prugh *et al.*, 2009). Abundance of mesopredators varies throughout the year (Kaufmann, 1982).

A number of studies have found that the highest predation risk to Blanding's Turtle (*Emydoidea blandingii*; Congdon *et al.*, 1983), Snapping Turtle (*Chelydra serpentina*; Congdon *et al.*, 1987; Robinson and Bider, 1988), and Alligator Snapping Turtle (*Macrochelys temminckii*, Holcomb and Carr 2013) eggs is within the first week after the eggs are laid (Tinkle *et al.*, 1981; Christens and Bider, 1987). Studies that tried to identify cues that mesopredators use to find nests monitored turtle nests for only two weeks (Marchand and Livaitis, 2004), two days (Strickland *et al.*, 2010), or up to one month (Rollinson and Brook, 2007) after the eggs were laid, which may have been too short a time period. Although high levels of predation occurs in the first week post-oviposition, other studies have found that depredation also occurs later in incubation for Painted Turtles (*Chrysemys picta*; Snow, 1982; Riley and Litzgus, 2014), Wood Turtles (*Glyptemys insculpta*; Brooks *et al.*, 1992), Diamondback Terrapins (*Malaclemys terrapin*; Burger, 1977), Northern Map Turtles (*Graptemys geographica*; Gillingwater, 2002), and Snapping Turtles (Riley and Litzgus, 2014). Congdon *et al.* (1983) found that a majority of Blanding's Turtle nest depredation occurred within the first week post-oviposition, and also observed a small spike of ~5% of Blanding's Turtle nests being depredated during hatchling emergence. The differences seen in turtle nest predation patterns may be attributed to the large variety of species that depredate turtle nests and cues available to potential mesopredators.

There are a number of nest cues mesopredators could use to find turtle nests throughout the incubation period. Mammal mesopredators may be able to smell oviposition fluid early in the season (Legler 1954; Congdon *et al.* 1983; Spencer 2002), rotten eggs throughout incubation, or embryonic fluids when the hatchlings hatch (Riley and Litzgus, 2014). Mesopredators may see turtles laying eggs (Congdon *et al.*, 1987; Eckrich and Owens 1995) or disturbed soil (Strickland *et al.*, 2010; Spencer 2002) during nesting season, and emerged hatchlings (Riley and Litzgus, 2014) during hatching season. Oddie *et al.* (2015) found that raccoons were more likely to depredate nests that had tactile cues, such as soft soil resulting from maternal digging and covering. Mesopredators may also hear hatchlings inside their nest cavity after they hatch (Ferrara *et al.*, 2013). Canines and other mammals seem to be the most common nest mesopredators in the later stages of incubation (Burger, 1977; Brooks *et al.*, 1992; Spotilla, 2011; Riley and Litzgus, 2014).

Globally, turtles are one of the most at risk taxa, and a contributing factor to population declines is unnaturally high levels of nest predation (Gibbons *et al.*, 2000). One rescue method that could be used would be to excavate eggs and incubate them indoors away from mesopredators, but this is time consuming, expensive, and requires specialized permits and trained technicians to handle turtle eggs (Kurtz *et al.*, 2011). A common conservation tool, used by trained technicians as well as informed members of the public, to protect turtle nests from mesopredators is a predator-exclusion nest cage (Riley and Litzgus, 2013). Unfortunately, this can become a learned visual cue for mesopredators, such as raccoons (Mroziak *et al.*, 2000) and *Corvus* sp. (Rollinson and

Brooks, 2007), to find nests. Some nest caging protocols recommend removing nest cages in the middle of the incubation period, but predation spikes have been observed in freshwater turtle (Rahman and Burke 2010) and sea turtle (Engeman *et al.*, 2006) nests after the cages were removed. One of my objectives is to identify a protocol that will be most beneficial to turtle egg survival thus helping to form a consensus on nest caging protocols.

Female turtles choose nesting sites based on environmental variables that aid in the successful development of hatchlings (Wilson 1998; Refsnider and Janzen 2010), but they may not have adapted their nest-site selections to account for subsidized mesopredators. Large numbers of turtles lay eggs in the gravel shoulder of roads in my study area, and roads connect human structures that can subsidize mesopredator populations (Prugh *et al.*, 2009). I monitored three roads in the South Muskoka area of Ontario in 2014 and 2015 that connected high anthropogenic densities to low anthropogenic densities and one remote location, for nesting turtles and nests monitored throughout incubation in order to determine the rate and pattern of nest depredation. To my knowledge, no studies have quantified nest depredation rates across an anthropogenic density gradient. I hypothesized that if human presence increases mesopredator abundance, then the frequency of depredation events on turtle nests will be greater and will occur over a longer period of time at field sites with a higher density of anthropogenic structures than at sites in more pristine habitats.

Methods

Study Area

The study area covers southern portions of the Parry Sound and Muskoka districts, in Central Ontario. The study area (roughly 44N 40' to 45N 20' latitude and -79W 50' to -79W 10' longitude) borders the southern boundary of the Boreal Shield Ecozone and the northern boundary of the Mixedwood Plains Ecozone, within the Great Lakes Watershed (Crins *et al.*, 2009). It extends across the Ecodistricts 551 and 552 in the Lake Simcoe-Rideau Ecoregion (6E) and Ecodistrict 413 in the Georgian Bay Ecoregion (5E; Crins *et al.*, 2009). There are many wetlands and lakes among rock barren outcrops and mixed forests (Crins *et al.*, 2009), which provide ideal habitat for the diverse herpetofauna that live in this area (Hecnar *et al.*, 2002). This region of Ontario experiences a humid continental temperate climate, with four distinct seasons (Baldwin, *et al.*, 2000).

Anthropogenic Density

I quantified anthropogenic density using QuantumGIS (QGIS) to calculate the density of buildings, roads, and railways in my study area. Southern Muskoka is an ideal study area because there is a wide range of anthropogenic densities, from low anthropogenic density, in conservation reserves and Crown land, to high anthropogenic density, in small town centers (e.g Gravenhurst and Bala). Moderately impacted areas include locations with few gravel roads and low building density. Southern Muskoka also simplifies a calculation of anthropogenic density because there are few agricultural and

logging practices present (personal observation).

