

**Diet: it's all about location, location, location! How urbanization  
influences endocrine stress, isotopic signatures, and fecal antioxidants  
in eastern chipmunks (*Tamias striatus*)**

by

Raven Ouellette

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Laurentian University

Sudbury, Ontario, Canada

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## Abstract

As cities continue to expand on a global scale, animals gain greater access to human food waste, and the consequences associated with the consumption of this food waste are poorly understood. Using eastern chipmunks (*Tamias striatus*) as a study species, I examined the differences in cortisol concentrations and body condition scores, as well as isotopic signatures of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ), and fecal antioxidants from eastern chipmunks on an urbanization gradient. I tested the hypotheses that: 1) chipmunks would have lower cortisol levels and better body condition scores if they are living in more urban areas because urban environments may contain higher amounts of human food waste relative to natural habitats, and 2) chipmunks would have higher carbon and nitrogen signatures and excrete more antioxidants in more urban environments compared to their natural counterparts because they may be consuming human food waste. Chipmunks were sampled across Sudbury, Ontario from 20 areas with varying levels of urbanization. Each time a chipmunk was captured, hair samples, fecal samples, and body measurements were collected. To quantify urbanization, I surveyed all study sites over a three-day period to score the level of human activity. I found that cortisol significantly differed among chipmunks across the gradient, such that chipmunks in more urban habitats experienced the highest levels of cortisol. I found chipmunks with poorer body condition in the most urban areas. Chipmunks in more urban habitats produced a higher nitrogen signature than their natural counterparts, while no significant difference was observed in carbon or fecal antioxidants. My results help us to understand differences in diet across as well as the physiological changes associated with urban habitats, which may help us understand how other species may respond to urbanization.

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## General Introduction

### *Urbanization and Stress*

Urbanization – the process of altering a landscape into an area for human settlement – is a global phenomenon (Shochat *et al.*, 2010; Faeth *et al.*, 2011; Saari *et al.*, 2016) that has forced many wildlife species to respond to rapid environmental change (McKinney, 2002; Partecke *et al.*, 2006). For example, the American mink (*Neovion vison*) has low colonization rates near urban areas because urban growth has decreased the amount of suitable habitat (Wolff *et al.*, 2015; Ahlers *et al.*, 2016). Moreover, many mammalian carnivores, such as bobcats (*Lynx rufus*), gray foxes (*Urocyon cinereoargenteus*), and mountain lions (*Puma concolor*) are becoming locally extinct in urban areas because of increased human disturbance and habitat loss (Crooks, 2002; Ordeñana *et al.*, 2010). However, some species are able to acclimatize to make physiological adjustments to respond to changes associated with urbanization (Kart *et al.*, 2007). Scavengers and herbivores can take advantage of new anthropogenic environments by consuming food waste from humans or crops due to their widespread and consistent availability (McKinney, 2002; Sih *et al.*, 2010; Birnie-Gauvin *et al.*, 2016). Over time, urban-living individuals may begin to phenotypically diverge from their counterparts living in natural habitats as they acclimatize to a resource-filled environment, such that urban-dwelling individuals may differ in body condition, colouration, or behaviour (McKinney, 2002; Hasegawa *et al.*, 2014; Lyons *et al.*, 2017; Baxter-Gilbert *et al.*, 2019).

For some species, however, acclimatizing to an urban environment may be challenging because of decrease in natural habitat (e.g. forest or grassland habitat) and food resources (e.g. nuts and seeds; Reijen and Foppen, 1997; Fernández-Juricic and Tellería, 2000; Lowry *et al.*, 2012). At the same time, there is an increase in automobile traffic, a source of direct mortality

(Reijen and Foppen, 1997), as well as an increase in noise and air pollution (Barber *et al.*, 2009). In other words, a new set of environmental conditions are present in an urban environment which may serve as stressors for individuals that originate from a natural habitat (Reijen and Foppen, 1997; Lowry *et al.*, 2012). Consequently, acclimatization is strategy to respond to unfamiliar and unnatural conditions as well as chronic stress (Luniak, 2004; Partecke *et al.*, 2006).

When an organism is initially introduced to a novel environment, such as urban space, it must maintain homeostasis. Initially, an individual will respond behaviourally to a new environment, however the individual will shift to physiological strategies which are more costly if the behavioural changes are not enough to maintain homeostasis (Tsigos *et al.*, 2016). Homeostasis, or the physiological balance of an individual, is maintained through various traits such as body temperature, blood glucose or pressure, and glucocorticoids hormones, which all increase when experiencing an increase in stress (Hers, 1990; Sheriff *et al.*, 2010; Coffman, 2011). Glucocorticoid hormones, like cortisol, aid individuals to acclimatize to the stress of a novel habitat through the hypothalamic-pituitary-adrenal (HPA) axis once glucocorticoids are elevated above baseline levels (Sheriff *et al.*, 2010; Mastromonaco *et al.*, 2014). Cortisol assists individuals in acclimatizing to a new environment by inhibiting the use of glucose in peripheral tissues which are not necessary for survival during a stressful event (Sapolsky *et al.*, 2000), using fat stores and stimulating gluconeogenesis to provide a source of energy (Sapolsky *et al.*, 2000; Romero and Wikelski, 2001; Partecke *et al.*, 2006). Although the activation of the HPA axis is beneficial when experiencing acute stress, some urban-dwelling individuals exposed to long-term or chronic stress experience a high allostatic load or pay a high cost for experiencing long-term stress which comes with the development of physiological consequences (Sapolsky, 1992; Partecke *et al.*, 2006; Ullmann *et al.*, 2019). For example, long-term exposure to elevated cortisol

can cause harm to the reproductive and immune systems, as well as the nervous system, such as decreases in the number of brain neurons found in the hippocampus (Sapolsky *et al.*, 1990; Sapolsky, 1992; Partecke *et al.*, 2006). Despite many studies concluding that cortisol levels are lower within urban environments in several species (Ditchkoff *et al.*, 2006; Lopucki *et al.*, 2019), there are some studies that suggest otherwise. For example, squirrel gliders (*Petaurus norfolcensis*) in Australia had higher levels of cortisol when they nested near major roads (Brearley *et al.*, 2012). In another study investigating vervet monkeys (*Chlorocebus aethiops*), male monkeys had higher cortisol levels near areas with high levels of human activity, compared to males exposed to less human activity (Fourie *et al.*, 2015). These studies highlight the effects of urbanization on an individual and suggest that an increase in cortisol is context or species-dependent (Brearley *et al.*, 2012; Fourie *et al.*, 2015; Iglesias-Merchan *et al.*, 2018).

Previous studies on cortisol levels in urban populations of species, such as eastern chipmunks (*Tamias striatus*; Lyons *et al.*, 2017), dark-eyed juncos (*Junco hyemalis*; Atwell *et al.*, 2012), and tree lizards (*Urosaurus ornatus*; French *et al.*, 2008) have suggested that individuals in urban habitats have lower levels of cortisol (or corticosterone, the stress response hormone in birds and reptiles) compared to their counterparts in natural habitats. This may be the end result of acclimatization, allowing those species to survive in an anthropogenic environment (French *et al.*, 2008; Atwell *et al.*, 2012; Lyons *et al.*, 2017). In contrast, the species' natural counterparts lack this stress response and therefore may not be as successful in an urban environment (Partecke *et al.*, 2006; Atwell *et al.*, 2012). As a consequence, natural and urban individuals may differ in life history traits such as the number of offspring produced or size at birth (Boal and Mannan, 1999; Sprau *et al.*, 2016).

## *Antioxidant Differences Between Urban and Natural Habitats*

Antioxidants are molecules that control the number of free radicals, such as reactive oxygen and nitrogen species, found within mammalian systems (Halliwell and Gutteridge, 2002). Free radicals are molecules with an unpaired electron in their outer orbital (Southorn and Powis, 1988). Antioxidants work by binding to the unstable, unpaired electron found on that free radical to prevent it from binding to biologically important molecules (e.g. enzymes and proteins; Southorn and Powis, 1988; Sies, 1997; Halliwell and Gutteridge, 2002). If free radicals bind to biologically important molecules, the consequences can include the development of diseases such as diabetes and cancer (Dominiqueti *et al.*, 2016; Poprac *et al.*, 2017). Some antioxidants are produced internally, such as vitamin C or uric acid, and are referred to as endogenous antioxidants (Surai, 2002). However, antioxidants can also be obtained through the diet (e.g. carotenoids, vitamin E, and glutathione; Böhm 1997; Surai, 2002), and are referred to as exogenous antioxidants. When antioxidants are low, they can no longer control the levels of free radical molecules present within a cell and damage can occur to various biomolecules within a system, and this is referred to as oxidative stress (Mittler, 2002). Damage can range from a change or inactivation of a protein's function within a cell (Schieber and Chandel, 2014), to damage to transcription or DNA function (Yakes and VanHouten, 1997), or even cell death (Ott *et al.*, 2007). As a consequence, oxidative stress has been linked to many diseases, including diabetes (Maritim *et al.*, 2003; Ullah *et al.*, 2015), cancer (Toyokuni *et al.*, 1995; Valko *et al.*, 2005), as well as obesity (Ozata *et al.*, 2002; Matsuzawa-Nagata *et al.*, 2008; Fernández-Sánchez *et al.*, 2011) among others.

Oxidative stress can be triggered by particular environment factors such as chemical pollution (Valavanidis *et al.*, 2006; Radwan *et al.*, 2010), noise and air pollution (Demirel *et al.*,

2009; Injaian *et al.*, 2018), infectious disease (Lykkesfeldt and Svendsen, 2007), and most notably, poor diet (Miller and Brzezinska-Slebodzinska, 1993; Noeman *et al.*, 2011). Each of these factors have been correlated with a decrease in antioxidants, suggesting that these individuals experience more oxidative stress (Demirel *et al.*, 2009; Injaian *et al.*, 2018). In urban habitats, animals may be consuming higher levels of exogenous antioxidants compared to their natural counterparts because of the increased consumption of an “unnatural” diet (Miller and Brzezinska-Slebodzinska, 1993; Valavanidis *et al.*, 2006; Noeman *et al.*, 2011; Isaksson, 2015). Some studies have investigated antioxidant levels in response to noise and chemical pollution between defined urban and rural habitats (Møller *et al.*, 2010; Costantini, 2014; Giraudeau and McGraw, 2014; Herrera-Duenas *et al.*, 2014; Isaksson, 2015). However, to my knowledge, there has yet to be a study investigating the use of exogenous antioxidants as biomarkers for diet on a continuous scale of urbanity.

Exogenous antioxidants may act as biomarkers to distinguish between individuals of urban and natural populations of animals because of the dietary differences that may exist between urban and natural habitats (Giraudeau *et al.*, 2015; Isaksson, 2015). For example, in a natural habitat many animals like golden mice (*Ochrotomys nuttali*; Davis *et al.*, 2013), house finches (*Carpodacus mexicanus*; Olson and Owens, 1998), and eastern chipmunks (*Tamias striatus*; Davis *et al.*, 2013), consume carotenoids and polyphenols (exogenous antioxidants) in their natural diet from berries and seeds (Brush and Power, 1976; Wrazen and Svendsen, 1978; Hercberg *et al.*, 1998; Salentik *et al.*, 2014). Thus, individuals consuming higher levels of exogenous antioxidants may benefit from decreasing oxidative stress, (Hercberg *et al.*, 1998; Salguero *et al.*, 2003; Andre *et al.*, 2010; Salentik *et al.*, 2014; Isaksson, 2015). Specifically, unabsorbed carotenoids and polyphenols decrease the number of free radicals present within the

intestine, which has been shown to decrease free radicals, the risk of cancer, and improve gut health (Garsetti *et al.*, 2000; Andre *et al.*, 2010). The remainder of antioxidants that are not absorbed are excreted, which may act as a marker of how many exogenous antioxidants individuals may be consuming (Salentik *et al.*, 2014). On the other hand, in urban habitats, animals may have access to human food waste, which may contain more exogenous antioxidants than their natural diets. Specifically, compost bins, gardens and garbage bins may provide a source of fruits and vegetables, which are high in exogenous antioxidants (Polidori *et al.*, 2009). If animals are consuming that food waste, it is possible that they may excrete more antioxidants than their natural counterparts, allowing the amount of antioxidants excreted (in feces) to provide insight into the diet of an urban-dwelling animal (Clauss *et al.*, 2006; Salentik *et al.*, 2014).

In urban habitats, food resources that are used by animals (e.g. bird feeders, garbage, or being fed directly from humans) provide nutrients that would not be found in their natural habitats (Marion *et al.*, 2008; Bateman and Fleming, 2011). Many human foods consist of vegetable oil (e.g. canola and peanut oil; Gunstone, 2000) that contains a fatty acid (omega-6 polyunsaturated fatty acid) that has been linked to increases in free radicals in humans (Patterson *et al.*, 2011). Additionally, added sugars (e.g. corn syrup, sucrose, and glucose) that are commonly found in human food waste (Giroto *et al.*, 2015), have also been found to induce oxidative stress (Prasad, 2014). If animals are consuming human food waste, it is possible that they too could experience negative effects associated with a poor diet (e.g. consuming a high-fat diet and increasing the chances of developing obesity, cancer, and diabetes; Matsuzawa-Nagata *et al.*, 2008; Patterson *et al.*, 2011). However, to my knowledge, there has yet to be a study using exogenous antioxidants left in fecal matter to understand diet on a natural-urban gradient within wild populations of mammals. By defining a series of measurable variables that could differ

between urban and natural areas, a gradient could be generated to accurately represent the transition from a more urban area into a more natural habitat.

### *Isotopes as Markers of Diet*

Isotopes can be used to identify dietary differences that exist among habitats (Sponheimer *et al.*, 2003; Fry, 2006; Auman *et al.*, 2011; Hopkins *et al.*, 2014; Savory *et al.*, 2014; Penick *et al.*, 2015). Dietary differences between individuals in natural and urban habitats may be reflected in two particular stable isotope signatures: carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ; Newsome *et al.*, 2010; Merkle *et al.*, 2011).  $\delta^{13}\text{C}$  is determined by the ratio of  $^{12}\text{C}$  and  $^{13}\text{C}$  that differs between samples of hair between individuals (Tykot, 2004; Brugnoli and Farquhar, 2000).  $^{12}\text{C}$  and  $^{13}\text{C}$  differ in the number of neutrons each atom has, whereby  $^{12}\text{C}$  has one less neutron than  $^{13}\text{C}$  (Smith, 1972; Kelly, 2000). Fractionation is the change in an isotope ratio within a medium due to various processes (e.g. chemical or biological), occurs between  $^{12}\text{C}$  and  $^{13}\text{C}$ , and it leads to differences in isotopic signatures between individuals (Criss, 1999, Hoefs, 2009).

