

Surviving the concrete jungle: The eco-physiological consequences of consuming anthropogenic food waste on raccoons (*Procyon lotor*)

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science (M.Sc.) in Biology

The Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

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THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian University/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	Surviving the concrete jungle: The eco-physiological consequences of consuming anthropogenic food waste on raccoons (<i>Procyon lotor</i>)		
Name of Candidate Nom du candidat	Mozzon, Christina		
Degree Diplôme	Master of Science		
Department/Program Département/Programme	Biology	Date of Defence Date de la soutenance	October 07, 2020

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Abstract

Urbanization is a process where natural landscapes are transformed to include structures like buildings and roads to accommodate the growing human population. Many species of wildlife are unable to adapt to these rapid and drastic landscape changes, resulting in declines to certain populations. However, species like mesocarnivores that have flexible diets are able to thrive in these urbanized environments due to the increased access to anthropogenic food waste. Food resources such as this are different in both quantity and quality, potentially leading to individual health effects. This research studied the physiological consequences associated with an urban diet in raccoons (*Procyon lotor*), a species that is opportunistic and omnivorous. Dietary patterns using C and N stable isotope analysis were measured, along with their effect on body condition, glycated serum protein (GSP), and leptin across an urbanization gradient from the Ontario and Québec regions during the fall. Stable isotope analysis revealed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were highest in agricultural and barren landscapes, exhibiting a diet highly reliant on C_4 plants such as corn and animal protein, respectively, instead of anthropogenic food waste as previous studies with summer sampling periods have shown. Body condition had a positive relationship with both $\delta^{13}\text{C}$ and leptin, providing evidence that increases in body condition is associated with fat mass. However, extremely high body condition with greater fat reserves may have negative health consequences such as hyperglycemia, as seen with increased GSP concentrations from raccoons in agricultural and barren landscapes. Broad scale analyses of body condition (Ontario and Québec) showed sex-specific results, where males were in better body condition in urban areas and females were in poorer body condition. Finer scale analyses of Ontario showed that raccoons

from agricultural and barren landscapes were in poorer body condition than those from natural landscapes, which may provide insight into how raccoons conserve energy for locomotion. Males were found to be in consistently better body condition than females in Ontario. Surprisingly, there was no effect of urbanization in Québec potentially due to differences in municipal waste management policies. Overall, this study suggests a shift in diet from anthropogenic food waste to resources from agricultural and barren landscapes throughout the fall, as it is more readily available food source.

Key words: urban ecology, stable isotope, body condition, glycated serum protein, leptin

Acknowledgements

There are many people in whom I want to extend my greatest thanks to for providing advice and support. First and foremost, I would like to express my deepest appreciation to my supervisor, Dr. Albrecht Schulte-Hostedde, for his endless guidance and encouragement throughout my degree. Your passion for research has inspired and pushed me to always be a better scientist. I also cannot thank you enough for your patience when answering my countless questions for the past two years.

I cannot begin to express my thanks to my committee members, Drs. Jeffrey Gagnon and Thomas Johnston, for involvement in so many different aspects of my project. Jeff, thank you for providing your expertise regarding the biomedical sections of my project, everything from help in the lab to interpreting my results. Your encouragement while watching many of my presentations always motivated me to strive further. Tom, thank you for providing me much needed guidance during my statistical analysis. Your thorough and detailed answers to my many questions have helped me complete the best thesis I possibly could. Your constant advice and support since completing my Honours thesis under your wing has pushed me academically and helped me achieve my dreams.

Special thanks to Dr. Sarah Jamieson and the crew at the Ontario Ministry of Natural Resources and Forestry Rabies Unit, as well as Dr. Fanie Pelletier from the Université de Sherbrooke, for assistance in sample collection. Many thanks to Dr. Keith Hobson for assistance in stable isotope analysis. I also had the pleasure of working with Dr. Rod Jouppi who I would like to thank for providing advice and support on various aspects of my project, as well as being my inspiration to pursue a career in veterinary

medicine. Additionally, I am extremely grateful towards the EBV lab for support both academically and personally. Thank you to my lab-mates for all the memories that were made the past two years that will never be forgotten, especially our weekly trivia nights at The Townhouse Tavern.

Lastly, I would like to extend my sincerest of thanks to all my family and friends. To my parents, for their unconditional love and continuous support throughout my journey of graduate school. You have always reminded me of the loving environment I come from, from coming home to a freshly cooked meal every night to cheering me on at any of my presentations you could attend – thank you. To my siblings, Nick and Angela, for the rivalry between us has always pushed me further, while also being a proud big sister to all of your accomplishments. Thank you immensely for helping me prepare for countless presentations by brainstorming potential questions audience members could ask. To my grandparents, Nana and Nono, for always believing in me and providing wise words of wisdom when I needed it most. To my boyfriend, Ryan, for always supporting me during my thesis and offering help in any way possible. Your continuous positive energy is truly inspirational. To my best friend, Adam, for being someone I can always count on through thick and thin; your endless support is greatly appreciated. Though we may push each other to our limits sometimes, our competitive nature with one another has pushed me to succeed and will continue to do so in my future endeavours.