Some studies have focused on animal responses to one measure of density or human influence (Gaddy and Kohlsaas, 1987; Crowley, 2006; Dorland *et al.*, 2014). In contrast, I calculated a number of anthropogenic density measures within 1 km of each nest (Table 2.1). This 1 km radius/diameter was based on the home range size of raccoons, known turtle nest predators (Snow, 1982) in rural eastern Ontario ($0.78 \text{ km}^2 \pm 0.46 \text{ km}^2$; Totton *et al.*, 2004). Roads were classified based on the National Road Network road layer categories (and used as a rough approximation of road quality): Gravel Road, Recreation/Resource Road, Local Streets, Arterial Roads, and Highway. I calculated the length (m) of roads within 1 km of turtles, including the road transects that were driven, using the 'Sum Line Lengths' function in QGIS. I used the building layer from the Muskoka Web Map and manually added points for buildings in the north part of my study area using the Bing Aerial Layer plugin for QGIS. The types of buildings in the Muskoka Web Map layer was not available and I could not determine types from the Bing Aerial Layer images. I calculated the number of buildings within 1 km of nests using the 'Points within Polygon' function in QGIS. I examined which combination of anthropogenic density variables best described the variation in nest predation patterns.

Field Data Collection and Study Species

Three road transects that spanned a range of anthropogenic densities were surveyed for nesting turtles. One remote site, without roads or buildings, was also surveyed and represented the lowest levels of anthropogenic densities. Turtle nests found

on gravel roads, gravel shoulders of paved roads, in moss and soil on rock outcrops beside roads, or on rock outcrops without roads, were used to compare nest predation patterns.

Captured female turtles were palpated to determine if they were gravid (had shelled eggs) and nest searching by researchers started once nesting behaviour was observed (2 June in 2014, 27 May in 2015). Nest searching by researchers ended after three consecutive days passed with no observed nesting behaviour (30 June in 2014 and 2015). Nests were marked with popsicle sticks and flagging tape after the female turtle finished nesting (Figure 2.1; a). Nests were monitored weekly throughout incubation to determine if the hatchlings emerged from the nest, the nest failed, or the nest was depredated (Figure 2.1: b). I did not excavate nests for egg measurements in my study, thus minimizing the effects of nest disturbance on the behaviour of predators (Riley and Litzgus, 2014). Wooden-sided and above-ground wire nest cages with hatchling escape holes (Figure 2.2; Riley and Litzgus, 2013) were opportunistically placed on top of Midland Painted Turtle and Snapping Turtle nests and placed on all Blanding's Turtle nests as soon as the female turtle had completed laying her eggs and covering her nest. Nest mesopredators learned that these type of markers indicate turtle nests over a number of years in some areas (Burke *et al.*, 2005; Rollinson and Brooks 2007; Spotila, 2011), but not in others (Mroziak *et al.*, 2000; Burke *et al.*, 2005; Strickland *et al.*, 2010; Kurz *et al.*, 2011). My study was two years in duration, so it is unlikely that mesopredators learned that nest cages indicated a food source (Riley and Litzgus, 2014). No Midland Painted Turtle nests and a number of Snapping Turtle nests were not caged as control

nests representing natural predator interactions.

The turtle species and sample sizes monitored in my study are listed in Table 2.2 and their associated federal and provincial conservation status designations can be found in Table 0.1. Due to the potential for illegal collection of turtles, specific location information is not disclosed.

Because of their difference in body size and nesting ecology (Ernst and Lovich, 2009; Riley and Litzgus, 2014), studying Midland Painted Turtles (*Chrysemys picta marginata*) and Snapping Turtles allowed me to compare and make predictions about the effects of anthropogenic density and mesopredator interactions applicable to multiple turtle species. Midland Painted Turtles dig relatively shallow nest cavities, with an average depth of 9.6 +/- 0.1 cm (Riley and Litzgus, 2013), and lay small clutch sizes, with a mean of eight eggs (Congdon *et al.*, 1981; Ernst and Lovich, 2009); Snapping Turtles dig relatively deep nest cavities, with an average depth of 20.7 +/- 0.4 cm (Riley and Litzgus, 2013), and lay large clutch sizes, with a mean of twenty-eight eggs (Congdon *et al.*, 1987; Ernst and Lovich, 2009); and Blanding's Turtles dig intermediate depth nest cavities and lay intermediate clutch sizes, with a mean of ten eggs (Congdon *et al.*, 1983; Ernst and Lovich, 2009). Snapping Turtle nests yielded the largest sample sizes for robust statistical analyses that could be made applicable to other turtle species at risk (Riley and Litzgus, 2013).

Thirty-two Camera traps in twenty-seven unique sites, including one at the remote site in 2014, were opportunistically set up at nesting sites to determine nest predation rates and species of mesopredators. Cameras were inspected weekly or biweekly, and

memory cards and batteries exchanged as needed. Nests were checked weekly, to determine mesopredator interactions (Riley and Litzgus, 2014) and to check for nest mesopredator tracks and scat, until October – when all viable hatchlings should have emerged (Ernst and Lovich, 2009). Predation attempts (e.g. holes dug close to nest chambers, disturbed soil, mesopredator scat) were recorded and any disturbed soil was replaced so that multiple interactions with each nest could be recorded. If a small hole in the soil was found in the location of the nest after 3-4 months, then it was concluded that the hatchlings had emerged (Ernst and Lovich, 2009) and the nests were excavated to count remaining egg shells and hatchlings. Nests were no longer checked if all the eggs had been predated, or all the hatchlings had emerged. Nests were excavated in October if the hatchlings had not emerged and the species' hatchlings could not overwinter in the nest (i.e Snapping Turtles; Ernst and Lovich, 2009).

Data Handling and Statistical Analyses

Because the same individual mesopredator could depredate all the nests in one location, I created a nesting site variable to help control for the clustering of nests. Using QGIS, I created 128 nesting site polygons around varying numbers of turtle nests in roughly geographically isolated areas.