Urban-dwelling individuals may produce higher carbon signatures than their counterparts in natural habitats because they may be consuming a diet that is higher in corn (Gregorich *et al.*, 1994; Newsome *et al.*, 2010). Corn is a plant that uses the  $\text{C}_4$  photosynthesis pathway, which is a pathway known for producing a high carbon signature. Corn is typically found in many human foods, which makes it accessible to wildlife in human food waste (Gregorich *et al.*, 1994; Newsome *et al.*, 2010). Because of the occurrence of fractionation in the  $\text{C}_4$  photosynthesis pathway, a high  $^{13}\text{C}$  signature is produced relative to plants that use the  $\text{C}_3$  photosynthesis pathway. Plants that are found in natural habitats, such as deciduous trees, tall grasses, and broad leaf herbs, are plants that use the  $\text{C}_3$  photosynthesis pathway (Franks and Farquhar, 1999; Patterson *et al.*, 1999). These plants produce a lower  $^{13}\text{C}$  signature because less fractionation

occurs in the C<sub>3</sub> photosynthesis pathway compared to the C<sub>4</sub> pathway, as mentioned previously (Franks and Farquhar, 1999; Patterson *et al.*, 1999; Newsome *et al.*, 2010). Therefore, depending on the type of plant consumed (C<sub>4</sub> or C<sub>3</sub>), we may see differences in the isotope signatures across individuals in urban and natural habitats (Penick *et al.*, 2015). As mentioned above, human food waste often consists of corn (*Zea mays*) or corn by-products such as corn syrup (Newsome *et al.*, 2010; Parker *et al.*, 2010). Not only do humans consume foods with high levels of corn products, but humans often provide corn-based diets to livestock, ultimately resulting in meat products that are high in corn content (Newsome *et al.*, 2010). Since these corn-based food products are readily available within urban food waste, animals are easily able to locate and consume them (Newsome *et al.*, 2010; Farr-Wharton *et al.*, 2014). Urban populations of mammals, like eastern chipmunks (Lyons *et al.*, 2017) and raccoons (Schulte-Hostedde *et al.*, 2018) are consuming these foods in their diets, and therefore they may differ from their conspecifics in natural habitats in terms of body condition and risk for metabolic diseases. The long-term impact of a diet based on human food waste for urban wildlife is still unknown, but may impact population dynamics, susceptibility to disease, and metabolic disorders (Bradley and Altizer, 2007; Murray *et al.*, 2018).

Similar to  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  is a ratio of  $^{14}\text{N}$  and  $^{15}\text{N}$ , which determines the amount of protein consumed by an individual, and can also indicate trophic level (Godley *et al.*, 1998; Lavin *et al.*, 2003; Newsome *et al.*, 2010). A trophic level represents a hierarchical position that an individual holds in a food chain or web (Thompson *et al.*, 2007). Typically, if an individual consumes more protein than another individual, they are at a higher position in the food chain (Robbins *et al.*, 2005; Bonhommeau *et al.*, 2013). If individuals occupy the same trophic level, they may use the same nutritional sources of energy (Cragg, 1967; Blaustein and Chase, 2007). Protein resources

may differ among urban and natural habitats, depending on the available food types, which could cause changes in the trophic position (and therefore nitrogen signature) of an individual.  $^{15}\text{N}$  originates from inorganic nitrogen, which is absorbed by plants from soil (O'Brien, 2015). When animals consume plant material,  $^{15}\text{N}$  remains within their systems, while  $^{14}\text{N}$  is excreted (Steele and Daniel, 1978; Minagawa and Wada, 1984). Therefore, if a predator consumes a herbivorous prey, that predator would have a higher  $\delta^{15}\text{N}$  than another species that consumed less prey. Not only does this suggest that the predator is consuming more protein, but also that the predator is at a higher trophic level than other predatory species. Therefore, by considering an individual's nitrogen and carbon signatures together on a natural-urban gradient, we can gain insight into any differences in the patterns of food consumption between urban and natural diets.

### *Project Overview*

To understand the physiological consequences of living within an urban environment, I investigated: 1) whether the concentration of cortisol found in the eastern chipmunks living along an urbanization gradient reflects the amount of endocrine stress experienced by individuals, and 2) whether variation exists in isotopic signatures and fecal antioxidant levels along an urbanization gradient. By determining the level of urbanization within a chipmunk's habitat, I can gain a greater understanding of their ability to acclimatize to stressors, which may differ across an urbanization gradient (Noeman *et al.*, 2011; Isaksson, 2015). Through the use of fecal antioxidants and isotopes, I can determine if variation in diet exists along the urbanization gradient. Finally, by considering cortisol and the isotope and antioxidant levels along the urbanization gradient, we can make inferences on how urbanization can impact the well-being of a small mammal species (Crago *et al.*, 2011; Isaksson, 2015; Hutton and McGraw, 2016).

I collected fecal and hair samples, as well as morphological measurements, from eastern chipmunks in habitats with varying degrees of urbanity within Sudbury, Ontario from April to August of 2019. Chipmunks are small diurnal rodents found throughout eastern North America (Banfield, 1974). Chipmunks typically have home ranges that overlap with each other, less than 0.4 hectares in size (Blair, 1942; Elliot, 1978). They use complex burrow systems where they cache food, overwinter, and raise their pups (Blair, 1942; Elliot, 1978). Chipmunks were an ideal study species for my project because there is an abundance of information about their life history and their populations are large in both urban and natural areas of Sudbury (Smith and Smith, 1971; Tryon and Snyder, 1973; Lyons *et al.*, 2017).

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# **Chapter One**

**Variation in cortisol of eastern chipmunks (*Tamias striatus*) along a natural-urban gradient**

## **Abstract**

Cities provide generous amounts of human food waste and have fewer predatory species compared to natural environments, making them ideal habitats for some organisms. A resource-filled, low-predatory environment may cause an individual to invest less energy into stress responses, and more energy into their body condition. I investigated whether chipmunks experienced differences in cortisol, a glucocorticoid hormone, and body condition across an urbanization gradient. I predicted that chipmunks in more urban environments would have lower levels of cortisol and a better body condition, compared to chipmunks living in more natural areas because of dietary differences that may exist across habitats. I studied chipmunks across 20 locations throughout Sudbury, Ontario. After capturing a chipmunk, fecal samples and body measurements were collected to determine cortisol concentrations and body condition, respectively. I surveyed all study sites over a three-day period to determine the level of human activity to generate an urbanization gradient scores. My findings show a significant difference in cortisol along an urbanization gradient, suggesting chipmunks in more urban habitats may be experiencing higher levels of stress compared their natural counterparts. My results show that body condition was affected by urbanization, which may be related to food availability. These findings highlight the importance of using an urbanization gradient to consider the effects of consuming a human diet on a small mammal species.

## Introduction

The rapid growth of cities often requires species to survive in novel urban habitats (Palmer, 2003). Once an animal is introduced to a new urban habitat, they may encounter unfamiliar, anthropogenic elements and human activity (Palmer, 2003; Tarlow and Blumstein, 2007). These elements can range from automobile and human traffic, noise and air pollution, destruction or alterations in habitat, and changes in predators and available diet (Sauvajot, 1998; Hamer and McDonnell, 2010; Fischer *et al.*, 2014; Giraudeau *et al.* 2014). Novel urban habitats contain many unfamiliar elements; therefore, an animal may begin to experience stress. Every individual has baseline levels of glucocorticoid hormones, like cortisol, which work to maintain homeostasis (Sapolsky, 2004). Stress occurs when an individual's body strays from homeostasis, or their physiological balance (Sapolsky, 2004). In the context of urbanization, a stressor, or an element that causes a deviation from homeostasis, would likely be from human disturbance or anthropogenic elements, such as automobile or human traffic. (Sapolsky *et al.*, 2000; Sapolsky, 2004). Once an individual experiences a stressor, the hypothalamic-pituitary-adrenal (HPA) axis is activated to divert energy away from maintaining homeostasis (e.g. maintaining body temperature or glucose levels) and eventually return the body to homeostasis to maintain physiological balance (Sapolsky *et al.*, 2000; Romero and Wikelski, 2001; Partecke *et al.*, 2006). The HPA axis is a negative feedback system that works when the hypothalamus releases corticotropin-releasing hormone (CRH), then triggering the anterior pituitary to release adrenocorticotrophic hormone (ACTH), followed by the adrenal cortex releasing a glucocorticoid hormone, such as corticosterone or cortisol (Sapolsky *et al.*, 2000; Sapolsky, 2004; Kudielka and Kirschbaum, 2005). Cortisol and corticosterone increase the concentration of glucose in the blood stream (therefore increasing energy availability) and promoting the function

of muscles or accelerating physiological processes that are required to cope with an immediate stressor (Sapolsky *et al.*, 2000; Sapolsky, 2004; Kudielka and Kirschbaum, 2005). The HPA axis is a mechanism that allows organisms to cope with short-term stress; however, over the long term, it can cause negative fitness consequences. Prolonged elevation of glucocorticoid hormones may decrease immunity, reproductive success, and overall well-being (Sapolsky *et al.*, 2000; Romero and Wikelski, 2001; Carlitz *et al.*, 2016).

Many species exhibit differences in cortisol concentrations depending on whether they live in an urban or natural habitat (Atwell *et al.*, 2012; Bonier, 2012; Brearley *et al.*, 2012; Deng *et al.*, 2014). For example, previous studies on cortisol levels in urban populations of various species, such as eastern chipmunks (*Tamias striatus*; Lyons *et al.*, 2017), dark-eyed juncos (*Junco hyemalis*; Atwell *et al.*, 2012), and tree lizards (*Urosaurus ornatus*; French *et al.*, 2008) have found that they experience lower levels of cortisol, compared to their natural counterparts. These species may be adapted to their urban habitats, allowing them to survive without experiencing the negative effects of long-term elevated cortisol levels (French *et al.*, 2008; Atwell *et al.*, 2012; Lyons *et al.*, 2017). However, in the short-term, the species' natural counterparts may not be as successful in an urban environment because they have not experienced urban stressors (Partecke *et al.*, 2006; Atwell *et al.*, 2012). As a consequence, individuals living in urban and natural habitats may differ in life history traits, such as the number of offspring produced or size at birth (Boal and Mannan, 1999; Sprau *et al.*, 2016). However, cortisol concentrations along an urbanization gradient are understudied, especially in small mammal species.

As a result of perceived stress, or an increase of glucocorticoids from baseline levels, an individual may experience a decrease in body condition (Cote *et al.*, 2010). Body condition

estimates an individual's energetic reserves (Schulte-Hostedde *et al.*, 2005). Individuals undergoing a stress response may experience a decrease in body condition because they are using available energy to try to maintain homeostasis and may not be replenishing fat reserves quickly enough to maintain their condition (Sapolsky *et al.*, 2000; MacBeth *et al.*, 2012; Zwijsz-Kozica *et al.*, 2013). These stress responses mediated by the HPA axis are energetically expensive because the body is using fat stores and undergoing gluconeogenesis to mobilize available energy to survive (Boissy 1995, Kitaysky *et al.*, 1999; Marin *et al.*, 2007). For example, Cabezas *et al.* (2007) found that wild rabbits (*Oryctolagus cuniculus*) experienced a decrease in body condition when experimentally exposed to long-term stress because of an increase in glucocorticoid blood levels. In the context of urbanization, however, little is known about how body condition may change over an urbanization gradient, and if this change may be correlated to cortisol.

Studies that have examined the effects of urbanization on glucocorticoids, body condition, and other physiological parameters (with the exception of few) have categorically defined urbanization as either urban or natural (Sears, 1989; Iglesias-Carrasco *et al.*, 2017; Lyons *et al.*, 2017; Shimamoto *et al.*, 2019; but see Meillère *et al.*, 2016; Price *et al.*, 2018). Conceptualizing habitat as either “urban” or “natural” is common in urban ecology but may not be the best approach. Rather, urbanization is a gradient, ranging from urban parks to the concrete and asphalt of buildings and roads. For example, Price *et al.* (2018) generated an urbanization gradient (referred to as rural-urban score) to examine their cortisol results in yellow-bellied marmots (*Marmota flaviventris*) and measured various anthropogenic variables to determine how urban or natural their study locations were. Some of the variables they accounted for were how many people, dogs, cars, and bikes that passed by each location every hour. However, this study

did not consider other important variables that could separate an urban habitat from a natural habitat, such as the amount of trash cans or food wrappers in a given area, or access to bird feeders. Defining and measuring all variables that may distinguish a more urban area from a more natural habitat could yield more accurate interpretations related to the physiological impacts an urban environment may have on a given species.

Anthropogenic stressors should be more evident in habitats that are more urban along an urban-natural gradient. Similarly, human food waste upon which chipmunks may feed should be more prevalent in more urban habitats. Thus, I predicted that chipmunks in more urban habitats would exhibit lower cortisol levels and greater energetic reserves (reflected by body condition) compared to chipmunks living in more natural habitats. In urban habitats, chipmunks may be consuming readily available and abundant human food waste, whereas chipmunks in natural habitats have to exert greater amounts of energy to forage for food (Matsuzawa-Nagata *et al.*, 2008; Lyons *et al.*, 2017). If chipmunks are consuming plenty of food and experiencing low levels of glucocorticoids, they may have more energy to invest into body condition in urban areas (Lyons *et al.*, 2017).

## Methods

### *Study Sites*

Adult eastern chipmunks were sampled from early April until late August of 2019 throughout Sudbury, Ontario, Canada (46.4917°N, 80.9930°W). A total of 20 study sites were sampled including four locations within a conservation area (Lake Laurentian), a single location on an extensive trail system (Kelly Lake Trail), three locations within a public park (Bell Park), four locations at Laurentian University, and eight backyards located throughout Sudbury (Appendix A). Sherman traps (H.B. Sherman Inc., Tallahassee, Florida) were set at all 20 locations, near areas with dense vegetation cover, and in areas where chipmunk burrows were found. Approximately 80-100 traps (10 traps per site, on average 2m apart from each other) were baited with peanut butter and oats every day at 07:00 and checked every two hours until 16:00. Traps were set at each location twice a week. If a chipmunk was caught and processed before the end of the day, the trap was baited again and reset.

### *Trapping and Sample Collection*

Upon capture, chipmunks were transferred into a handling bag and the reproductive state and age were assessed. Males were classified as non-reproductive or reproductive (scrotal), while females were classified as non-reproductive or reproductive (either lactating or pregnant). Age categories were determined by mass, such that a chipmunk under 80g at the time of the initial capture was considered a juvenile, while over 80g was considered an adult (Patterson and Schulte-Hostedde 2011). Each chipmunk was weighed using a Pesola® scale ( $\pm 0.1$ g), and morphometric measurements were obtained using a ruler ( $\pm 1$ mm) and caliper ( $\pm 0.1$ mm). The morphometric

data that was collected included skull length and width, hind foot length, and body mass (Schulte-Hostedde and Millar, 2000; Lyons *et al.*, 2017).

At initial capture, each chipmunk was given a metal ear tag with a unique number sequence (National Band and Tag Company, Newport, Kentucky), allowing individual identification. Fecal samples were collected by removing feces from traps or collecting them directly from the individual during handling. Following the protocol of Palme *et al.* (2005), the fecal samples were placed in 3 mL centrifuge tubes with 1 mL of 80% methanol per 0.1g of fresh feces to preserve the sample for cortisol analysis. The samples were refrigerated at -20°C until they were used for analysis.

#### *Hormone Extraction*

The stored fecal samples were transferred from 2 ml polypropylene tubes into glass tubes. One millilitre of 100% methanol was used to rinse the polypropylene tube and the remainder was pipetted into the glass tube. All samples were left to evaporate under a fume hood for approximately 48 hours.

Once the methanol was evaporated and the extract was in the glass tube, the samples were analyzed for cortisol concentrations using the methods completed by Majchrzak *et al.* (2015) and Terwissen *et al.* (2013). However, the cortisol antiserum and horseradish peroxidase conjugate in our analysis was diluted to 1:10 000 and 1:33 500, respectively. Similarly to Majchrzak *et al.* (2015), cortisol standards were used if they were in the range of 78-20,000 pg/ml.