Thank you again to everyone involved with my thesis. I could not have done it without you.

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General Introduction

Urbanization is increasing globally, resulting in changes to natural habitats to which various species of wildlife have successfully evolved (Reichert et al. 2017, Stark et al. 2020). It involves land use changes in which natural landscapes composed of vegetation, soil, and water are converted to manmade landscapes featuring cement, chemical materials, metal, and asphalt (Haverland and Veech 2017). Drastic and rapid changes to habitats are a challenge for many wildlife species, as processes that occur within urban and natural habitats vary significantly and are cause for concern regarding the ecological and evolutionary consequences associated with human-induced changes (Shochat et al. 2006, Gibbs et al. 2019).

Urban landscapes influence various ecological factors. Urbanization impacts predator-prey interactions by introducing anthropogenic food resources, as predators will preferentially consume readily available anthropogenic food resources instead of hunting for prey (Fischer et al. 2012, Eötvös et al. 2018). This results in the predation paradox, where the number of predators in an urban area increases as predation rates decrease (Fischer et al. 2012). Urban environments may produce mosaic landscapes as well, where interfaces between various habitat types influence the spatial distribution patterns of wildlife and their interactions (Ehlers Smith et al. 2018, Zungu et al. 2020). For example, areas with a high density of houses had a negative impact on community and species responses of most urban wildlife (Villaseñor et al. 2014). Spatial distribution of wildlife may also lead to variation in the spread of pests and diseases, where transmission rates

will increase with greater contact rates between individuals (Gordon et al. 2016, Soulsbury and White 2016).

Alterations in ecological processes such as predator-prey interactions, spatial distribution patterns, and transmission of pathogens may have a negative influence on biodiversity. Disturbances associated with urbanization, such as habitat fragmentation or unfavorable conditions, may lead to extinctions of sensitive species (Łopucki and Kitowski 2017, Schmidt et al. 2020). Biological homogenization of species occurs as sensitive native species decline in urbanized environments (Ofori et al. 2018, Colléony and Shwartz 2020) while others thrive by taking advantage of the availability of anthropogenic food resources, ultimately leading to a decline in biodiversity (Červinka et al. 2014, Stark et al. 2020).

Availability of food resources may change depending on the urbanization of the area (Bateman and Fleming 2012, Murray et al. 2015, Magioli et al. 2019). However, one challenge in urban ecology is the ability to characterize this ‘urbanization’ and determine methods to quantify how urban a habitat is, instead of classifying habitats categorically such as ‘urban’, ‘agriculture’, and ‘natural’ (Seress et al. 2014, Suarez-Rubio and Krenn 2018). A common method for creating an urbanization gradient is by incorporating both landscape characteristics and demographics to capture differences in abundance and nutritional qualities of food (i.e. carbohydrate, lipid, and protein composition) provided in varying habitats (Seress et al. 2014, Suarez-Rubio and Krenn 2018). Landscape characteristics include physical elements of the land, including buildings, roads, agriculture, vegetation, water, and barren land. Demographics can be measured by human population density and socioeconomic status, which serve as indices of food resources

available in the area. Human population density reflects the availability of anthropogenic food resources, as greater populations of humans produce more garbage (Mckinney 2001, Luck 2007). Differences in socioeconomic status, measured by median household income, can capture how wealthier areas can provide food resources more nutritionally similar to natural environments in terms of carbohydrate, lipid, and protein composition (Magle et al. 2016).

Raccoons (*Procyon lotor*) are an opportunistic and omnivorous species that are densely populated in many habitat types across North America, making them an ideal species to study the effects of urbanization (Thornton et al. 2020). The diet of a raccoon generally consists of small mammals, invertebrates, fish, nuts, and berries in natural habitats, or corn and corn-based food waste in urban areas (Bartoszewicz et al. 2008). The macronutrient composition between these diets are different, where it is assumed that an urban diet is composed of greater carbohydrate and lipid content than a natural diet (Murray et al. 2015, 2018). Though a natural diet contains carbohydrates as well, the key difference is in the type of carbohydrate in each diet; the natural diet is speculated to have higher proportions of roughage carbohydrates (i.e. fiber) whereas the urban diet would contain more energy carbohydrates (e.g. refined starches, sugars). Previous studies have shown that raccoons are impacted by urbanization at the population level (Prange et al. 2003, 2004, Prange and Gehrt 2004), but a knowledge gap exists on the individual effects of an urban diet. Therefore, this research will examine the eco-physiological consequences of consuming anthropogenic food waste by comparing differences in dietary patterns and how that impacts their body condition, glucose metabolism, and fat metabolism.