Not all nests were included in all analyses. I found 494 nests, but not all were monitored. I either knew the date they were laid, their fate, or both. Unknown fates included Midland Painted turtle nests that had hatchlings that presumably overwintered in the nest cavity, or nests that were lost (often when a road-side grader removed nest

markers). The nests with known dates laid and fates were considered the most valuable to my analysis (see Table 2.2 for sample sizes); the nests with unknown dates laid and known fates were the second most valuable (see Table 2.2 for sample sizes). The nests with unknown fates were the least valuable (see Table 2.2 for sample sizes). I used only the most valuable data in statistical analyses. All statistical analyses were performed with R (R version 2.0.3; R Core Team 2013). Certain outliers were determined by an adjusted boxplot (Hubert and Vandervieren, 2008) using the “adjboxplot” function in R for skewed data in the robustbase package (Maechler, 2015).

I monitored predator behaviours and nest survival. Mesopredator interactions were defined as either the first depredation attempts on caged nests or successful depredations. It is assumed that the nest cages were protecting the nests from a successful depredation, so a depredation attempt on a caged nest was considered a successful depredation for analysis and no subsequent depredation attempts on that nest were included in analyses. Subsequent depredation attempts were still recorded and used to create a monthly summary of predator sign.

I performed a non-parametric product-limit survival analysis (Kaplan and Meir, 1958; Engeman *et al.*, 2006), with the “survfit” function in R in the survival package (Therneau and Lumley, 2015) to create a survival curve for all the turtle nests with known dates laid and fates. The survival time was days from oviposition to mesopredator interaction. Nests were censored (removed from analysis without being depredated) if they had hatchlings emerge, failed to complete incubation, or were still intact on their last nest check. I used cox-proportional hazards regression modeling (similar to linear mixed

effects modeling), using the “coxph” function in R in the survival package (Therneau and Lumley, 2015), and model selection to compare the survival curves of nests between candidate independent fixed variables (Table 2.1). I used Akaike's Information Criterion (AIC) to evaluate the support of potential cox-proportional hazards models, and chose models with high support ($\Delta AIC < 2$; Zuur *et al.*, 2009). Due to the large study area, there were forty nesting sites with 1 - 14 nests with known dates and fates per site. Site was included as a random independent factor (or frailty term; Therneau and Lumley, 2015). The dependent variable was the survival curve object from the “survfit” function that was a mathematical representation of survival time until the nest it was either predated or censored (Therneau and Lumley, 2015) .

I compared the percent of mesopredator interactions that occurred per week post-oviposition in my study to the average percent of mesopredator interactions expected based on previous studies (Table 2.4 adapted from Table 1 in Riley and Litzgus, 2014), using the “ks.test” function in the dgof R package (Arnold *et al.*, 2015). Most mesopredator interactions occurred on Snapping Turtle nests and the frequencies of interactions with nests of the other two species were highly variable, so data for all species were pooled to compare distributions (5 on Blanding's Turtle nests, 8 on Painted Turtle nests, and 62 on Snapping Turtle nests). I also ran a generalized linear mixed effects model using the “glmer” function in the lme4 R package (Bates *et al.*, 2013) to compare the mesopredator interactions between candidate independent variables (Table 2.1). Species were not pooled for this model Site was included as a random independent factor. The dependent variable was week post-oviposition of mesopredator interaction.

I examined the relationships among anthropogenic density, predator abundance, and seasonal patterns in nest predation using trail cameras. I recorded the date of each trail camera photo of each mesopredator species per month of the field season (May, June, July, August, and September) to determine if nest mesopredators were more abundant in higher anthropogenic densities. I recorded the number of days at least one photo was taken of any mesopredator species and the number of days at least one photo was taken per mesopredator species. If more than one species of mesopredator was recorded on the same date, it was recorded as one count of “any mesopredator species”. Because I was comparing relative rates of photos among anthropogenic densities, the time between independent photos was more valuable than unique identification of each individual mesopredator (Jenks *et al.*, 2011). The length of time each trail camera was on, functioning properly, and taking photos without anything obscuring the camera's view was calculated for each trail camera. I used generalized linear mixed effects modeling in the poisson family (for count data), using the “glmer” function in the lme4 R package (Bates *et al.*, 2013), and model selection to compare relative abundances of mesopredators between candidate independent variables (Table 2.3), for each month. I used AIC to evaluate the support of potential linear and linear mixed effects models, and chose models with high support ($\Delta AIC < 2$; Zuur *et al.*, 2009). The candidate independent fixed variables are shown in Table 2.3. Site was included as a random independent factor. The length of time, in weeks, that each trail camera was functioning was included as an offset (or number of trials) variable to ensure that data represented a relative mesopredator abundance rather than a static number of photos of mesopredators. The

offset creates a relative abundance by taking the time the trail camera was functioning into account. The dependent variable (or number of successes) was the number of photos per month.

Tracks or scat were found at some depredation attempts and successful depredations. The presence of tracks or scat does not necessarily mean that they represent the species that first dug up the nest (Larviviére, 1999), but they do give a general indication of mesopredator activity. The number of nests disturbed by each mesopredator species, based on successful depredations and all depredation attempts (including those after the initial interaction), were summarized per month. The potential temporal patterns of mesopredator presence were qualitatively compared to the relative abundance of nest mesopredators per month and the patterns of mesopredator interactions.

Results

More than half (65.3%) of the uncaged monitored nests were depredated. The majority (80.4%) of the nests with predator exclusion cages survived the full incubation period, regardless of predation attempts that affected 28.6% of those nests. The year the nest was laid, presence of a cage, length of paved roads within 1 km of each nest, site, number of buildings within 1 km of each nest, and length of railway tracks within 1 km of each nest, were strong explanatory variables for the probability of survival of turtle nests (Table 2.5, Figure 2.3). There was no difference in probability of survival of turtle nests between turtle species (Table 2.5). Nests without predator-exclusion cages were 69.7%

more at risk of being depredated than nests with cages ($\chi^2(1) = 15.63, p < 0.001$; Table 2.6). Nests in 2015 were 123.4% more at risk of being depredated than nests in 2014 ($\chi^2(1) = 4.64, p = 0.03$; Table 2.6). Nests were 0.3% less at risk of predation with each kilometre of all roads within 1 km of the nest ($\chi^2(1) = 3.83, p < 0.05$; Table 2.6).