Each plate was coated with antiserum and left overnight to cool at 4°C in a fridge. The following day, the plates were washed and loaded with the standards, samples, controls, and

horseradish peroxidase conjugate. The plate was then left to sit for two hours at room temperature and 200µl substrate solution was subsequently added. Using a spectrophotometer (MRX microplate reader, Dynex Technologies, Chantilly, VA), absorbance was measured at 405 nm.

#### *Natural-Urban Score (NUS) Data Collection*

Using the methods outlined in Price *et al.* (2018), I completed surveys and used land cover data to generate a score that represented how urban or natural each study site was (the natural-urban score - NUS). While conducting surveys, I monitored the number of people, dogs, cars, and bikes that passed by all study sites in an hour, the number of trash pieces found within a 100m radius of the trapline, the number of trash cans found within a 100m radius of the trapline, and the number of bird feeders found within a 100m radius of the trapline. Trash was defined as any item on the ground that could be held in a trash can, ranging from wrappers and recyclables to peanut shells. Trash cans were defined as any bin that could hold food waste, such as typical garbage bins or compost bins. For locations near busy major roads and highways, such as Bell Park and Kelly Lake Trail, an average number of cars per hour was determined from traffic data supplied from the Ontario Ministry of Transportation to the City of Greater Sudbury (Greater Sudbury Statistics, 2017). All variables were collected from 07:00-13:00 over a three-day period at each study site. After the three-day observation period concluded, averages of all measured variables were determined, which generated a single number for each variable measured per location (Price *et al.*, 2018).

I determined the type of land cover found at all study sites within a 100 m radius using the 2015 Land Cover of Canada map (Latifovic *et al.*, 2017; Price *et al.*, 2018; Latifovic, 2019).

The 100m radius ensured that land cover was consistently and equally measured across study sites. The categories of land cover found at various study sites included: sub-polar taiga needleleaf forest, temperate or sub-polar shrubland, temperate or sub-polar broadleaf deciduous forest, mixed forest, barren land, and urban sites (Appendix B).

### *Population Size and Density*

In order to determine chipmunk population size at each study site, the Lincoln-Peterson Index was used because chipmunks were captured, marked, and had the opportunity to be recaptured (Lancia *et al.*, 1994; Tenzin *et al.*, 2015). The formula is  $N = \frac{MS}{R}$ , where N represents the population size estimate, M is the number of marked chipmunks released, S is the size of the next sample, and R is the number of marked chipmunks recaptured (Lancia *et al.*, 1994; Tenzin *et al.*, 2015). Next, the area of each trap site (in m<sup>2</sup>) was calculated using Google Maps (2020) using the minimum convex polygon method (Burgman and Fox, 2002). Then, the population size estimate was divided by the area of the trapline to determine population density (chipmunks per m<sup>2</sup>; Appendix C).

### *Statistical Analysis of Cortisol Concentrations, Body Condition, and NUS*

All statistical analyses were performed using R (version 1.2.1335, R Core Team, 2019). First, the land cover data, as well as surveyed observations were combined in a base R principal component analysis (PCA) that generated a natural-urban score (PC1 and PC2) for each site. Next, I used the average skull length, skull width, and hind foot length of each individual to determine body size, and each length and width was log-transformed for normality (Schulte-Hostedde *et al.*, 2005; Lyons *et al.*, 2017). A PCA was performed to determine if all lengths and

widths were positively related in the same direction, and then the PC1 scores were extracted. Log-transformed values for body mass and PC1 scores were inputted into a simple linear regression and the residuals were extracted to represent the body condition score of each individual (Fig. 1.2; Schulte-Hostedde *et al.*, 2005; Lyons *et al.*, 2017). Lastly, cortisol concentrations were log-transformed to meet normality assumptions.

I selected models to investigate based on their ecological relevance to answer my central questions. Once the models of best fit were determined for both cortisol concentrations and body condition as response variables, they were examined further by performing generalized linear models using base R. Lastly, both models that were selected were plotted using ggplot2 (Wickham, 2016 [v3.2.1]).

## Results

### *Sample Size*

I trapped and sampled a total of 59 chipmunks - 41 females and 18 males. Of the 59 chipmunks sampled, 32 chipmunks were from backyards, 16 chipmunks were from Bell Park, 5 chipmunks were from Lake Laurentian Conservation Area, 2 chipmunks were from Kelly Lake Trail, and 4 chipmunks were from Laurentian University.

### *Natural-Urban Score (NUS) PCA*

PC1 explained 44.9% of the variation within the surveying data and land cover map data (Appendix D), while PC2 explained 20.3% of the variation (Appendix D). PC1 tended to distinguish the degree of urbanity among locations (e.g. a more positive factor represents a variable associated with a more urban area, while a more negative factor represents a variable associated with a natural area), while PC2 tended to discriminate food resources from non-food resources (Appendix D). The average number of people per hour was the factor had the strongest relationship to PC1 (0.423; Appendix D), while vegetation cover related the least to PC1 (-0.297; Appendix D). The average number of trash cans found within a 100m radius of the trapline had the strongest relationship to PC2 (0.521, Appendix D), while the average number of bikes per hour had the weakest relationship to PC2 (-0.478; Appendix D). Because PC1 had the greatest variation and distinguished the degree of urbanity between variables, it was used to determine as the NUS for each site.

### *Body Condition Residuals*

PC1 explained 44.7% in the body condition variation, and all loadings were positive (skull length =0.653, skull width = 0.674, and hind foot length 0.345). The linear regression of log-transformed body mass and PC1 scores displayed a positive relationship, thus all residuals could be extracted to represent body condition in subsequent analyses (Figure 1.1).

#### *Body Condition Generalized Linear Model*

Body condition was significantly affected by NUS, PC2 and the interaction between NUS and PC2 ( $t=-2.238$ ,  $p=0.02$ ; Table 1.1; Figure 1.2). Additionally, body condition was significantly affected by reproductive condition ( $t=2.23$ ,  $p=0.02$ , Table 1.1). However, body condition was not significantly affected by  $\log_{10}$ (cortisol;  $t=0.048$ ,  $p=0.633$ , Table 1.1) or  $\log_{10}$ (cortisol), NUS, or the interaction of NUS: $\log_{10}$ (cortisol;  $t=-0.836$ ,  $p=0.407$ , Table 1.1). The interactions between NUS, PC2, and population density were excluded from analysis because there was no significant relationship amongst those variables.

#### *Cortisol Generalized Linear Model*

Cortisol was significantly affected by NUS ( $t=2.23$ ,  $p=0.02$ ; Table 1.4; Figure 1.3). In other words, chipmunks captured in more urban habitats had elevated fecal cortisol compared to chipmunks from more natural habitats. However, it should be noted that the adjusted  $r^2$  was 0.06, suggesting there was unexplained variance. Additionally, cortisol was not affected by the interaction of NUS and PC2 ( $t=-0.732$ ,  $p=0.467$ , Table 1.2), population density ( $t=0.529$ ,  $p=0.599$ , Table 1.2), or PC2 ( $t=-0.381$ ,  $p=0.705$ , Table 1.2). The interactions between NUS, PC2, and population density were excluded from analysis because there was no significant relationship amongst those variables.



## Discussion

I found a significant effect of the urbanization gradient on cortisol concentrations from chipmunks across the urbanization gradient, such that chipmunks from the most urban areas exhibited the highest levels of cortisol. However, I observed no significant effect of the urbanization gradient on the body condition of chipmunks. These results do not support my prediction that chipmunks may experience decreased levels of cortisol and a better body condition in more urban habitats, relative to their counterparts in more natural habitats.

Body condition was poorer in chipmunks from more urban areas relative to more natural environments. At the same time, PC2 seemed to be lowest in the most urban and most natural study sites, while PC2 scores were highest in intermediate levels of urbanization. Since PC2 differentiated food sources (people, trash cans, and bird feeders) from non-food sources (e.g. dogs, cars etc.), it is possible that chipmunks may experience poorer body condition because of their interactions with cars and bikes (which could be stressful), rather than the access to food waste. Body condition also shared a positive relationship to reproductive condition. Prior research has shown that glucocorticoids, like cortisol, increase energy uptake (Kenagy and Place, 2000; Marin *et al.*, 2007). Therefore, I expected that chipmunks that had high cortisol would have a decrease in body condition because of an increased use of energy for processes such as gluconeogenesis (Boissy 1995, Kitaysky *et al.*, 1999; Marin *et al.*, 2007). It is possible that chipmunks in urban habitats may have access to enough food resources to maintain body condition, regardless of cortisol concentrations (Kitaysky *et al.*, 1999, Bonier *et al.*, 2007). For example, a study examining the diet of herring gulls (*Larus argentatus*) found that the diet was in urban areas was of poorer quality than their natural diet, but had a higher caloric value, protein content, and fat content (Pierotti and Annett, 1990; Pierotti and Annett, 1991). Like herring gulls,

chipmunks may be consuming a diet that allows them to maintain body condition, despite experiencing higher levels of cortisol than their natural counterparts.

These results contradict previous findings, in which chipmunks in urban habitats had lower cortisol levels than those in natural habitats, and in which females had elevated body condition in urban habitats (Lyons *et al.*, 2017). I quantified the level of urbanity at each study site, while Lyons *et al.* (2017) categorically defined an urban versus natural habitat. It is possible that because my study accounted for the degree of urbanization and possible sources of food (e.g. trash pieces and garbage bins), I observed different results in both cortisol and body condition. Additionally, Lyons *et al.* (2017) sampled chipmunks from two cities, while my study was conducted in a single city. Chipmunks in Sudbury may not experience a large urbanization gradient that leads to differences in body condition scores, especially in females. Despite Sudbury having many forested areas, by not including natural habitats like Algonquin Park, or comparative urban habitats outside of Sudbury, like Huntsville, ON as was included by Lyons *et al.* (2017), the gradient I generated may be more constrained. In other words, all chipmunks were found within Sudbury city limits, and the interpretation of the results is limited to the relative degree of urbanization within the city. This highlights the importance of considering a gradient relative to multiple cities to interpret results because other cities may differ in the level of urbanity, and therefore show different results not only from Lyons *et al.* (2017) study, but my results as well. However, future research is needed to determine whether using a gradient and considering areas beyond city limits would have influenced these results.

Chipmunks experience higher levels of cortisol in urban sites; therefore it is possible that urban habitats may be considered stressful by chipmunks, compared to their natural habitats. By comparing Lyons *et al.* (2017) cortisol concentrations to my values, it appears that chipmunks in

the most urban areas of Sudbury experience higher levels of cortisol than those in the urban areas in the prior study. Although, it is clear that cortisol was affected by NUS, there was still some unexplained variation within the model. Based on the natural-urban score, it appears that the variables representing people, cars, bikes, trash pieces, and dogs were the factors that loaded heaviest on PC1, perhaps explaining why chipmunks may experience an increase in cortisol. Human presence and activity have been shown to increase stress levels in many species, such as wildcats (*Felis silvestris*; Pinerio *et al.*, 2012), swamp rats (*Scapteromys aquaticus*) and Azara's grass mice (*Akodon azarae*; Gomez-Villafane *et al.*, 2012) as well as woodland caribou (*Rangifer tarandus*; Ewacha *et al.*, 2017). Young marmots may perceive dogs as predators in urban habitats based on their fecal glucocorticoid results (Price *et al.*, 2018). For this reason, it is not surprising that chipmunks experience an increase in cortisol levels near the most urban area within the gradient, as these areas would be expected to have the most human and dog presence.

There are other factors that could influence cortisol levels in an urban setting including disease or social interaction amongst chipmunks (Reperant *et al.*, 2009; Morand *et al.*, 2015; Haigh *et al.*, 2017). Because urban environments may have plenty of food resources and shelter, there may be more chipmunks present in those areas. If larger chipmunk populations are present in urban habitats, there is a chance their home ranges could overlap, increasing contact or social interaction between individuals. Disease rates amongst rodents increases in urban habitats because rodent populations are in high densities, allowing disease to spread easily between individuals (Bradley and Altizer, 2006; Himsworth *et al.*, 2013). Although I did not examine parasite loads or diseases, future studies should investigate whether parasitism or infectious disease affect cortisol concentrations in urban populations. Additionally, high social interactions amongst rodents have been linked to increased glucocorticoids (Wolff and Sherman, 2007;

Schoepf and Schradin, 2013; Haigh *et al.*, 2017). Haigh *et al.* (2017) found that human traffic was not linked to increased levels of cortisol in red squirrels (*Sciurus vulgaris*) as predicted; rather the interactions amongst squirrels themselves were responsible for the increase in cortisol. However, I found no effect of population density on cortisol, which may be because of the study sites that were chosen. Population density may not have varied among my sites, or it is possible that I was not able to not re-capture or capture enough chipmunks to get a true estimation of the population density at each site.

### *Conclusions*

My results suggest that higher levels of cortisol are found in eastern chipmunks inhabiting areas with a high urban score suggesting greater levels of stress are perceived than their natural conspecifics. Chipmunks in urban habitats may experience higher stress levels due to increased human disturbance and presence of dogs. My study shows that despite the higher levels of cortisol in chipmunks from more urban areas, they do not differ in body condition along the urbanization gradient. Rather, they experience changes in body condition depending on the access to food waste. Furthermore, the slight difference in body condition only depends on reproductive condition, suggesting reproductively active individuals have more energetic reserves than those are non-reproductive. These findings highlight the importance of considering the degree of urbanization in a particular area to better understand how animals are responding to the presence of anthropogenic elements within their habitats, since urbanization is impacting wildlife globally.

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## Tables and Figures

**Table 1.1.** Generalized linear models examining body condition for eastern chipmunks (*Tamias striatus*; n=59) in response to NUS, PC2, cortisol, sex, and reproductive condition in Sudbury, Ontario.