Stable isotope analysis is used to determine species long-term feeding strategies and is used in urban ecology to quantify access to, or consumption of, anthropogenic food waste (DeNiro and Epstein 1978, 1981). Ratios of heavy to light isotopes for carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$), denoted by delta (δ), are commonly used to determine the original sources of dietary carbon and trophic level position in the food web, respectively (DeNiro and Epstein 1978, 1981). In terrestrial ecosystems, carbon isotopic signatures ($\delta^{13}\text{C}$) are used to distinguish between individuals consuming C_4 (e.g. corn) or C_3 (e.g. woody trees) plants, as plants using C_4 photosynthetic pathways have ^{13}C -enriched isotopic signatures compared to those using C_3 photosynthetic pathways (DeNiro and Epstein 1978, Tieszen et al. 1983). If raccoons are consuming food waste in urban environments and natural forage in non-urban environments, this may be reflected in their isotopic signatures (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019). Nitrogen ($\delta^{15}\text{N}$) isotopic signatures are used to determine an individual's trophic level position in the food web ranging from primary producers to carnivores, where individuals in higher trophic levels will exhibit enriched nitrogen isotopic signatures compared to those beneath (DeNiro and Epstein 1981, Minagawa and Wada 1984). This pattern may be reflected in raccoons from urbanized environments, which should exhibit greater nitrogen isotopic signatures due to the consumption of food waste containing animal products, compared to those feeding on natural forage in non-urbanized environments (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019).

Body condition refers to an individual's energy reserves and is measured as the observed mass of an individual in relation to their predicted mass for a specific structural size (Schulte-Hostedde et al. 2005). Indices of body condition are important in wildlife

research as they provide insight into overall fitness, as individuals in better body condition generally carry more energy reserves, and are expected to have greater survival and reproductive success (Fløjgaard et al. 2017, Coon et al. 2019). Consuming an urban diet can alter an individual's body condition due to differences in macronutrient abundance and composition of the food resources offered in urbanized landscapes (Lyons et al. 2017, Murray et al. 2019). However, whether these anthropogenic resources provide a better or poorer body condition overall remains unclear (Meillère et al. 2015).

Urbanized environments provide predictable food resources, so species may be in better body condition as they consume greater amounts of food to attain the fitness benefits of survival and reproduction (Schulte-Hostedde et al. 2005, Lyons et al. 2017). Increased fat reserves act as a buffer against periods of food shortages as well, but are also associated with metabolic or locomotive stress (Schulte-Hostedde et al. 2005, Meillère et al. 2015). Therefore, wildlife may be in poorer body condition instead in urbanized environments that have a predictable food source to avoid these unnecessary stresses (Schulte-Hostedde et al. 2005, Meillère et al. 2015).

Leptin, a hormone considered as a lipostat, is released by adipose cells and interacts with the central nervous system through the blood-brain barrier to regulate feeding behavior (Zhang et al. 1994, Friedman and Halaas 1998). As leptin increases, appetite is suppressed and metabolic rate is increased (Ahmadi et al. 2016, Pandit et al. 2017). At chronically elevated leptin levels, leptin resistance occurs where leptin interactions with the brain are inhibited and diet-induced obesity arises (Münzberg et al. 2005, Myers et al. 2012). Leptin is associated with adiposity in various species (Banks et al. 2001, Shibata et al. 2005, Spady et al. 2009) and is therefore expected to increase in

areas with greater urbanization due to elevated fat content found in anthropogenic food waste (Banks et al. 2003, Schulte-Hostedde et al. 2018).

Glycated serum proteins (GSP) are used to measure average blood glucose concentrations over a 2-3 week period and can provide insight into glycemic control (Welsh et al. 2016). These proteins are formed through a nonenzymatic reaction between a monosaccharide (most commonly glucose) and the amino group of a protein (Welsh et al. 2016). GSPs have been used previously to measure changes in glucose metabolism with increasing urbanization; they were found to be in highest concentrations in individuals consuming an urban diet and consequently, greater amounts of carbohydrates (McCain et al. 2008, Schulte-Hostedde et al. 2018).

Overall, this study aims to determine the dietary patterns of raccoons and their associated physiological consequences seen across an urbanization gradient. It can be applied to various species globally and provides a preliminary model for researchers to better understand the effects of urbanization on wildlife populations, as research in urban ecology becomes increasingly more essential. Research should further investigate whether changes in diet have associated evolutionary consequences, or if there is evidence of raccoons genetically adapting to the consumption of anthropogenic food waste in urbanized landscapes (Harris and Munshi-South 2017). For example, recent work with white-footed mice (*Peromyscus leucopus*) provided evidence of genetic adaptation that enabled for the metabolism of lipids and carbohydrates in urban areas (Harris and Munshi-South 2017).