The percent of mesopredator interactions that occurred per week post-oviposition in my study was significantly different than the average expected based on previous studies ($D = 0.56, p < 0.01$; Figure 2.4). The majority of nests were depredated in the first week post-oviposition and there were small spikes in depredation in the second, fifth, and sixteenth weeks post-oviposition. Mesopredator interactions were concentrated on the egg laying and hatching periods of incubation. Site, the presence of a cage, the turtle species, and the interaction between the turtle species and the presence of a cage were strong explanatory variables for the week of mesopredator interaction (Table 2.7), but no significant relationships were found. There was no pattern shown in relation to the site.

Foxes and raccoons were the most common and only confirmed nest mesopredators in my study area (Figure 2.5). Therefore, they were the two types of species used in the relative mesopredator abundance analysis. None of the anthropogenic density variables were strong explanatory variables of relative mesopredator abundance (Table 2.8). The species of nest mesopredator, the month the trail camera was on, the length of time in weeks the trail camera was on, site, and year were strong explanatory variables for relative mesopredator abundance (Table 2.8). More mesopredators were observed in June compared to any other month based on trail cameras ($\chi^2(4) = 62.17, p < 0.001$; Table 2.9) and sign (Figure 2.5). There is a spike in mesopredator sign found in

September that coincides with the spike in mesopredator interactions in the final months of incubation (Figure 2.5). More raccoons than foxes were observed based on trail camera photos ($\chi^2(2) = 22.72, p < 0.001$; Table 2.9) and sign (Figure 2.5). The species of bird that punctured turtle eggs was never determined (Figure 2.5).

Discussion

Turtle nests seemed to have a greater chance of survival near more roads in Muskoka, but I did not find a difference in relative nest mesopredator abundance based on close proximity to roads. Also, I did not find a difference in either based on close proximity to buildings in Muskoka. These findings contradict my predictions that higher anthropogenic density areas will have higher mesopredator abundance and higher rates of nest predation. Marchand and Litvaitis (2004) also found that simulated turtle nests close to roads were less likely to get depredated. Predators in Muskoka that survive near larger road densities may have learned that roads are dangerous and should be avoided, while predators near smaller road densities may be less familiar with them and get run over more frequently. It was very difficult to find nests in remote areas with very low road densities and the vast majority of the nests found in my study in Muskoka were found in the gravel shoulder of a road. Future studies should target more remote nesting locations to determine nest survival away from roads. In contrast to my study, other studies have found that mesopredator abundance is higher closer to higher anthropogenic density areas, especially near agricultural fields (Oehler and Litvaitis, 1996; Fordham *et al.*,

2008; Prugh, 2009).

In my study a nest was considered 'depredated' if a mesopredator dug at the side of a nest cage (in reality, a predation attempt). I assumed that the mesopredator would have eaten the eggs if the cage was not protecting the nest. Taking 'depredation' into consideration, the nest cage still increased the chances of a nest surviving. Many of the caged nests that were coded as 'depredated' were in fact subject to a predation attempt, but survived to have the eggs hatch. My results indicate that mesopredators were less likely to investigate a nest if there was a cage over the nest. Nests in the second year of my study were more at risk of being depredated than nests in the first year, but there was no difference in relative mesopredator abundance between the years. Mesopredators in some areas of my study area may have started to learn that a nest cage meant a turtle nest (i.e food source) was underneath (Mroziak *et al.*, 2000; Rollinson and Brooks, 2007), but that was not enough to remove the benefit a nest cage provided on nest survival. Raccoons and foxes were the most common nest mesopredator in my study and previous studies have found that they use tactile cues, such as soft soil, to investigate potential turtle nests (Oddie *et al.*, 2015; Dawson *et al.*, 2014). Putting a nest cage on a nest removes the tactile cue and may prevent a predator from gaining access to the nest chamber, which may explain why nest cages increased the chances of survival of the nests in my study.

Like Riley and Litzgus (2014), but in contrast to many other studies (Snow, 1982; Congdon *et al.*, 1983; Burke *et al.*, 2005; Geller, 2012; Wirsing *et al.*, 2012), I found that nest depredation was not restricted to the early days of egg incubation. A high percentage

of nests were indeed depredated in the first week post-oviposition, but there also seemed to be spikes in depredation later in incubation. These spikes indicate that if increased recruitment is a target of recovery actions, then turtle nests should be protected until the hatchlings emerge from the nest, not only for two weeks post-oviposition, as some protocols recommend (Moriarty and Linck, 1994; Endangered Species Environmental Review Coordinator, 2008; Turtles for Tomorrow, 2011). Protecting the nests with cages for the full incubation period can also prevent predation spikes like those that occurred in freshwater turtles (Rahman and Burke 2010), sea turtles (Engeman *et al.*, 2006), and anecdotally in freshwater turtles in South Muskoka, Ontario, in 2014, after the cages were removed (personal communication). Future studies determining nest survival and mesopredator interactions should monitor nests for the full incubation period rather than a short period of time at the beginning of incubation. Researchers should not assume that they can identify depredation patterns by only monitoring nests at the beginning of incubation.

The highest overall relative mesopredator abundance was in June, the peak month of turtle nesting season (Ernst and Lovich, 2009), as indicated by the highest percent of mesopredator interactions and the largest amount of mesopredator sign. The main predators appeared to be raccoons. The timing of mesopredator interactions did not differ among turtle species or anthropogenic density, indicating that the mesopredators may be cueing to nest characteristics that I did not measure. There are four types of cues mesopredators can use to find a nest: tactile, chemical, auditory, and visual (Congdon *et al.*, 1987; Oddie *et al.*, 2015). Raccoons largely use tactile cues to find nests (Oddie *et al.*,

2015), so the presence of strong tactile cues may be an important predictor of nest survival. Spikes in the percent of mesopredator interactions during egg laying and hatching could indicate that the mesopredators are cuing into characteristics similar to both events. Mesopredators, especially mammals, could be using chemosensory cues, such as musk or water from the wetland from which the female travelled to nest (Oddie *et al.*, 2015), the smell of female fluids when laying (Legler 1954; Congdon *et al.*, 1983; Spencer 2002) or embryonic fluid when hatching (Riley and Litzgus, 2014). Rotten eggs may emit a stronger smell closer to nests hatching than being laid (Riley and Litzgus, 2014). Mesopredators could be using the visual cues of seeing the mother laying (Congdon *et al.*, 1987; Eckrich and Owens 1995) and disturbing soil (Strickland *et al.*, 2010; Spencer 2002) or of hatchlings emerging (Riley and Litzgus, 2014) and disturbing soil. Mesopredators could also be using auditory cues from the noises of hatchlings hatching (Ferrara *et al.*, 2013) and digging out of the nest (Oddie *et al.*, 2015).