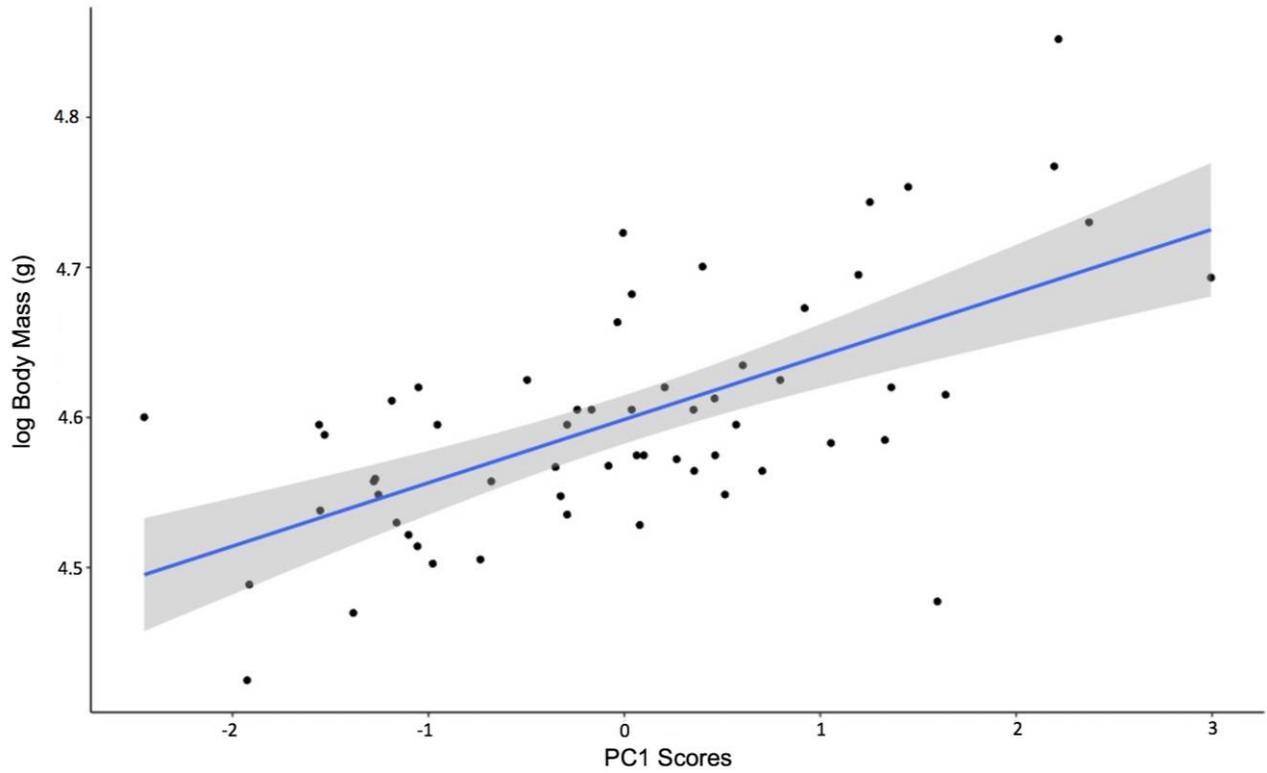
Model	Estimate	Standard Error	t - value	p-value	Multiple R <sup>2</sup>	Adjusted R <sup>2</sup>
Body condition ~ NUS + PC2 + NUS:PC2	Intercept: -0.0007 NUS: -0.0105 PC2: 0.0265 NUS:PC2: -0.0102	Intercept: 0.0084 NUS: 0.0049 PC2: 0.0107 NUS:PC2: 0.0045	Intercept: -0.088 NUS: -2.121 PC2: 2.458 NUS:PC2: -2.238	Intercept: 0.9303 <b>NUS: 0.0384</b> <b>PC2: 0.0171</b> <b>NUS:PC2: 0.0293</b>	0.1254	0.0771
Body condition ~ NUS	Intercept: 0.0034 NUS: -0.0054	Intercept: 0.0085 NUS:0.0041	Intercept:0.408 NUS: -1.297	Intercept: 0.685 NUS: 0.200	0.0286	0.0116
Body condition ~ log <sub>10</sub> (Cortisol)	Intercept: -0.0226 log <sub>10</sub> (Cortisol):0.0037	Intercept: 0.0466 log <sub>10</sub> (Cortisol):0.0078	Intercept: -0.484 log <sub>10</sub> (Cortisol):0.480	Intercept: 0.630 log <sub>10</sub> (Cortisol):0.633	0.0040	-0.0134
Body condition ~ Sex + Reproductive condition + Sex:Reproductive condition	Intercept: -0.0199 Sex: 0.0220 Reproductive condition: 0.0424 Sex:Reproductive. Condition: -0.0443	Intercept: 0.0126 Sex: 0.-237 Reproductive condition: 0.0190 Sex:Reproductive. Condition: 0.0342	Intercept: -1.582 Sex: 0.929 Reproductive condition: 2.231 Sex:Reproductive. Condition: -1.295	Intercept: 0.119 Sex: 0.357 <b>Reproductive condition: 0.029</b> Sex:Reproductive. Condition: 0.200	0.0833	0.0333
Body condition ~ log <sub>10</sub> (Cortisol) + NUS + log <sub>10</sub> (Cortisol):NUS	Intercept: -0.0462 log <sub>10</sub> (Cortisol): 0.0089 NUS: 0.0149 log <sub>10</sub> (Cortisol):NUS: -0.0036	Intercept: 0.0485 log <sub>10</sub> (Cortisol): 0.0083 NUS: 0.0260 log <sub>10</sub> (Cortisol):NUS: 0.0043	Intercept: -0.955 log <sub>10</sub> (Cortisol): 1.070 NUS: 0.573 log <sub>10</sub> (Cortisol):NUS: -0.836	Intercept: 0.344 log <sub>10</sub> (Cortisol):0.290 NUS: 0.569 log <sub>10</sub> (Cortisol):NUS: 0.407	0.0549	0.0025

\*NUS, natural-urban score representing the values used to generate the urbanization gradient.

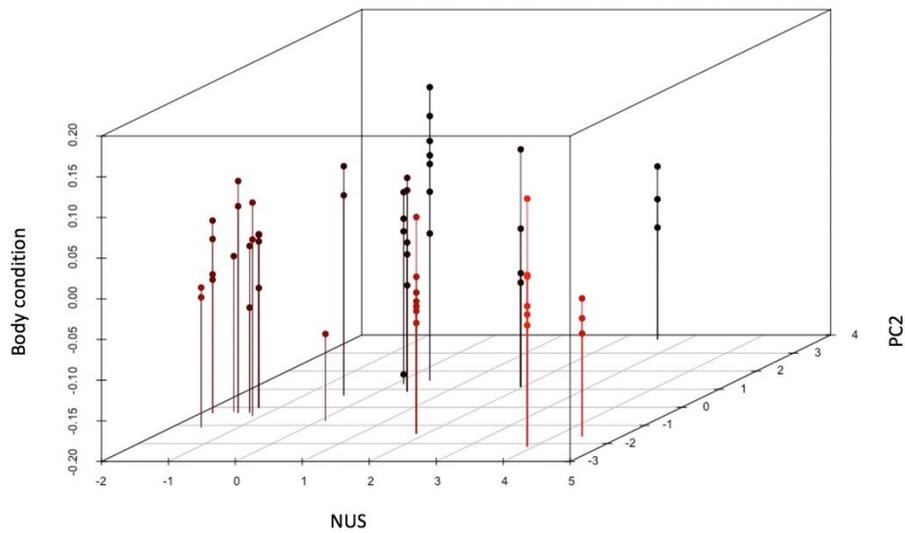
**Table 1.2.** Generalized linear model examining log-transformed cortisol concentrations for eastern chipmunks (*Tamias striatus*; n=59) in response to NUS, PC2, and population density in Sudbury, Ontario.

Model	Estimate	Standard Error	t - value	p-value	Multiple R <sup>2</sup>	Adjusted R <sup>2</sup>
log <sub>10</sub> (Cortisol) ~ NUS	Intercept: 5.769 NUS: 0.1537	Intercept: 0.1405 NUS: 0.0687	Intercept: 41.056 NUS: 2.237	<b>Intercept: &lt;0.01</b> <b>NUS: 0.0292</b>	0.080	0.064
log <sub>10</sub> (Cortisol) ~ NUS + PC2 + NUS:PC2	Intercept: 5.749 NUS: 0.1210 PC2: 0.13122 NUS:PC2: -0.0576	Intercept: 0.1455 NUS: 0.0861 PC2: 0.1862 NUS:PC2: 0.0787	Intercept: 39.505 NUS: 1.405 PC2: 0.705 NUS:PC2: -0.732	<b>Intercept: &lt;0.01</b> NUS: 0.166 PC2: 0.484 NUS:PC2: 0.467	0.0286	0.0116
log <sub>10</sub> (Cortisol) ~ Population density	Intercept: 6.0038 Population density: -6.230	Intercept: 0.2642 Population density: 11.788	Intercept: 22.721 Population density: -0.529	<b>Intercept: &lt;0.01</b> Population density: 0.599	0.0048	-0.0125
log <sub>10</sub> (Cortisol) ~ PC2	Intercept: 5.886 PC2: -0.0350	Intercept: 0.1364 PC2: 0.0919	Intercept: 43.140 PC2: -0.381	<b>Intercept: &lt;0.01</b> PC2: 0.705	0.0025	-0.0149

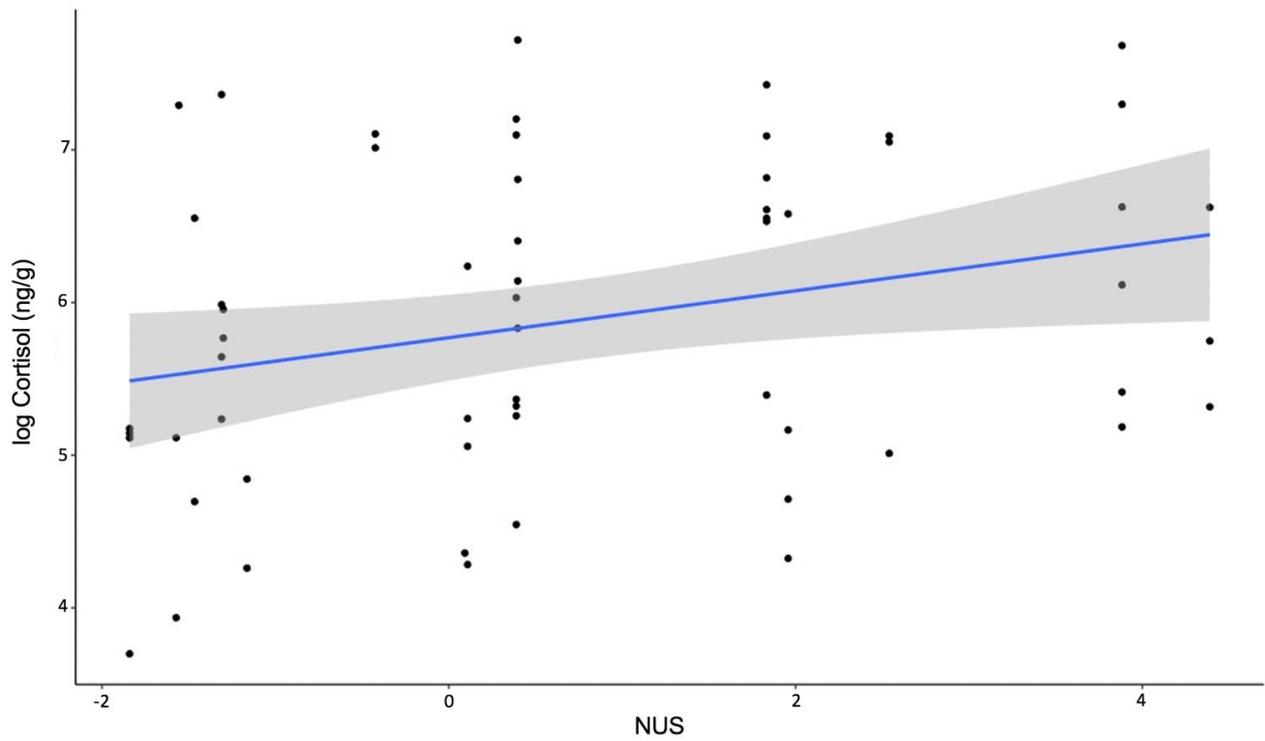
\*NUS, natural-urban score representing the values used to generate the urbanization gradient.



**Figure 1.1.** Linear regression of PC1 and log-transformed body mass used to determine the residuals which represents body condition scores for eastern chipmunks (*Tamias striatus*;  $p < 0.01$ , adjusted  $r^2 = 0.59$ ,  $n = 59$ ). Positive residuals indicate good body condition and negative residuals indicate poor body condition. The shaded area denotes a 95% confidence interval.



**Figure 1.2.** 3D scatterplot of body condition scores from eastern chipmunks (*Tamias striatus*) over the natural-urban score (NUS) in relation to PC2 ( $p=0.01$ ,  $r^2=0.07$ ,  $n=59$ ). On the x axis, positive values indicate more urban areas and negative values indicate more natural areas. Each grey circle reflects PC2 scores indicating the access to sources of food waste, such as people, trash cans, and bird feeders. Positive PC2 values indicate the greatest access to sources of food waste, while negative PC2 values indicate the least access to sources of food waste. Brightest red indicates the highest values, while darkest red indicates the lowest values amongst PC2, NUS and body condition.



**Figure 1.3.** Linear regression of log-transformed cortisol concentrations from eastern chipmunks (*Tamias striatus*) over the natural-urban score (NUS;  $p=0.02$ ,  $r^2=0.06$ ,  $n=59$ ). On the x axis, positive values indicate more urban areas and negative values indicate more natural areas. The shaded area denotes a 95% confidence interval.

## **Chapter Two**

**Dietary analysis of eastern chipmunks (*Tamias striatus*) on a natural-urban gradient**

## Abstract

Urban habitats provide wildlife with reliable access to human food waste that is vastly different in nutrient content compared to natural food resources. Human food subsidies may influence the physiology of eastern chipmunks (*Tamias striatus*) living in urban habitats because a diet in an urban habitat is atypical. I tested whether chipmunks in urban habitats were consuming more corn-based foods, high-protein foods, and antioxidant-rich foods than their natural counterparts by studying  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures in hair and feces, respectively. If chipmunks are consuming a human diet in urban habitats, then these individuals are expected to have higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures, and excrete more antioxidants compared to their natural counterparts because of the increased access to human food waste. I collected hair and fecal samples from individual chipmunks across 20 different locations throughout Sudbury, Ontario that varied in degree of urbanization. To quantify urbanization, we surveyed all study sites over a three-day period to determine the level of human activity. Chipmunks from more urban habitats had higher  $\delta^{15}\text{N}$  signatures than those from more natural habitats, indicating that chipmunks from urban habitats are consuming a diet higher in protein compared to their natural counterparts. There was no effect of urbanization on  $\delta^{13}\text{C}$  signatures, and thus there was no significant difference in the consumption of corn-based foods among the gradient. Additionally, sex differences were found in both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . We also found no difference in fecal antioxidants across the urbanization gradient. As a consequence, chipmunks in urban environments may be at a higher risk for metabolic diseases associated with protein-rich diet because they are consuming human foods. My results highlight the importance of considering the degree of urbanization of a given area to account for dietary differences amongst habitats, which may be useful for understanding how diets of other species may be impacted by urbanization.

## **Introduction**

### *Urbanization and Human Food Waste*

Urbanization, or the rapid growth of cities, provides many unique opportunities for urban-dwelling animals to consume human food waste (Srivastava, 2009; Newsome and van Eeden, 2017). Trash cans and compost bins account for large sources for food waste, while bird feeders, gardens, and direct feeding by humans provide additional opportunities for consumption of food waste (Orams, 2002; Osterback *et al.*, 2015; Tryjanowski *et al.*, 2015). By consuming human food waste, animals living in urban areas may experience physiological consequences associated with consuming a diet that is unnatural (Riley *et al.*, 2003; Lyons *et al.*, 2017; Schulte-Hostedde *et al.*, 2018). Human food waste often consists of corn (*Zea mays*) or corn by-products such as corn syrup or corn starch (Newsome *et al.*, 2010; Parker *et al.*, 2010). Common foods, such as dairy (e.g. yogurt) and wheat (e.g. bread) products all contain high levels of corn syrup (Keating and White, 1990; Parker *et al.*, 2010). Not only do humans consume foods with high levels of corn products, humans also provide corn-based meals to livestock, allowing carbon to assimilate into meat products (Jahren and Kraft, 2008; Newsome *et al.*, 2010).