The majority of turtle nests get predated in a given year in many turtle populations (Congdon *et al.*, 1983, 1987; Fordham *et al.*, 2008), which reduces population recruitment (Reed, 2009). Installing cages over nests helps prevent depredation (Kurz *et al.*, 2011; Riley and Litzgus, 2013). Nest mesopredators are present and depredate nests throughout the egg incubation period, so nest cages should be kept on nests until the eggs hatch. Because nest caging and conservation projects are constrained by time and financial resources (Wilson *et al.*, 2006; McDonald-Madden *et al.*, 2008), according to my findings, prioritizing the installation of nest cages in low road density areas may be more beneficial than in high road density areas to improve the survival of turtle nests.

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Figures



a)

b)

Figure 2.1: Intact (a) and depredated (b) nests.



a)

b)

Figure 2.2: Predator exclusion cages used to protect nests with known dates laid; wooden-sided (a) and wire (b).

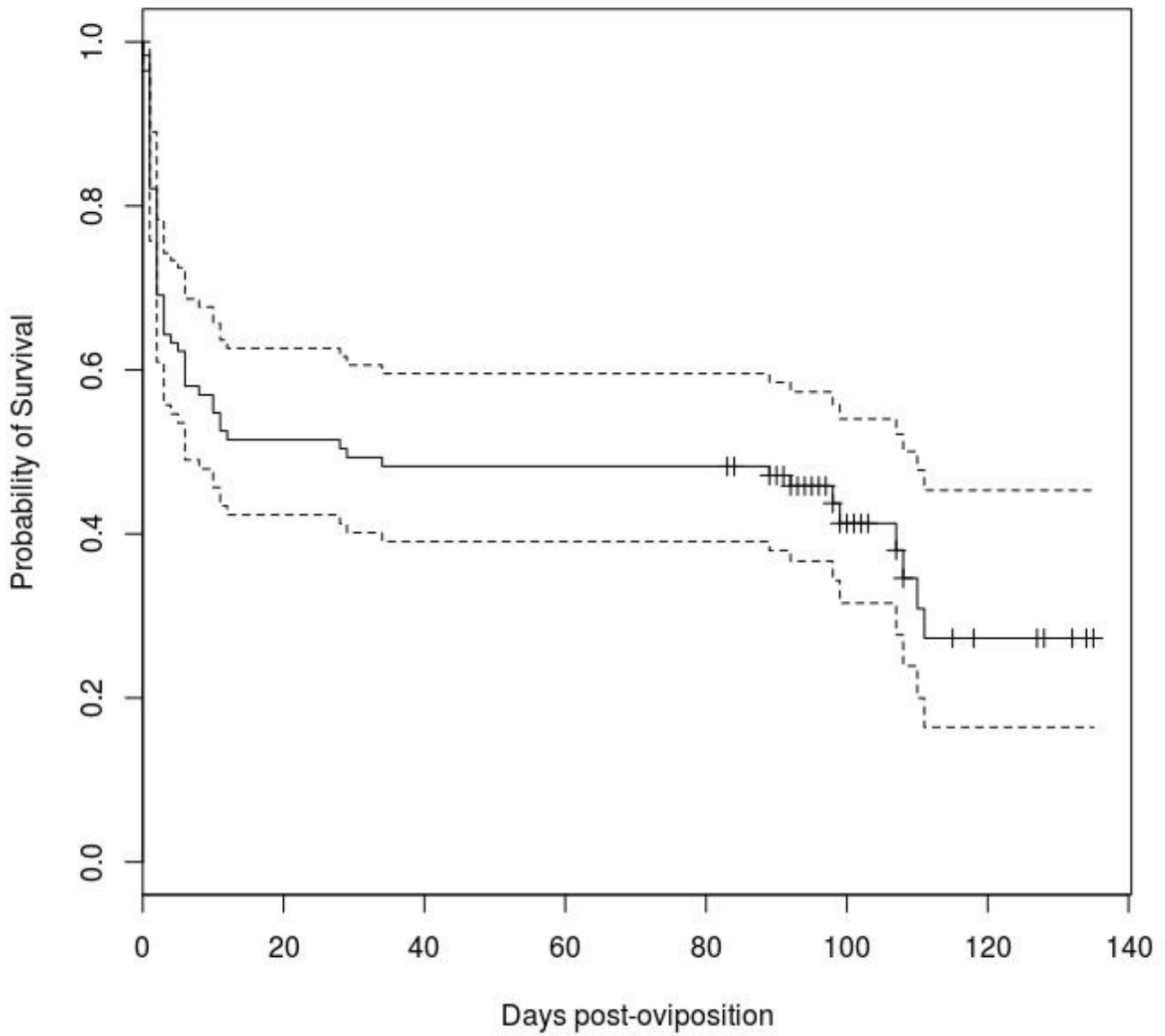


Figure 2.3: Survival curve (solid line) of turtle nests over two years of monitoring over the incubation period in central Ontario. Vertical tick marks represent when a nest hatched and was “censored” from analysis. Dashed lines represent 95% confidence intervals.

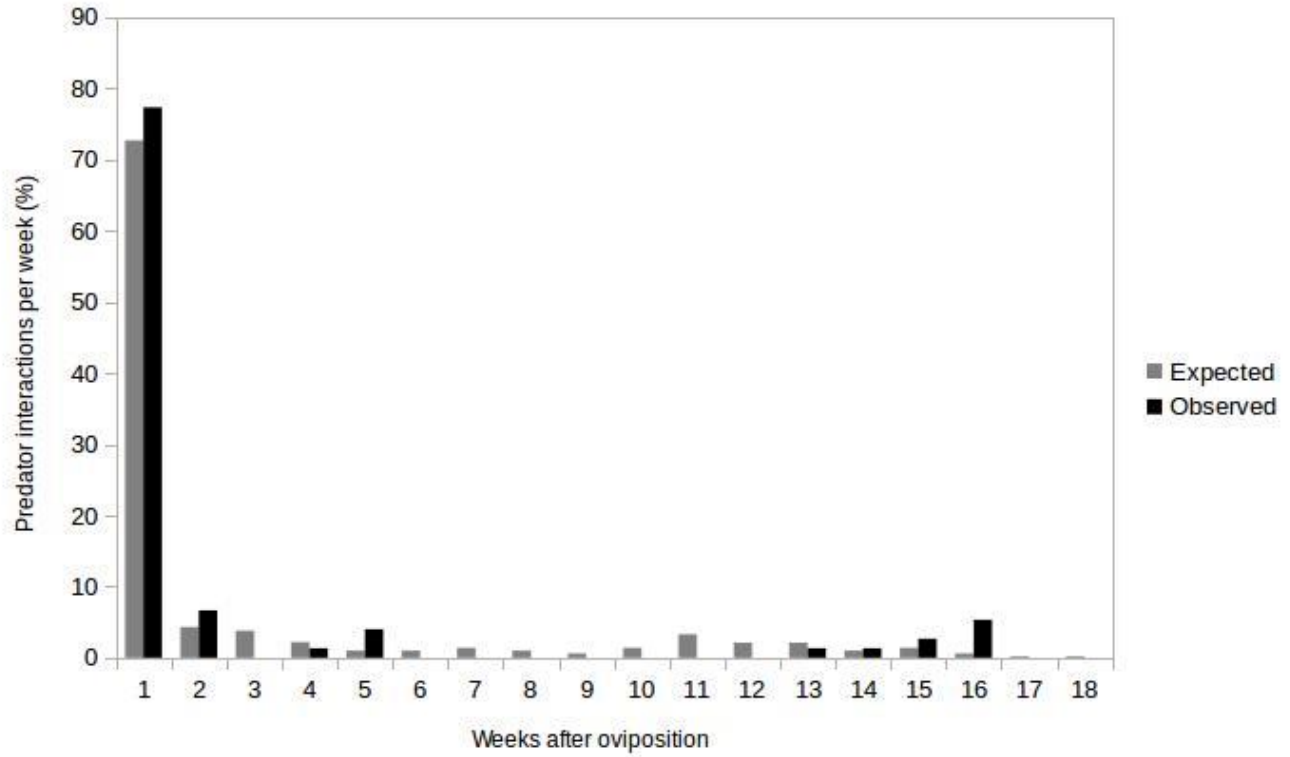


Figure 2.4: Observed percent of mesopredator interactions per week post-oviposition on turtle nests in Muskoka were significantly different than those expected based on previous studies ($D = 0.56$, $p < 0.01$; see Table 2.4 for list of studies).

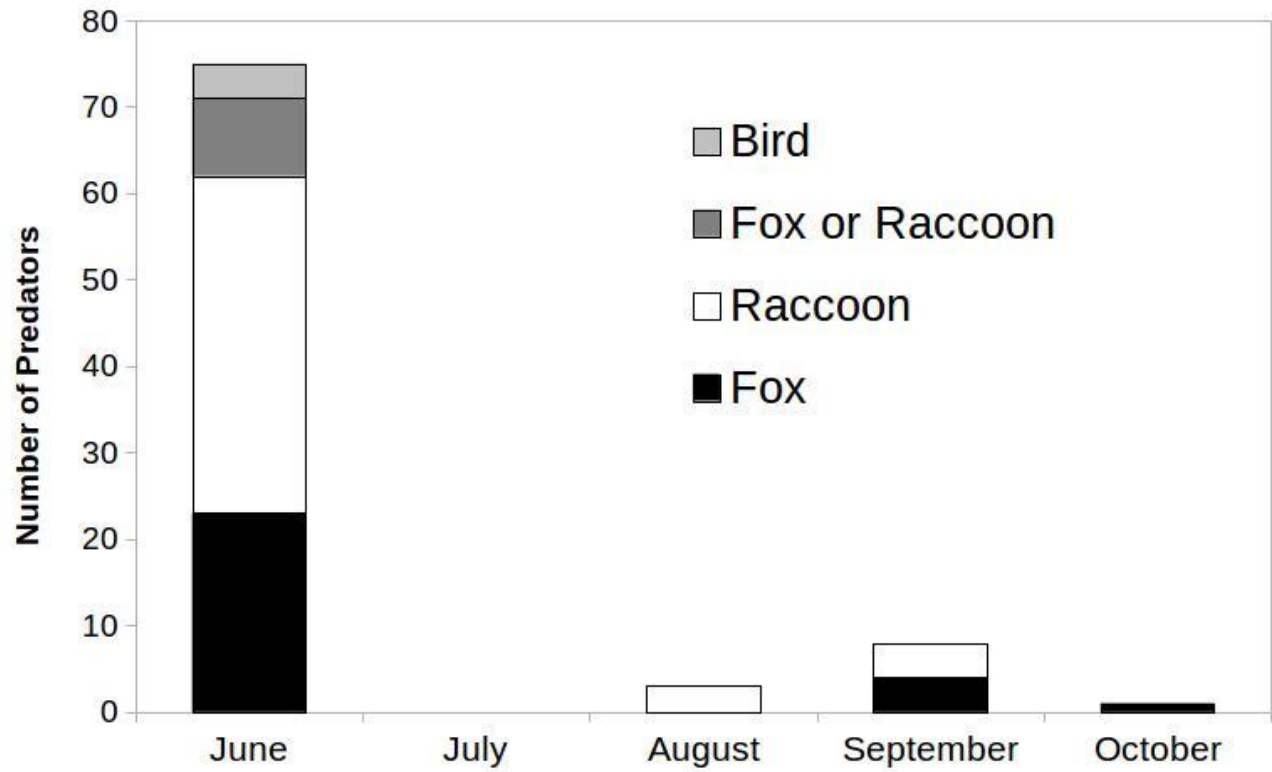


Figure 2.5: Number of nests with mesopredator interactions by species of mesopredator by month of incubation. Mesopredators were identified by tracks or scat at turtle nests.