### *Human Food Waste and Body Condition*

Body condition reflects an animal's energetic state and is calculated as individual body mass after correcting for structural size (Schulte-Hostedde *et al.*, 2005). Because food availability and quality may vary between individuals from urban and natural habitats, individual body condition may be influenced by diet (Pothaven *et al.*, 2001; Haug *et al.*, 2002; Sanchez *et al.*, 2008). An individual in better body condition has greater energetic reserves relative to an individual in poor body condition (Millar and Hickling, 1990; Schulte-Hostedde *et al.*, 2005). For

example, Lyons *et al.* (2017) found that female eastern chipmunks (*Tamias striatus*) in urban habitats were in better body condition than their conspecifics in natural habitats, perhaps because urban chipmunks had access to human food waste. Human food waste is readily available in urban habitats, allowing urban-dwelling wildlife to maintain a better body condition relative to their natural counterparts because of the quality and consistent availability of human food (Eley *et al.*, 1989; Tauler-Ametlller *et al.*, 2019). For example, some studies have found that many urban populations of animals have experienced an increase in body condition relative to their natural counterparts, such as palmate newts (*L. helveticus*; Igelias-Carrasco *et al.*, 2017) yellow-legged gulls (*Larus michaehellis*; Steigerwald *et al.*, 2015), and olive baboons (*Papio anubis*; Eley *et al.* 1989) likely because of the access to human food waste. However, the effects of specific dietary differences on body condition between urban and natural habitats are understudied.

#### *Oxidative Stress and Antioxidants in Food Waste*

Oxidative stress occurs when there is a high number of free radicals present in a cell (Southorn and Powis, 1988; Sies, 1997; Halliwell and Gutteridge, 2002; Mittler, 2002). Free radicals are unstable molecules with an unstable electron in their outer orbital that bind to other molecules (e.g. proteins, enzymes, DNA) to become stable (Southorn and Powis, 1988; Halliwell and Gutteridge, 2002; Mittler, 2002). Once free radicals bind to another molecule, they can alter or inhibit the molecule's function, which can lead to an increased risk of diseases like diabetes, obesity, and cancer (Yakes and VanHouten, 1997; Valko *et al.*, 2005; Ott *et al.*, 2007; Schieber and Chandel, 2014). Antioxidants work by binding to free radicals and preventing them from binding to other molecules, therefore preventing oxidative stress (Böhm, 1997; Surai, 2002).

Two types of antioxidants are responsible for preventing oxidative stress: endogenous and exogenous antioxidants. Endogenous antioxidants are produced by the body (e.g. glutathione or coenzyme Q10; Hoppe *et al.*, 1999; Marí *et al.*, 2009), whereas exogenous antioxidants are consumed through diet (e.g. vitamin A or E; Böhm, 1997; Surai, 2002).

Urban habitats are likely to provide a diet that contains different nutrients than a natural diet, as human food waste typically consists of corn by-products, high fats, and fruits and vegetables (Beckmann and Lackey, 2008; Bateman and Fleming, 2011). Thus, chipmunks and many other species may be at a lesser risk for oxidative stress in urban habitats, as exogenous antioxidants may be more readily available than in natural habitats because of the access to compost bins (e.g. vegetables and fruits; Bouayed and Bohn, 2010; Guerra-Araiza *et al.*, 2013). Prior research has shown that exogenous antioxidants may play a larger role than endogenous antioxidants in limiting free radicals within cells or reducing the risk of oxidative stress (Magwere *et al.*, 2006; Bouayed and Bohn, 2010; Haung *et al.*, 2013). For example, Bloomer *et al.* (2010) found that consumption of foods high in fats result in increased oxidative stress, which can be reduced by consuming exogenous antioxidants. However, it remains to be investigated if individuals are consuming different amounts of exogenous antioxidants in urban habitats compared to natural ones. If individuals are consuming more exogenous antioxidants in more urban habitats, then individuals may be at a decreased risk for oxidative stress (Valko *et al.*, 2006; Huang *et al.*, 2015; Ullah *et al.*, 2016).

Exogenous antioxidants have been detected within fecal matter, suggesting that: 1) antioxidants may limit oxidative stress within the intestine, and 2) antioxidants can be used as a marker for gut contents and provide insight into diet (Garsetti *et al.*, 2000; Halliwell *et al.*, 2000; Wu *et al.*, 2006; Bianchi *et al.*, 2010). Prior research has found that mice (*Mus musculus*) and

rats (*Rattus norvegicus*) that had consumed foods rich in flavonoids and polyphenols – exogenous antioxidants – had less intestinal free radicals than conspecifics that did not consume such foods (Zhao *et al.*, 2005; Zhao and Shah, 2016). Because of the differences in available food between urban and natural habitats, chipmunks may have access to more phenols and decrease the risk of free radicals in their guts in urban habitats. Therefore, it is possible that urban animals that consume more fruits or vegetables may pass more antioxidants through their intestine, ultimately excreting more antioxidants in their feces. This would suggest that chipmunks inhabiting urban environments could have lower oxidative stress in their intestinal tracts than their urban counterparts and antioxidants found in the feces could be indicative of variation in diet.

#### *Isotopes ( $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ) as Markers of Diet*

Urban diets may vary in quality and quantity relative to natural diets, and so carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope signatures may also vary (Newsome *et al.*, 2010; Merkle *et al.*, 2011).  $\delta^{13}\text{C}$  is derived from the ratio of  $^{12}\text{C}$  and  $^{13}\text{C}$  (Tykot, 2004; Brugnoli and Farquhar, 2000).  $^{12}\text{C}$  has one neutron less than  $^{13}\text{C}$  (Smith, 1972; Kelly, 2000). Fractionation, or the change of an isotope ratio because of a chemical or biological process, occurs between  $^{12}\text{C}$  and  $^{13}\text{C}$  resulting in differences in  $\delta^{13}\text{C}$  amongst individuals (Criss, 1999; Hoefs, 2009). Corn uses the  $\text{C}_4$  photosynthesis pathway which results in a high  $^{13}\text{C}$  signature because of fractionation that occurs during  $\text{C}_4$  photosynthesis, relative to the  $\text{C}_3$  photosynthesis pathway (Franks and Farquhar, 1999). Other plant-based foods that are found in natural habitats, such as black oak acorns (*Quercus velutina*), common hackberries (*Celtis occidentalis*), and red maple samaras (*Acer rubrum*), are from  $\text{C}_3$  plants, meaning they have a low carbon signature due to the photosynthesis

pathway that they use (Koziol and Whatley, 1984; Siemann and Rogers, 2003; Newsome *et al.*, 2010). The C<sub>3</sub> photosynthesis pathway results in less <sup>13</sup>C produced because of the amount of fractionation that occurs during the pathway (Franks and Farquhar, 1999; Patterson *et al.*, 1999). It is unlikely that a C<sub>4</sub> plant like corn would exist in a natural habitat because most plants in forested areas follow the C<sub>3</sub> pathway, allowing a carbon signature to act as an indicator of how much human food waste an animal is consuming (Wullschleger *et al.*, 1993; Reich *et al.*, 1995).

$\delta^{15}\text{N}$  reflects the amount of protein consumed by an individual (Sponheimer *et al.*, 2003; Newsome *et al.*, 2010), therefore reflecting the trophic level that an individual occupies (Sponheimer *et al.*, 2003; Rakontondranary *et al.*, 2011). A trophic level refers to the position an individual occupies in a food web or chain (Marczak *et al.*, 2007; Thomsson *et al.*, 2007; Saigo *et al.*, 2015). The further the position is away from primary producers (e.g. plants), the higher the trophic level that an individual occupies (Marczak *et al.*, 2007; Thomsson *et al.*, 2007; Saigo *et al.*, 2015). In general, <sup>15</sup>N is derived from inorganic nitrogen, which is present in soil and is absorbed by plants (O'Brien, 2015). Once an animal has consumed the plants that have absorbed the inorganic nitrogen, <sup>15</sup>N remains within their systems, while <sup>14</sup>N (created by the organism) is released (Steele and Daniel, 1978; Minagawa and Wada, 1984). This is a cumulative effect, whereby the more prey a predator consumes, the higher their  $\delta^{15}\text{N}$  is expected to be (Steele and Daniel, 1978; Minagawa and Wada, 1984). In urban habitats, there may be an increase in protein and energy resources from bird feeders or meat waste, which animals often consume (Eagle and Pelton, 1983; Pierotti and Annett, 1987; Machovsky *et al.*, 2016). Although individuals may consume more protein in an urban habitat, this may not change the trophic level an individual occupies, since they are not consuming a species established at a higher trophic level (Penick *et al.*, 2015). For example, in a study examining ants (*Tetramorium* sp. E), researchers found  $\delta^{15}\text{N}$

in urban individuals did not differ from that of their natural counterparts, suggesting that ants were located in the same trophic level, even though the ants were consuming a human diet based on the  $\delta^{13}\text{C}$  signature (Penick *et al.*, 2015).

Studies that have examined dietary differences of animals between urban and natural habitats have often defined their study locations categorically, as either urban, natural, or rural areas, rather than as a gradient (Mizukami *et al.*, 2005; Newsome *et al.*, 2010; Penick *et al.*, 2015). By defining urbanization as a categorical variable, factors that could influence the urbanity of an area, such as the number of people or garbage bins found in that particular location, are not accounted for. Without distinguishing the urbanity of an area by a set of measurable criteria, there may be an inaccurate interpretation of results. For example, Price *et al.* (2018) defined their rural-natural score by counting the number of people, dogs, cars and bikes that passed by every hour. However, the Price *et al.* (2018) study did not consider the sources of human food waste (e.g. garbage cans, compost bins etc.), which could be an important factor for determining the degree of urbanization of a study site. For this reason, using an urbanization gradient to understand urban ecology is valuable.

### *Chapter Goal*

I tested the hypothesis that if urban chipmunks are eating human food then fecal antioxidants should be lower and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures in hair should be higher in chipmunks than the signatures of chipmunks living in natural areas along the gradient, because human food-based diets are likely to contain higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures and lower antioxidants compared to natural diets. A chipmunk's diet in a natural habitat is not typically high in protein from animal sources (with the exception of insects and bird eggs), as they are primarily seed

eaters (Wrazen and Svendsen, 1978; Pyare *et al.*, 1993), and there may be more protein resources available in an urban habitat due to the access of meat waste (Lavin *et al.*, 2003; Merkle *et al.*, 2011). Additionally, a chipmunk in its natural habitat may be less likely to encounter a food item high in  $^{13}\text{C}$ , as food items high in carbon come from corn-based resources, more likely to be found in an urban habitat (Jacquot and Buchanan, 1981; Tafuri *et al.*, 2009; Newsome *et al.*, 2010). Therefore, if chipmunks are found to be consuming resources high in protein and carbon, it is possible they have access to an anthropogenic diet, which could influence their body condition (Veloso *et al.*, 2003; Pollock *et al.*, 2017). Specifically, chipmunks who consume plenty of human food waste may experience an increase in energetic reserves.

## **Methods**

### *Study Sites*

Adult eastern chipmunks were sampled in 2019 from early April to late August throughout Sudbury, Ontario, Canada (46.4917°N, 80.9930°W). Chipmunks were sampled from a total of 20 study sites including: three locations within a public park (Bell Park), a single location on a trail system (Kelly Lake Trail), four locations at Laurentian University, four locations within a conservation area (Lake Laurentian) and eight front yards and backyards located throughout the city (Appendix A). Sherman traps (H.B. Sherman Inc., Tallahassee, Florida) were set specifically near areas with dense vegetation cover and where chipmunk burrows were present. Approximately 80-100 traps (10 traps per site, on average 2m apart from each other) were baited with peanut butter and oats every day at 07:00 and checked every two hours until 16:00. Traps were set at each location twice per week. If a chipmunk was caught and processed before the end of the day, the trap was baited and reset again.

### *Trapping and Sample Collection*

Following capture, chipmunks were transferred into a handling bag and their reproductive states and ages were assessed. Males were classified as non-reproductive or scrotal, while females were classified as non-reproductive or either lactating or pregnant. Age was categorically determined by mass, such that a chipmunk under 80g at the time of the initial capture was considered a juvenile, while over 80g was considered an adult (Patterson and Schulte-Hostedde 2011). Each chipmunk was weighed using a Pesola® scale ( $\pm 0.1$ g), and morphometric measurements were obtained using a ruler ( $\pm 1$ mm) and caliper ( $\pm 0.1$ mm). Morphometric data

included skull length and width, hind foot length, and body mass (Schulte-Hostedde and Millar, 2000; Lyons *et al.*, 2017).

At initial capture, each chipmunk was given a metal ear tag with a unique number sequence (National Band and Tag Company, Newport, Kentucky), allowing individual identification. Fecal samples were collected by removing feces from traps or collecting them directly from the individual when handling. Following the protocol of Palme *et al.*, (2005), the fecal samples were placed in 3 mL centrifuge tubes with 1 mL of 80% methanol per 0.1g of fresh feces to preserve the sample for antioxidant analysis. The samples were refrigerated at -20°C until they were used for analysis. Chipmunk hair was tweezed from above the base of the tail on the rump and placed in 2 mL centrifuge tubes to avoid contamination (Mosbacher *et al.*, 2016). All hair samples were stored at room temperature until analysis.

#### *$\delta^{13}C$ and $\delta^{15}N$ Analysis*

Hair samples from 47 adult chipmunks were sent to Dr. Keith Hobson at Western University (London, Ontario, Canada) for analysis of  $\delta^{13}C$  and  $\delta^{15}N$  signatures. Hair was cleaned using distilled water and an ultrasonic cleaner, and later air dried. Hair was then cut into small pieces using sterile stainless-steel scissors, and then rinsed using a 2:1 chloroform: methanol solution to remove any surface oils (Hobson *et al.*, 2000). Subsequently, 1 mg of hair was placed into precombusted tin capsules. The hair was then combusted at 1030°C in a Eurovector 3000 elemental analyser (EuroVector S.p.A, Milan, Italy).  $N_2$  and  $CO_2$  were separated chromatographically and were analyzed using an Elementar Isoprime isotope ratio mass spectrometer (Elementar, Langenselbold, Germany). Two reference materials were used to normalize the results to Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (AIR): 1)

BWBIII keratin ( $\delta^{13}\text{C} = -20.18$ ,  $\delta^{15}\text{N} = +14.31$  per mil), and 2) PRCgel ( $\delta^{13}\text{C} = -13.64$ ,  $\delta^{15}\text{N} = +5.07$  per mil).

#### *Total-Antioxidant Capacity (TAC) Analysis*

A single fecal sample was used per individual for the TAC assay (n=114 samples). Following the protocol of Salentik *et al.* (2014), 0.1g of fecal sample was added to 0.9 ml of phosphate-buffered saline and centrifuged to generate the supernatant and pellet. The supernatant was extracted with the Total Antioxidant Capacity assay kit (ab65329) from Abcam (Cambridge, United Kingdom) to determine the amount of exogenous antioxidants found within each fecal sample.

#### *Natural-Urban Score (NUS) Data Collection*

I completed surveys and used land cover data to generate a score that represented the urbanity of each site based on the methods outlined in Price *et al.* (2018). I monitored the number of people, dogs, cars, and bikes that passed by all 20 locations within an hour, the number of trash pieces found within a 100m radius of the trapline, the number of trash cans found within a 100m radius of the trapline, and the number of bird feeders found within a 100m radius of the trapline. Trash was defined as any item on the ground that could be held in a trash can, such as wrappers and recyclables to peanut shells. Trash cans were defined as any bin that could hold food waste, such as garbage or compost bins. For locations near busy major roads and highways, such as Bell Park and Kelly Lake Trail, an average number of cars per hour was determined from traffic data supplied from the Ontario Ministry of Transportation to the City of Greater Sudbury (Greater Sudbury Statistics, 2017). All variables were collected from 07:00-

13:00 over a three-day period at each study site. After the three-day period came to an end, averages of all variables were calculated, which generated a single number for each variable measured per location (Price *et al.*, 2018).