Tables

Table 2.1: Independent variables used in cox-proportional hazards and linear mixed effects model selection with the codes used in the results. Anthropogenic Structures are the categories used to calculate anthropogenic densities within 1 km of turtles. QGIS was used to calculate the number of buildings and length (m) of roads within 1 km of turtles. ' * ' indicates a random variable. The site variable was created to help control for the clustering of nests.

Anthropogenic Structure	Code	Non-Anthropogenic Variables	Code
Buildings	build	Turtle Species	tspecies
Railway tracks	rt	Year (2014 or 2015)	year
Unpaved roads	unp	Nest Cage Installed (yes or no)	cage
Paved roads	pave	Julian Date Nest Laid	start
Recreational roads and trails	rec	Site*	site
Local roads	local		
Arterial roads	art		
Highways	high		
All roads	allrd		
All linear features (roads and railway tracks)	rdrt		

Table 2.2: Sample sizes of turtle species nests that were found in South Muskoka, Ontario and used in non-parametric product-limit survival analysis.

Turtle Species	Known Dates Laid and Fates	Unknown Dates Laid and Known Fates	Known Dates Laid and Unknown Fates
Midland Painted Turtle (<i>Chrysemys picta marginata</i>)	10	26	11
Snapping Turtle (<i>Chelydra serpentina</i>)	111	209	17
Blanding's Turtle (<i>Emydoidea blandingii</i>)	7	10	0
Unknown Species	0	73	0

Table 2.3: Independent variables used in linear mixed effects model selection with the codes used in the results. Anthropogenic Structures are the categories used to calculate anthropogenic densities within 1 km of turtle nests. QGIS was used to calculate the number of buildings and length (m) of roads within 1 km of turtle nests. ' * ' indicates a random variable. The site variable was created to help control for the clustering of nests.

Anthropogenic Structure	Code	Non-Anthropogenic Variables	Code
Buildings	build	Mesopredator Species	mspecies
Railway tracks	rt	Year (2014 or 2015)	year
Unpaved roads	unp	Month (May, June, July, August, September)	month
Paved roads	pave	Site*	site
Recreational roads and trails	rec		
Local roads	local		
Arterial roads	art		
Highways	high		
All roads	allrd		
All linear features (roads and railway tracks)	rdrt		

Table 2.4: Percent depredation interactions per week post-oviposition. The distribution of the average expected percent depredation interactions per week post-oviposition was used in a Kolmogoroc-Smimov goodness-of-fit test to compare to the percent depredation interactions per week post-oviposition in this study. The mean percent depredation interactions per week post-oviposition were estimated for weeks 4+ in the first 6 studies because they did not distinguish between each week.

Study	Species	Percent depredation interaction per week (%)																	
		1	2	3	4 (+)	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Congdon <i>et al.</i> , 1983	Blanding's Turtle	87.0	5.0	4.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Christens and Bider 1987	Painted Turtle	86.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Burke <i>et al.</i> , 2005	Diamond-backed Terrapin	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wirsing <i>et al.</i> , 2012	Snapping Turtle	98.0	1.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Painted Turtle	98.0	1.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geller 2012	Map Turtle spp.	90.0	7.0	0.0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Snow 1982	Painted Turtle	64.0	21.0	12.0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Riley and Litzgus 2014	Painted Turtle	14.3	3.6	17.9	17.9	3.6	0.0	7.1	3.6	0.0	0.0	14.3	7.1	0.0	3.6	3.6	3.6	0.0	0.0
	Snapping Turtle	17.2	0.0	0.0	0.0	3.5	6.9	3.5	3.5	3.5	10.3	13.8	10.3	17.2	3.5	6.9	0.0	0.0	0.0
Mean expected distribution used in Kolmogorov-Smirnov test		72.7	4.3	3.8	2.2	1.0	1.0	1.4	1.0	0.6	1.4	3.3	2.1	2.1	1.0	1.4	0.6	0.2	0.2

Table 2.5: Akaike's Information Criterion (AIC) for selection of best cox-proportional hazard regression models for explaining turtle nest survival. Abbreviations of independent variables are defined in the Methods and in Table 2.1.

Model	Number of estimated parameters (K)	AIC	Δ AIC	Akaike weight (w_i)
cage+year+site+pave	3	573.43	0.00	0.56
cage+year+site+build	3	575.13	1.70	0.24
cage+year+site+build+rt	4	575.35	1.92	0.21

Table 2.6: Influence of effects of top Akaike's Information Criterion (AIC) model on survival of turtle nests. 'df' is the degrees of freedom. Significant effects are shown as bold P values. Abbreviations of independent variables are defined in the Methods in Table 2.1.

Parameter	Effect	df	χ^2	<i>p</i> values
Survival	cage	1	15.63	<0.001
	year	1	4.64	0.03
	pave	1	3.83	0.05
	site	19.9	38.37	0.008

Table 2.7: Akaike's Information Criterion (AIC) for selection of best linear mixed effects model for week of mesopredator interaction. Abbreviations of independent variables are defined in the Methods in Table 2.1. ':' indicates an interaction term.

Model	Number of estimated parameters (K)	AIC	Δ AIC	Akaike weight (w_i)
site	3	208.56	0.00	0.31
site+cage	4	208.60	0.03	0.31
site+cage+tspecies	6	209.32	0.76	0.21
site+cage+tspecies+tspecies:cage	7	209.85	1.29	0.16

Table 2.8: Akaike's Information Criterion (AIC) for selection of best linear mixed effects model for relative mesopredator abundance. Abbreviations of independent variables are defined in the Methods in Table 2.1.

Model	Number of estimated parameters (K)	AIC	Δ AIC	Akaike weight (w_i)
mspecies+month+site	8	642.57	0	0.52
mspecies+month+site+year	9	642.75	0.18	0.48

Table 2.9: Influence of effects of top Akaike's Information Criterion (AIC) model on relative mesopredator abundance based on trail camera photos. 'df' is the degrees of freedom. Significant effects are shown as bold P values. Abbreviations of independent variables are defined in the Methods in Table 2.1.

Parameter	Effect	df	χ^2	P values
Relative mesopredator abundance	mspecies	2	22.72	<0.001
	month	4	62.17	<0.001

General Conclusion

I investigated the indirect impacts of human development on freshwater turtles by examining chronic stress levels (Chapter 1) in areas of varying anthropogenic densities. Snapping Turtles (*Chelydra serpentina*) were more likely to have above-minimum CORT than Midland Painted Turtles (*Chrysemys picta marginata*) or Blanding's Turtles (*Emydoidea blandingii*); however, for turtles that had above-minimum CORT, male Midland Painted Turtles and Blanding's Turtles had relatively high CORT levels. Surprisingly, anthropogenic density did not seem to affect CORT levels, although variation in the date turtles were captured and the date enzyme immunoassays were run complicated my interpretations.

I investigated the indirect and direct impacts of human development on freshwater turtles by examining rate and patterns of nest predation (Chapter 2) in areas of varying anthropogenic densities. Nest depredation was not restricted to the early days of egg incubation, but occurred late in incubation when eggs were hatching as well. Predator exclusion nest cages and higher road densities close to a turtle nest increased the nests' survival. Relative nest mesopredator abundance indices did not differ among sites with varying anthropogenic densities, but were highest during the nesting season. Of the two major nest mesopredators in my study area, raccoons were more abundant than foxes.

I found that male Midland Painted Turtles and Blanding's Turtles had higher chronic CORT than females. Corticosterone is associated with processes in the body that are not solely due to stress, such as reproductive status, season, and disease (Sheriff *et al.*, 2011; Sapolsky, 1990). Many of these relationships are understood in other taxonomic groups, but not in reptiles (Sheriff *et al.*, 2011). Immune function may be more directly related to CORT than a body condition index and future studies should further explore the relationships (Sheriff *et al.*, 2011). Research is still required, in the form of adrenocorticotrophic hormone (ACTH) challenges (Mastromonaco *et al.*, 2014), comparing CORT in

blood to CORT in claws to determine if a strong relationship exists (Berkvens *et al.*, 2013). Claw growth rates should be measured for individual claws of each species to determine the amount of time represented in the claw samples (Baxter-Gilbert *et al.*, 2014). Stress responses are species-specific and it would be beneficial to study and validate enzyme immunoassay methods for claws in more turtle species with more detail to help understand the stress response in turtles. Future studies should also investigate other potential sources of stress, such as land use, vehicle encounters, sound, light and chemical pollution, and natural disasters (White, 1979; Hopkins *et al.*, 1997; Longcore and Rich, 2004). Caution must be taken when interpreting chronic CORT levels before more is understood about CORT in turtles.

My study indicated that turtles do not seem to have higher chronic CORT around roads or buildings, but they are still subject to road mortality, so mitigating road effects will still benefit populations. Road ecology is the study of the interactions between wildlife and roads (Forman and Alexander, 1998; Fahrig and Rytwinski, 2009; vander Ree *et al.*, 2011). Up to 98-100% of individual turtles can be killed when they first cross a road (Aresco, 2005) and mitigation structures help reduce the negative effects of roads on wildlife (Huijser *et al.*, 2007). Mitigation structures can be placed in two general categories: exclusion structures (e.g fencing, gravity walls) and connectivity structures (e.g ecopassages, bridges; Dodd *et al.*, 2004; Aresco, 2005). The effectiveness of mitigation structures should be assessed with a Before-After-Control-Impact experimental design (Forman *et al.*, 2003; Baxter-Gilbert *et al.*, 2015). Contractors need to understand how and why particular fencing needs to be installed, while taking the likelihood of flooding into account (Baxter-Gilbert *et al.*, 2015), and ecopassages should let in light to encourage turtles to use them (Woltz *et al.*, 2008). Road mitigation projects need to take into account that snapping turtles can climb chain-link fences and hatchling turtles are small enough to pass through many gauges of fencing.

Gravid females are at an increased risk of road mortality when travelling to nesting sites and while laying their eggs (Ashley and Robinson, 1996; Paterson *et al.*, 2013), but I found that nests had a better chance of survival when they were near larger densities of roads. Mitigation structures, including underpasses, overpasses, and fencing (Ashley and Robinson, 1996; Baxter-Gilbert *et al.*, 2015) should be built to facilitate turtle movements around roads, while preventing them from walking on the road surface. Because fencing cannot be placed close to the paved portion of a road, artificial nesting mounds could be placed outside fencing in the most likely path females would take to travel to nesting sites (Paterson *et al.*, 2013).

The majority of turtle nests we found were in the gravel shoulders on the sides of roads. Future studies should target more remote nesting locations to determine nest survival away from roads. Future studies should also select smaller and fewer independent sites along a specific gradient of road density to patrol in more detail than we were able to in this project. Traffic volume may be a better indicator of the type of road than density of roads based on a particular category. Future studies should incorporate measures of agricultural practices, logging activities, and building use. Mesopredators depredated most nests while turtles were laying eggs and others while the eggs were hatching, as found by Riley and Litzgus (2014), but in contrast to the findings of many other studies (Snow, 1982; Congdon *et al.*, 1983; Burke *et al.*, 2005; Geller, 2012; Wirsing *et al.*, 2012). The abundance of mesopredators did not differ among locations with varying densities of roads or buildings, and we did not determine what cues mesopredators were using to find nests. Future studies should investigate tactile, chemical, and auditory cues that predators use to find nests (Oddie *et al.*, 2015), and determine if these cues are impacted by anthropogenic density.

Conservation, government, and private groups should concentrate on protecting more key habitats (like nesting sites, hibernacula, and summer wetland complexes) that are important to all life stages of turtles in parks and conservation reserves (Gibbons *et al.*, 2000). Predator exclusion nest

cages should be kept on nests until the turtles hatch and priority should be given to caging nests in low road density areas to help increase population recruitment. Experts also need to engage the public in outreach and conservation activities to improve political and societal attitudes and awareness toward turtles in order to improve their conservation status and population recruitment (Gibbons *et al.*, 2000).

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