I determined the type of land cover found at all study sites within a 100m radius using the 2015 Land Cover of Canada map (Latifovic *et al.*, 2017; Price *et al.*, 2018; Latifovic, 2019). The 100m radius ensured that land cover was consistently and equally measured across study sites. The categories of land cover found at various study sites included: sub-polar taiga needleleaf forest, temperate or sub-polar shrubland, temperate or sub-polar broadleaf deciduous forest, mixed forest, barren land, and urban sites (Appendix B).

#### *Population Size and Density*

Chipmunk population size was calculated using the Lincoln-Peterson Index (Lancia *et al.*, 1994; Tenzin *et al.*, 2015). The formula is  $N = \frac{MS}{R}$ , where N represents the population size estimate, M is the number of marked chipmunks released, S is the size of the next sample, and R is the number of marked chipmunks recaptured. Subsequently, the area of each trap site (in m<sup>2</sup>) was calculated using Google Maps (2020) using the minimum convex polygon method (Burgman and Fox, 2002). Then, the population size estimate was divided by the area of the trapline to determine population density (chipmunks per m<sup>2</sup>; Appendix C).

#### *Statistical Analysis of Body Condition, Antioxidants, Isotopes, and NUS*

All statistical analyses were performed using R (version 1.2.1335, R Core Team, 2019). First, the land cover data and surveyed observations were combined in a base R principal component analysis (PCA) that generated a natural-urban score (NUS; PC1 and PC2) for each

site. To assess body condition, body size was calculated using log-transformed values of average skull length, skull width, and hind foot length of each individual in a PCA (Schulte-Hostedde *et al.*, 2005; Lyons *et al.*, 2017). Factor loadings were in a consistent direction, and so PC1 scores were used as an index of overall body size. Residuals from a simple linear regression of log-transformed body mass and on PC1 scores were used to represent individual body condition (Fig. 2.1; Schulte-Hostedde *et al.*, 2005; Lyons *et al.*, 2017). Lastly, TAC values were log-transformed to meet normality assumptions.

I selected models to investigate based on their ecological relevance to answer my central questions. Once the models of best fit were determined for both cortisol concentrations and body condition as response variables, they were examined further by performing generalized linear models using base R. Lastly, both models that were selected were plotted using ggplot2 (Wickham, 2016 [v3.2.1]).

## Results

### *Sample Size*

I trapped and sampled a total of 114 adult chipmunks - 62 females and 52 males. Of the 114 chipmunks sampled, 54 chipmunks were from backyards, 34 chipmunks were from Bell Park, 8 chipmunks were from Lake Laurentian Conservation Area, 3 chipmunks were from Kelly Lake Trail, and 15 chipmunks were from Laurentian University. A total of 47 samples were used for isotope analysis, 114 samples for body condition analysis, and 112 samples for TAC analysis.

### *Body Condition Residuals*

PC1 explained 42.1% in the variation in body size, and all loadings were found to be positive (skull length =0.710, skull width = 0.692 and hind foot length 0.129). The linear regression of log-transformed body mass and PC1 scores showed a positive relationship (Figure 2.1).

### *Natural-Urban Score (NUS) PCA*

PC1 explained 44.9% of the variation within the dataset while PC2 explained 20.3% (Appendix D; Appendix E). PC1 tends to distinguish the level of urbanity between variables, while PC2 distinguishes sources of food from non-food sources (Appendix D) The variable that provided the greatest contribution to PC1 was the average number of people per hour (0.423; Appendix D), while vegetation cover contributed the least to PC1 (-0.297; Appendix D). The average number of trash cans found within a 100m radius of the trapline had the greatest contribution to PC2 (0.521, Appendix D), while the average number of bikes per hour contributed the least to PC2 (-0.478; Appendix D). Because PC1 had the greatest variation and

distinguished the degree of urbanity between variables, it was used to determine the NUS for each site.

#### *Body Condition Generalized Linear Model*

Body condition was significantly affected by chipmunk population density ( $t=2.649$ ,  $p=0.009$ ; Table 2.1; Figure 2.2). In other words, chipmunks experienced an increase in body condition at higher population densities (Figure 2.2). However, body condition was not significantly affected by NUS ( $t=-0.009$ ,  $p = 0.921$ , Table 2.1), PC2 ( $t=1.478$ ,  $p=0.147$ , Table 2.1), TAC ( $t=-1.146$ ,  $p=0.259$ , Table 2.1),  $\delta^{13}\text{C}$  ( $t=0.094$ ,  $p=0.926$ , Table 2.1), or  $\delta^{15}\text{N}$  ( $t=0.089$ ,  $p=0.930$ , Table 2.1).

#### *$\delta^{13}\text{C}$ Generalized Linear Model*

$\delta^{13}\text{C}$  was significantly affected by sex ( $t=-2.089$ ,  $p=0.042$ ; Table 2.2; Figure 2.3). Specifically, female chipmunks had higher levels of  $\delta^{13}\text{C}$  than males (Figure 2.3). However,  $\delta^{13}\text{C}$  was not affected by NUS ( $t=0.825$ ,  $p=0.414$ , Table 2.2), PC2 ( $t=-0.411$ ,  $p=0.683$ , Table 2.2), or NUS:PC2 ( $t=-0.621$ ,  $p=0.538$ , Table 2.2). Multiple interactions between various variables such as PC2, NUS, population density, and body condition were also tested, but were not significant relationships, and therefore were not included in results.

#### *$\delta^{15}\text{N}$ Generalized Linear Model*

$\delta^{15}\text{N}$  was significantly affected by NUS ( $t=4.563$ ,  $p<0.01$ ; Table 2.3; Figure 2.4) and sex ( $t=-2.162$ ,  $p=0.03$ , Table 2.3; Figure 2.4). Particularly, both male and female chipmunks generally had higher  $\delta^{15}\text{N}$  values in more urban areas than their natural counterparts, however,

females had significantly higher  $\delta^{15}\text{N}$  than males. However,  $\delta^{15}\text{N}$  was not affected by population density ( $t=0.901$ ,  $p=0.373$ ; Table 2.3) or PC2 ( $t=0.759$ ,  $p=0.452$ , Table 2.3). Interactions between PC2, NUS, and population density were also tested, but were not significant relationships, and therefore were not included in results.

#### *TAC Generalized Linear Model*

TAC was not significantly affected by Julian date ( $t=-1.661$ ,  $p=0.099$ ; Table 2.4). Furthermore, TAC was not affected by sex ( $t=-1.51$ ,  $p=0.296$ , Table 2.4), NUS ( $t=-0.488$ , NUS = 0.655, Table 2.4), or PC2 ( $t=-0.058$ ,  $p=0.954$ , Table 2.4). Multiple interactions between various variables such as PC2, NUS, population density, and body condition were also tested, but were not significant relationships, and therefore were not included in results.

## Discussion

I initially predicted that  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , fecal antioxidants (TAC), and body condition would be affected by the urbanity of a given area, such that all variables would be higher in chipmunks living in more urban habitats, relative to more natural ones. However, I observed a significant effect of population density on body condition of chipmunks. More specifically, the body condition of chipmunks was better when population densities were higher, compared to chipmunks living in low density areas. I also found that  $\delta^{13}\text{C}$  was not affected by NUS, though it was affected by sex. Particularly, female chipmunks had higher levels of  $\delta^{13}\text{C}$  than males. I also found that  $\delta^{15}\text{N}$  was affected by both NUS and sex, such that generally, chipmunks in more urban areas consumed more animal protein compared to chipmunks in more natural areas, however females consumed significantly more protein than males. Finally, I found no difference in fecal antioxidants among the urbanization gradient. These results provide insight into the diet a chipmunk consumes along an urbanization gradient.

Surprisingly, body condition was not affected by NUS,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , or antioxidants. Rather, chipmunks had a better body condition at higher population densities compared to chipmunks living at lower population densities. Although, it is clear that body condition was affected by population density, there was still some unexplained variation within the model. Additionally, population density was not affected by NUS. Only a few studies have found body condition to be affected by population density, specifically in salamanders (*Hydromantes flavus*; Lunghi *et al.*, 2008) and hares (*Lepus europaeus*; Jennings *et al.*, 2006). Hares at high densities were larger and tended to have more fat than those at lower densities (Jennings *et al.*, 2006). The habitat with low hare population density was of low quality, thus the hares may have used more energy to survive, compared to those living in high densities in suitable habitat. It is possible that

the chipmunks experience the same cost at lower population densities, whereby chipmunks living in less populated areas also happen to be living in areas that are of low quality (e.g. have more predators, not ideal food resources), and hence, are unable to maintain their body condition compared to chipmunks in highly populated areas.

I expected that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures would both be higher in chipmunks living in more urban habitats relative to more natural habitats; however, my results did not support this prediction. Rather, females consume more  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  compared to males. However,  $\delta^{15}\text{N}$  was affected by NUS, although there was still some unexplained variation within the model.

Chipmunks generally consumed more protein in more urban habitats compared to chipmunks in more natural areas. However, there were significant differences between sexes, such that females consumed more protein-based foods than males which indicates that females forage differently than males, perhaps to pay for reproductive costs (Awkerman *et al.*, 2007; Cherel *et al.*, 2009; Goncharov *et al.*, 2015). It is possible that female chipmunks may be consuming high-protein food resources in urban habitats, such as seeds and nuts from bird feeders, meat in garbage, or from being hand-fed and may rely more heavily on those resources than corn-based food waste. Depending on the type of protein consumed, chipmunks may experience different health consequences. For example, one study found that the protein type consumed could greatly influence obesity level (Huang *et al.*, 2008). Urban-dwelling chipmunks may be consuming not only more proteins, but proteins of a greater variety, which may put them at risk for obesity or high cholesterol (Pick *et al.*, 1959; Trichopoulou *et al.*, 1992; Adam *et al.*, 2016). Future research is needed to confirm the types of proteins chipmunks are consuming in urban habitats to determine the consequences associated with protein consumption over the long-term.

Based on my results, chipmunks living in more urban habitats may consume food from a higher trophic level than their counterparts living in more natural habitats. This may be because chipmunks have access to protein resources that they do not typically have access to in their natural habitats. Chipmunks in their natural habitats typically consume protein resources from bird eggs or insects, however urban-dwelling chipmunks may gain access to meat waste from garbage (Wrazen and Svendsen, 1978; Pyare *et al.*, 1993). It is also possible chipmunks may have a greater access to bird eggs in habitats if there is a higher population density of birds in a given urban area, which may help to explain the differences observed in  $\delta^{15}\text{N}$  values. However, whether this is a true trophic level shift is questionable. It is possible that chipmunks may have access to more animal protein resources and consume them because they are available causing an increase in  $\delta^{15}\text{N}$ , rather than an actual change or switch to consuming prey at a higher trophic level. For example, in urban-dwelling silver gulls (*Chroicocephalus novaehollandiae*), Auman *et al.* (2011) found they increased in trophic rank based on  $\delta^{15}\text{N}$  values compared to their natural conspecifics. However, upon examining the their regurgitant contents, Auman *et al.* (2011) found there was no indication of a drastic change in diet to suggest the urban gulls were at a true higher trophic level than natural gulls.

Carbon and nitrogen isotopes may not provide complete insight into the human food waste chipmunks are consuming. For example, without examining the isotopic signatures of the food waste itself, I cannot determine the amount of fruit and vegetables consumed by a chipmunk (Merckle *et al.*, 2011; Carter and Chesson, 2017). Compost bins are easily accessible to chipmunks living in more urban habitats, which would be an excellent resource for fruits and vegetables. Therefore, it is possible that a chipmunk diet in an urban habitat may be comprised of fruits and vegetables in addition to protein resources (e.g. bird seed). This may explain why no

significant difference was observed for  $\delta^{13}\text{C}$  signatures among urban and natural samples of chipmunks; they may not be relying on corn-based food waste as a source of energy. This idea is consistent with Merkle *et al.* (2011), where Merkle *et al.* (2011) found no significant difference in  $\delta^{13}\text{C}$  between urban and natural American black bear (*Ursus americanus*) populations. Merkle *et al.* (2011) mentions that analyzing the contents of refuse/rubbish could have been useful to better understand what the bears were consuming. Additionally, by examining the food types consumed by chipmunks, we may be able to make inferences on the evolution of urban chipmunks. For example, Munshi-South and Harris (2017) located genes responsible for the metabolizing of carbohydrates and lipids differed from urban and natural populations of white-footed mice (*Peromyscus leucopus*), such that urban mice could potentially digest carbohydrates and lipids more efficiently than natural populations of mice. It would be worthwhile to investigate if such differences exist in eastern chipmunks to understand the consequences of a high protein diet, and if there is selection for specific genes.

Isotopes may be used as markers to determine the type of food waste consumed by an individual, which may reflect the degree of urbanity of a particular area (Newsome *et al.*, 2010; Penick *et al.*, 2015). Unlike my results, prior research in species such as ants (Penick *et al.*, 2015), kit foxes (*Vulpes macrotis mutica*; Newsome *et al.*, 2010), and silver gulls (Auman *et al.*, 2008) have found differences in  $\delta^{13}\text{C}$  signatures between individuals living in more urban versus more natural habitats. However, I did find that female chipmunks had higher  $\delta^{13}\text{C}$  values than males, suggesting that females may consume more  $\text{C}_4$ -based foods, regardless of the level of urbanity in a given habitat. It is possible that females may exhibit higher  $\delta^{13}\text{C}$  values because of the expenses of reproduction or because of differences in foraging patterns, such that females may travel further for food (Hobson *et al.*, 2000; Cherel *et al.*, 2009; Goncharov *et al.*, 2015). In

general however, my findings contribute to this growing literature that uses isotope differences to better understand the types of human food waste consumed by animals. Based on my results, isotopes may differ depending on species and location. For instance, I observed no differences in carbon signatures across individuals along the urbanization gradient, which could be exemplified by the types of food waste available depending on the NUS. In other words, it is possible that chipmunks may actually be consuming more corn-based resources in more urban areas, however because the difference among the gradient in Sudbury is not strong enough, I was not able to find a significant effect of urbanity. If the study was replicated in another city that was more urban than Sudbury, or perhaps if more urban study sites were added, the carbon signatures may significantly differ. On the other hand, it is also possible that chipmunks preferentially consume less corn-based resources because other more energetically valuable food waste items (e.g. composted fruits and vegetables) are available to them. For example, a study examining fecal matter of coyotes (*Canis latrans*) found that coyotes living in urban areas consumed a more diverse diet and less animals, and also tended to be very opportunistic when compared to their natural counterparts (Murray *et al.*, 2015). The same case could be true for chipmunks, whereby urban-dwelling chipmunks may opportunistically feed on food items close to their natural diet (e.g. fruits and vegetables), rather than corn-based ones.

I found no difference in fecal antioxidants among chipmunks along the natural-urban gradient. Fecal antioxidants may not differ because the availability of antioxidants is similar amongst habitats. In urban habitats, chipmunks may have access to plenty of fruits and vegetables by entering compost bins, while natural-dwelling individuals have access to their natural diets, presumably also high in antioxidants. Additionally, chipmunks that live in natural habitats may have access to fruit and vegetable waste by travelling to compost bins nearby their

natural habitats. For example, some yards or areas in Bell Park were densely forested, presenting a more “natural” habitat for a chipmunk. Chipmunks may travel to use both spaces for food resources, which could have affected the antioxidant concentrations in the feces. Moreover, the gradient may not have been a full spectrum of urban to natural habitat, therefore showing no differences in fecal antioxidants across Sudbury. Lastly, the samples used for the TAC analysis were only a snapshot of what a chipmunk had consumed that day, suggesting that there is a chance chipmunks consumed foods low in antioxidants at that time and may not be reflective of their true day-to-day diet. Because the fecal samples used for TAC are representative of the food consumed that day, my results may be limited and may not provide complete insight into how many antioxidants a chipmunk may consume over a long-term scale.

### *Conclusions*

My results suggest that chipmunks in more urban areas consume more protein in their diet than their more natural-dwelling counterparts; this is especially true in the case of females based on  $\delta^{15}\text{N}$  values. While there appears to be no difference in  $\delta^{13}\text{C}$  or fecal antioxidants along the gradient, females consume more  $\delta^{13}\text{C}$  than males. Chipmunks may not excrete different amounts of antioxidants between habitats because: 1) they are consuming enough antioxidants from both urban and natural sources of foods resulting in no difference, 2) the NUS is not broad enough to include the most urban and natural locations to see differences, or 3) the samples collected do not reflect the true diet of a chipmunk over the long-term. My findings show that the access to protein varies depending on the degree of urbanity in a given area, which may help to understand the types of human food waste animals are consuming other cities.

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## Tables and Figures

**Table 2.1.** Generalized linear model examining body condition for eastern chipmunks (*Tamias striatus*; n=112) in response to NUS, PC2,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , TAC and population density in Sudbury, Ontario.

Model	Estimate	Standard Error	t - value	p-value	Multiple R <sup>2</sup>	Adjusted R <sup>2</sup>
Body condition ~ $\delta^{13}\text{C} + \delta^{15}\text{N} + \text{TAC}$	Intercept: -0.007 $\delta^{13}\text{C}$ : 0.0005 $\delta^{15}\text{N}$ : 0.0003 TAC: -0.0106	Intercept: 0.1450 $\delta^{13}\text{C}$ : 0.0059 $\delta^{15}\text{N}$ : 0.0040 TAC: 0.0092	Intercept: -0.051 $\delta^{13}\text{C}$ : 0.094 $\delta^{15}\text{N}$ : 0.089 TAC: -1.146	Intercept: 0.960 $\delta^{13}\text{C}$ : 0.926 $\delta^{15}\text{N}$ : 0.930 TAC: 0.259	0.0332	-0.0384
Body condition ~ NUS	Intercept: 0.0025 NUS: -0.0002	Intercept: 0.0043 NUS: 0.0023	Intercept: 0.590 NUS: -0.099	Intercept: 0.558 NUS: 0.921	0.0002	-0.022
Body condition ~ PC2	Intercept: 0.0009 PC2: 0.004	Intercept: 0.004 PC2: 0.002	Intercept: 0.219 PC2: 1.478	Intercept: 0.828 PC2: 0.147	0.0472	0.0256
Body condition ~ Population density	Intercept: -0.010 Population density: 0.5602	Intercept: 0.0048 Population density: 0.2115	Intercept: -2.266 Population density: 2.649	<b>Intercept: 0.025</b> <b>Population density:</b> <b>0.009</b>	0.0599	0.0514

\*NUS, natural-urban score representing the values used to generate the urbanization gradient.

**Table 2.2.** Generalized linear model examining  $\delta^{13}\text{C}$  values for eastern chipmunks (*Tamias striatus*; n=112) in response to NUS, PC2, sex, and population density in Sudbury, Ontario.

Model	Estimate	Standard Error	t - value	p-value	Multiple R <sup>2</sup>	Adjusted R <sup>2</sup>
$\delta^{13}\text{C} \sim \text{NUS}$	Intercept: -23.087 NUS: 0.0506	Intercept: 0.1135 NUS: 0.0613	Intercept: -203.32 NUS: 0.825	<b>Intercept: &lt;0.01</b> NUS: 0.414	0.0152	-0.007
$\delta^{13}\text{C} \sim \text{Sex}$	Intercept: -22.825 Sex: -.0449	Intercept: 0.1585 Sex: 0.2149	Intercept: -144.04 Sex: -2.089	<b>Intercept: &lt;0.01</b> <b>Sex: 0.042</b>	0.0905	0.069
$\delta^{15}\text{N} \sim \text{Population density}$	Intercept: -23.348 Population density: 14.26	Intercept: 0.182 Population density: 7.537	Intercept: -127.92 Population density: 1.893	<b>Intercept: &lt;0.01</b> Population density: 0.06	0.0752	0.0542
$\delta^{13}\text{C} \sim \text{PC2}$	Intercept: -23.058 PC2: -0.030	Intercept: 0.115 PC2: 0.074	Intercept: -199.62 PC2: -0.411	<b>Intercept: &lt;0.01</b> PC2: 0.683	0.0038	-0.0188

\*NUS, natural-urban score representing the values used to generate the urbanization gradient.

**Table 2.3.** Generalized linear model examining  $\delta^{15}\text{N}$  values for eastern chipmunks (*Tamias striatus*; n=112) in response to NUS, PC2, Sex, and population density in Sudbury, Ontario.

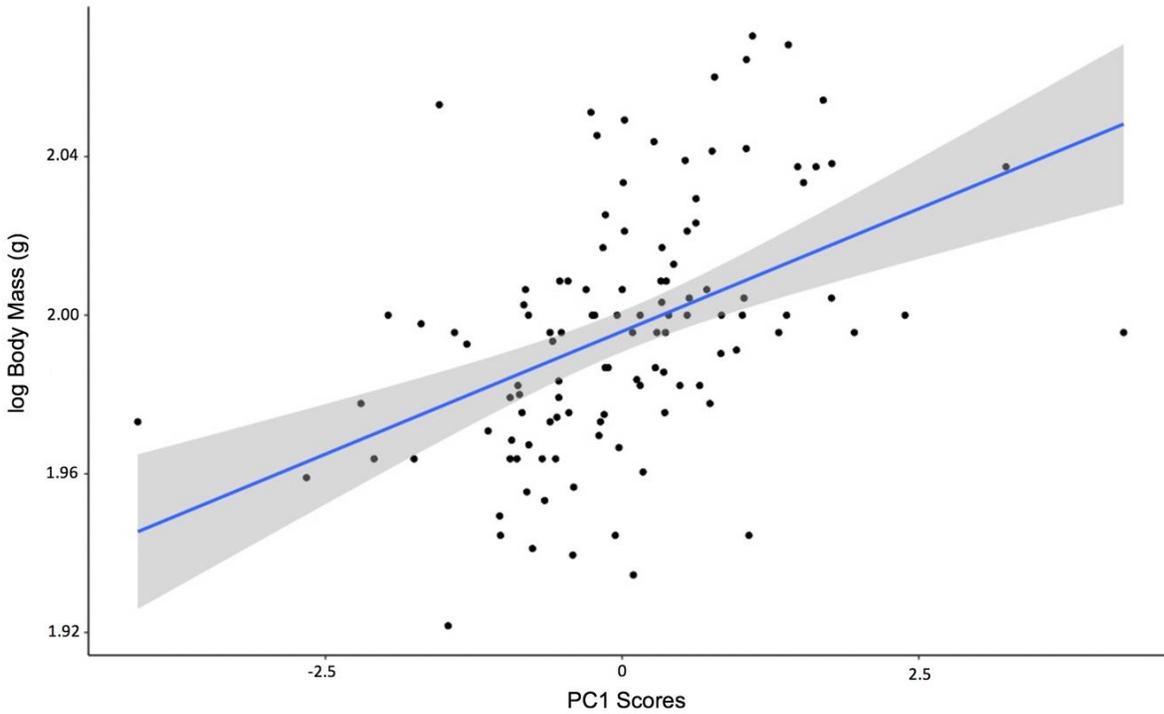
Model	Estimate	Standard Error	t - value	p-value	Multiple R <sup>2</sup>	Adjusted R <sup>2</sup>
$\delta^{15}\text{N} \sim$ Population density	Intercept: 4.758 Population density: 10.495	Intercept: 0.2822 Population density: 11.652	Intercept: 16.866 Population density: 0.901	<b>Intercept: &lt;0.01</b> Population density: 0.373	0.0181	-0.0042
$\delta^{15}\text{N} \sim$ PC2	Intercept: 4.931 PC2: 0.084	Intercept: 0.1725 PC2: 0.111	Intercept: 28.59 PC2: 0.759	<b>Intercept: &lt;0.01</b> PC2: 0.452	0.0129	-0.0095
$\delta^{15}\text{N} \sim$ Sex	Intercept: 5.341 Sex: -0.695	Intercept: 0.237 Sex: 0.3214	Intercept: 22.541 Sex: -2.162	Intercept: <0.01 <b>Sex: 0.03</b>	0.0960	0.0755
$\delta^{15}\text{N} \sim$ NUS	Intercept: 4.834 NUS: 0.3486	Intercept: 0.1414 NUS: 0.076	Intercept: 34.21 NUS: 4.563	<b>Intercept: &lt;0.01</b> <b>NUS: &lt;0.01</b>	0.3212	0.3058

\*NUS, natural-urban score representing the values used to generate the urbanization gradient.

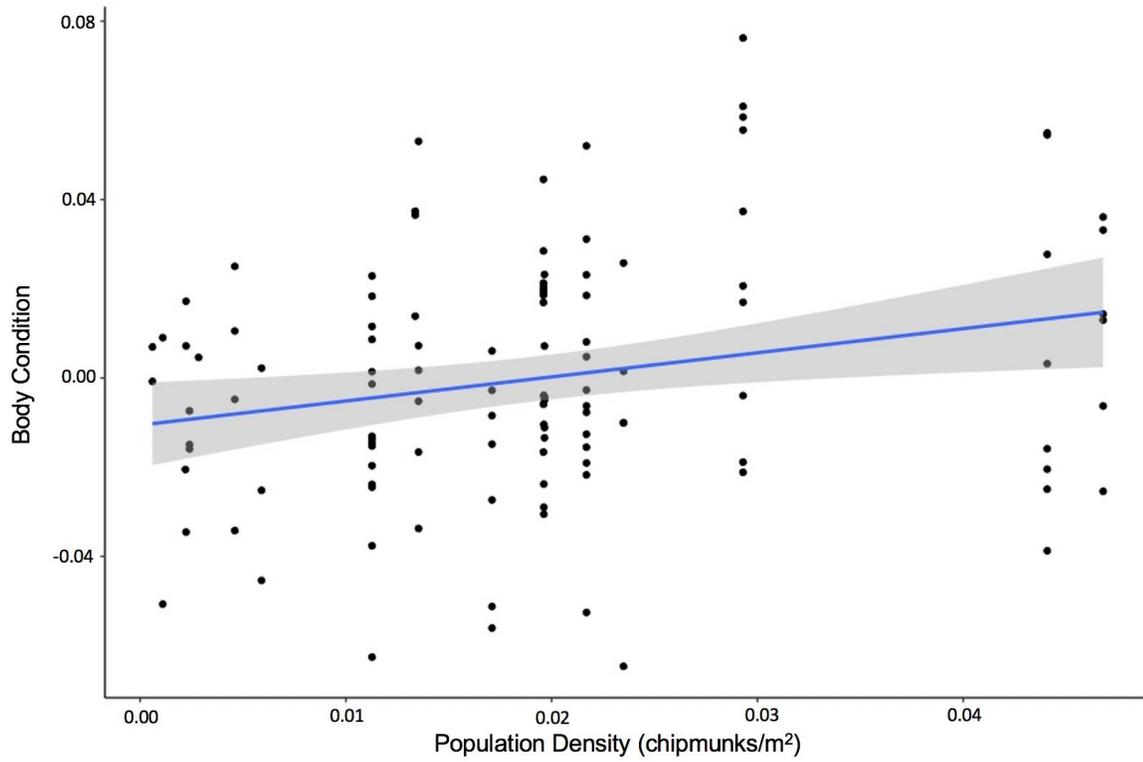
**Table 2.4.** Generalized linear model examining TAC values for eastern chipmunks (*Tamias striatus*; n=112) in response to NUS, PC2, Julian date and sex in Sudbury, Ontario.

Model	Estimate	Standard Error	t - value	p-value	Multiple R <sup>2</sup>	Adjusted R <sup>2</sup>
log <sub>10</sub> (TAC) ~ Sex	Intercept: -1.910 Sex: -0.099	Intercept: 0.0644 Sex: 0.094	Intercept: -29.623 Sex: -1.051	<b>Intercept: &lt;0.01</b> Sex: 0.296	0.009	<0.01
log <sub>10</sub> (TAC) ~ Julian date	Intercept: -1.6291 Julian date: -0.001	Intercept: 0.2023 Julian date: 0.0010	Intercept: -8.050 Julian date: -1.661	<b>Intercept: &lt;0.01</b> Julian date: 0.09	0.0244	0.0156
log <sub>10</sub> (TAC) ~ NUS	Intercept: -1.947 NUS: -0.010	Intercept: 0.0512 NUS: 0.0241	Intercept: -37.99 NUS: -0.488	<b>Intercept: &lt;0.01</b> NUS: 0.655	0.0018	-0.0072
log <sub>10</sub> (TAC) ~ PC2	Intercept: -1.956 PC2: -0.001	Intercept: 0.047 PC2: 0.033	Intercept: -40.941 PC2: -0.058	<b>Intercept: &lt;0.01</b> PC2: 0.954	<0.01	<0.01

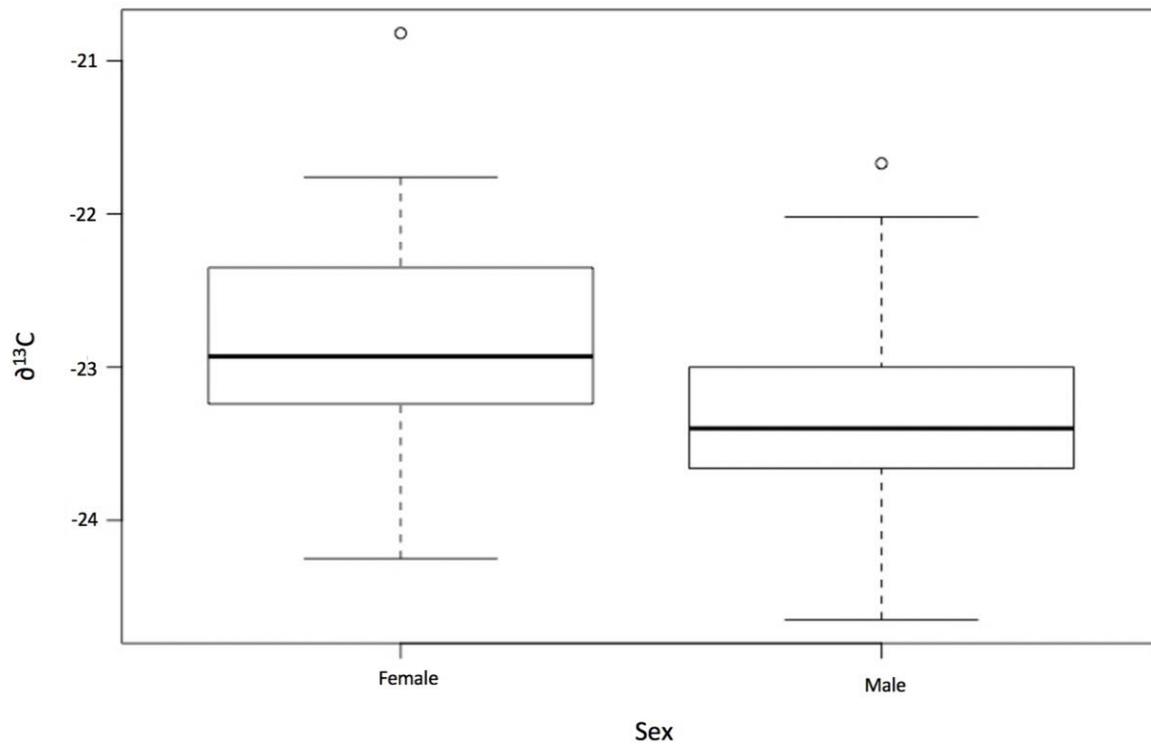
\*NUS, natural-urban score representing the values used to generate the urbanization gradient.



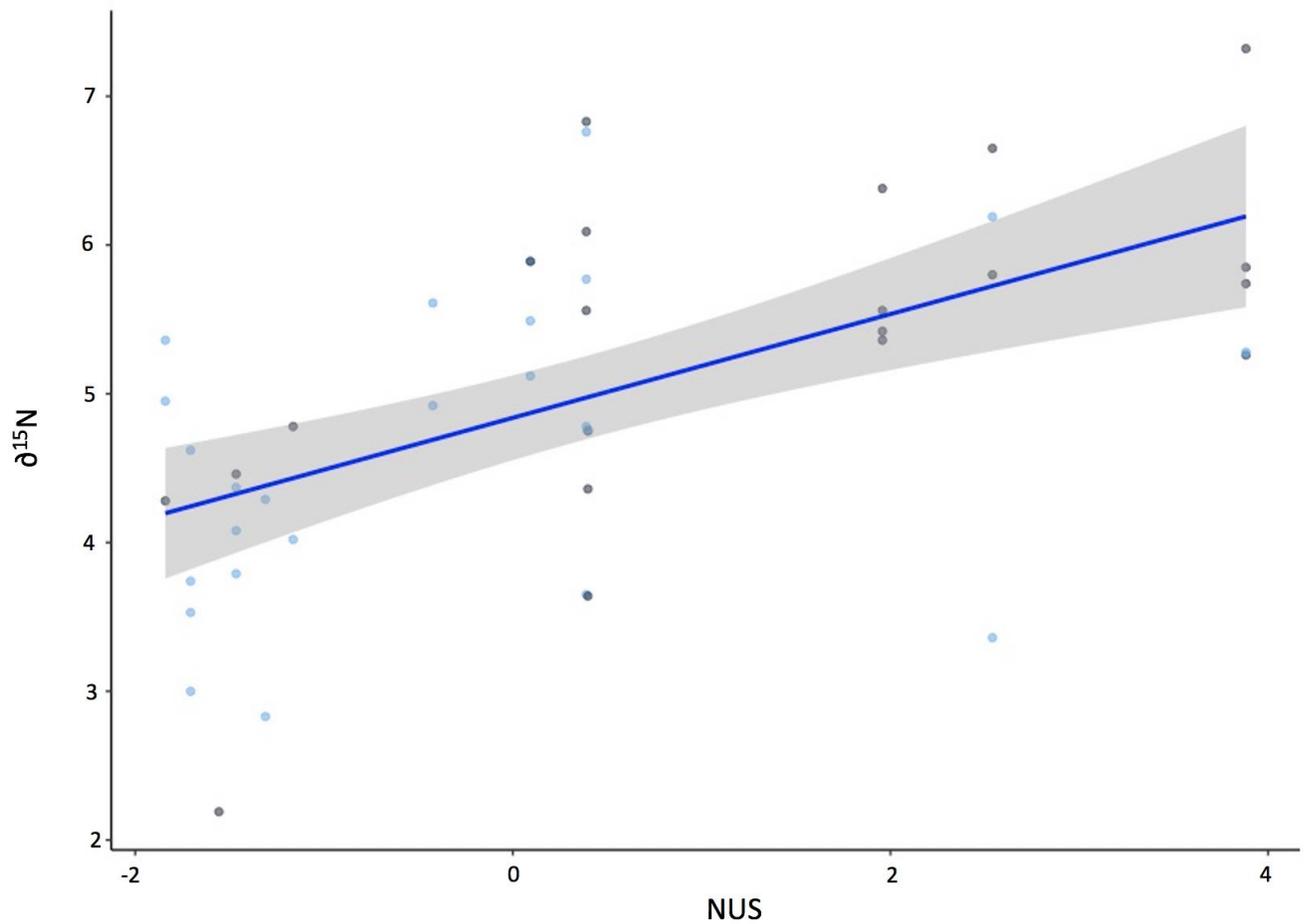
**Figure 2.1.** Linear regression of PC1 scores of body size and log-transformed body mass used to determine the residuals which represents body condition scores for eastern chipmunks (*Tamias striatus*;  $p < 0.01$ , adjusted  $r^2 = 0.194$ ,  $n = 114$ ). Positive residuals indicate good body condition and negative residuals indicate poor body condition. The shaded area denotes a 95% confidence interval.



**Figure 2.2.** Linear regression of body condition in response to population density of eastern chipmunks (*Tamias striatus*;  $p=0.010$ , adjusted  $r^2=0.048$ ,  $n=114$ ). The shaded area denotes a 95% confidence interval.



**Figure 2.3.** Box plot comparing  $\delta^{13}\text{C}$  differences based on sex of eastern chipmunks (*Tamias striatus*; ( $p=0.042$  adjusted  $r^2=0.069$ ,  $n=47$ )). The thick bar within the box represents the median, while the whiskers represent the highest and lowest observations.



**Figure 2.4.** Linear regression of  $\delta^{15}\text{N}$  depending on sex (male = light blue, female = dark blue) of eastern chipmunks (*Tamias striatus*) across the natural-urban gradient ( $\delta^{15}\text{N} \sim \text{NUS}$ :  $p < 0.01$  adjusted  $r^2 = 0.305$ ,  $n = 47$ ;  $\delta^{15}\text{N} \sim \text{sex}$ :  $p = 0.036$ , adjusted  $r^2 = 0.075$ ,  $n = 47$ ). On the x axis, positive values indicate more urban areas and negative values indicate more natural areas. The shaded area denotes a 95% confidence interval.

## General Discussion

The growth of cities provides wildlife with abundant access to human food waste, which may influence the amount of endocrine stress an individual experiences, as well as their health (Shochat *et al.*, 2010; Faeth *et al.*, 2011; Isaksson, 2015; Saari *et al.*, 2016). The overall objective of my research was to determine the dietary differences of eastern chipmunks along a gradient of urbanization. To investigate my objectives, using a natural-urban score, I examined: 1) the concentration of cortisol found within feces, and 2) carbon and nitrogen isotope signatures found in hair, and the amount of antioxidants in feces.

In Chapter One, I tested the hypothesis that chipmunks in urban habitats experience lower levels of cortisol and had a better body condition than their conspecifics in natural habitats because chipmunks have access to human food waste. Opposed to my predictions, chipmunks in urban habitats experienced the highest levels of cortisol compared to their natural counterparts. I also found that body condition decreased across the urban-natural gradient, while PC2 was highest at intermediate levels of urbanization. Based on these findings, in urban habitats, chipmunks may experience higher cortisol because: 1) they have increased interaction with humans and dogs, or 2) they have increased social interactions with each other.

In Chapter Two, I tested the hypothesis that chipmunks from urban habitats would produce higher carbon and nitrogen isotope signatures and more fecal antioxidants compared to chipmunks in natural habitats because they are consuming a diet consisting of human food waste. I found that chipmunks living in urban habitats did in fact have a higher nitrogen signature, but that unexpectedly, carbon signatures did not differ along the urbanity gradient. I also found that fecal antioxidants did not differ across the gradient. Chipmunks in urban habitats may have access to abundant protein resources, such as bird feed or meat food waste, which may have

consequences for their health, such increases in cholesterol (Pick *et al.*, 1959; Trichopoulou *et al.*, 1992; Adam *et al.*, 2016). Since carbon signatures did not differ between among habitat types, chipmunks may be consuming less corn-based food waste than I initially predicted. This suggests, along with the antioxidant results, that chipmunks in urban habitats may be consuming other types of food waste that may not be accounted for in the isotope analysis, such as fruits and vegetables. Based on my findings, dietary nitrogen varies depending on how urban an area is, and isotope signatures are a useful tool to better understand what chipmunks are consuming, and consequently the potential impact that a particular diet has on an individual.

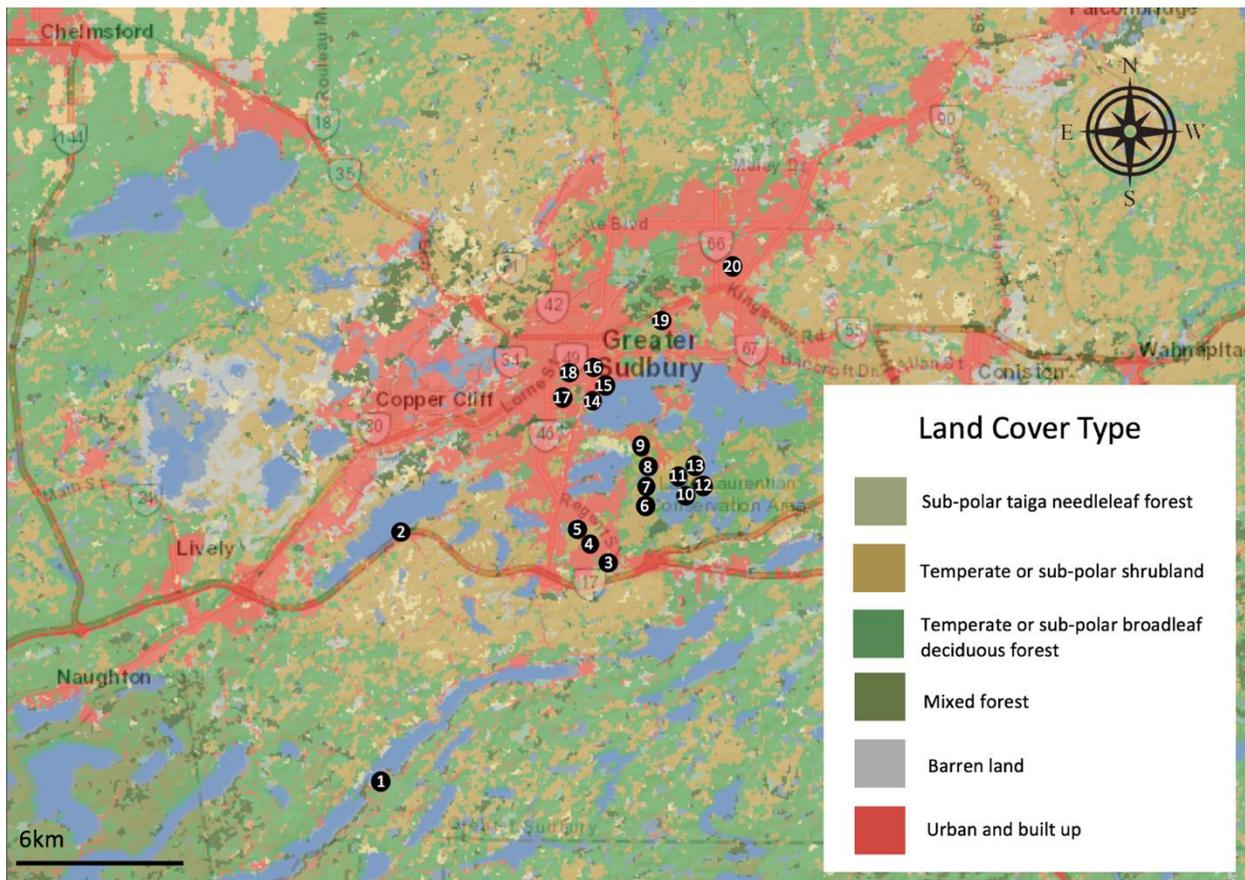
Overall, my study highlights dietary difference of living within an urban environment. Chipmunks may benefit the most from urban habitats because they may have greater access to food waste than their natural counterparts, at the expense of experiencing more endocrine stress. Diets consisting of human food waste could have long-term impacts on a population, such as increasing density, increasing metabolic disorders like diabetes, or influence the rates of disease spreading.

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## Appendices



**Appendix A.** Map of the 20 study sites visited and the respective land cover types found at each study site for eastern chipmunks (*Tamias striatus*) generated from the 2015 Land Cover of Canada map (Latifovic *et al.*, 2017; Latifovic, 2019). Study sites included 8 backyards (1, 3-5, 17-20), Bell Park (14-16), Laurentian University campus (6-9), Lake Laurentian Conservation Area (10-13), and Kelly Lake Trail (2).

**Appendix B.** Land cover present at each study site in Sudbury, Ontario determined from the 2015 Land Cover of Canada map (Latifovic *et al.*, 2017; Latifovic, 2019).

Land Cover Type	Description	Locations with land cover
Sub-polar taiga needleleaf forest	Forests taller than 3m and accounting for more than 20% of the vegetation cover; 75% of the species present have needles.	1, 2
Temperate or sub-polar shrubland	Woody perennial plants with persistent woody stems; accounts for more than 20% of the vegetation cover.	2, 3, 9-14
Temperate or sub-polar broadleaf deciduous forest	Forests taller than 3m and accounting for more than 20% of the vegetation cover; 75% of the species are deciduous.	2-5, 8, 10-13
Mixed forest	Forests taller than 3m accounting for 20% of vegetation; needleleaf and deciduous are codominant and make up 75% of the cover.	1

Barren land	Bare rock, gravel, or other mineral material that has very little plant material; less than 10% of total cover.	15, 16
Urban and built up	Areas that have at minimum 30% of constructed materials for human use	1, 3-5, 8, 9, 14-20

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**Appendix C.** Eastern chipmunk (*Tamias striatus*) population densities present at each study site in Sudbury, Ontario.

Study Site	Population Density (chipmunks per m <sup>2</sup> )
1	0.013
2	0.002
3	0.019
4	0.046
5	0.019
6	0.006
7	0.001
8	0.004
9	0.016
10	0.002
11	0.002
12	0.002
13	0.005
14	0.002
15	0.019
16	0.011
17	0.023
18	0.044
19	0.029
20	0.013

**Appendix D.** A PCA analysis was conducted to create the scores for the urbanization gradient based on the habitats of eastern chipmunks (*Tamias striatus*). A summary of PC1 and PC2 loadings consisting of all variables surveyed at each study site and land cover data in Sudbury, Ontario. PC1 explains 44.9% of the variation in the dataset, while PC2 explains 20.3%.

Variable	PC1	PC2
	Loadings	Loadings
Average number of people per hour	0.423	0.315
Average number of cars per hour	0.381	-0.097
Average number of bikes per hour	0.377	-0.478
Average number of trash pieces found within 100m radius of the trapline	0.375	-0.332
Average number of dogs per hour	0.362	-0.338
Average number of trash cans found within 100m radius of the trapline	0.332	0.521
Average number bird feeders found within 100m radius of the trapline	0.251	0.312
Vegetation cover	-0.297	-0.274