From a management perspective, this study could be used to develop effective management policies to improve human-wildlife interactions. For example, a recent study

found that improving management efforts towards unsecure garbage and ornamental fruits should reduce the number of coyotes (*Canis latrans*) living in urban environments (Larson et al. 2020). In my study, it was found that a raccoons diet in the fall primarily consists of food resources within agricultural areas, demonstrating the need to establish effective monitoring efforts to avoid destruction of crops and small farm animals. The land sharing (i.e. integrating conservation and farming) and land sparing (i.e. separating conservation and farming) policies involved in agricultural landscape management should be reconsidered, and involve a collaboration between city planning, land-use policy makers, and ecologists to ensure the development of a program that is most beneficial (Phalan et al. 2011). For example, techniques with land sharing (e.g. agroforestry, organic farming) or land sparing (e.g. sustainable intensification of agriculture) may need to be improved based on current species population densities and crop yields in the area (Phalan et al. 2011). Ultimately, future studies should investigate if species are adapting to live within human-modified landscapes and how policies can be implemented to reduce conflicts with wildlife.

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Chapter 1: Changes in body condition of raccoons (*Procyon lotor*) along an urbanization gradient

Abstract

Urbanization involves drastic changes to natural landscapes that present challenging circumstances to wildlife as they strive to adapt to these alterations. Many species that thrive in urban environments feed on anthropogenic food waste, but the physiological consequences associated with consumption of this type of food have rarely been studied. I examined the impact of an urban diet on the body condition of raccoons (*Procyon lotor*) across multiple years and regions. Morphometric measurements were collected from raccoons throughout southern Ontario and Québec, across a natural-urban gradient. I calculated an ‘urbanization score’ from various landscape characteristics using land use classification (i.e. buildings, roads, agriculture, vegetation cover, water, barren land) and demographics (i.e. human population density and average household income). Results showed the effect of urbanization on body condition was sex-specific, where males were in better body condition in urban environments and females were in poorer body condition. Finer-scale analyses showed raccoons were in better body condition in natural environments, compared to environments of agriculture and barren land in Ontario. Body condition was not related to any landscape or demographic variable in Québec, nor did it have an effect of sex. This study shows that urbanization can impact body condition, but further research is required to determine if these changes in individual health are negatively impacting the raccoon or if raccoons are adapting to urban landscapes. From a management perspective, this study can be used to develop more effective waste management policies to improve human-wildlife interactions.

Keywords: urban ecology, anthropogenic food waste, mesocarnivore, mass-size residual

Introduction

Urbanization occurs as cities expand to accommodate increasing human populations globally, and causes environmental change in landscape characteristics (Grimm et al. 2008, Liu et al. 2014). As humans modify landscapes, natural features such as soil, vegetation, and water are altered to include manmade structures composed of cement, metal, asphalt, and chemical materials (Haverland and Veech 2017). Some species are unable to adapt to these rapidly changing habitats, leaving many ecologists and conservationists concerned for the ecological and evolutionary consequences associated with human-induced changes (Shochat et al. 2006, Gibbs et al. 2019).

Urban landscapes can influence various ecological factors, including predator-prey relationships (Eötvös et al. 2018, Gallo et al. 2019), spatial distribution patterns (Ehlers Smith et al. 2018, Zungu et al. 2020), and the spread of pests and diseases (Gordon et al. 2016, Soulsbury and White 2016). Changes in these processes may have a negative effect on biodiversity as sensitive native species face declines in their populations due to environmental disturbances, such as habitat fragmentation or unfavourable climates (Łopucki and Kitowski 2017, Schmidt et al. 2020). However, other species, such as mesocarnivores, tend to prosper in urbanized areas in part due to their flexible diet and ability to consume anthropogenic food waste (Červinka et al. 2014, Stark et al. 2020).

Available food resources can vary with urbanization (Bateman and Fleming 2012, Murray et al. 2015, Magioli et al. 2019), with its changes being best defined along a gradient rather than broad classifications such as ‘urban’, ‘agriculture’ and ‘natural’ (Seress et al. 2014, Suarez-Rubio and Krenn 2018). Incorporating information from

landscape characteristics and demographics is a common method to capture differences in food quantity and quality (i.e. carbohydrate, lipid, and protein composition) across a variety of habitats (Seress et al. 2014, Suarez-Rubio and Krenn 2018). Landscape characteristics provide insight into the physical elements of the land, which include buildings, roads, agriculture, vegetation, water, and barren land. Human population density and socioeconomic status are demographic measurements that can serve as indices of available food resources in an area. Human population density reflects food resource accessibility, as areas that are occupied by more people will produce greater amounts of anthropogenic food waste available for consumption by surrounding wildlife (Mckinney 2001, Luck 2007). Variation in socioeconomic status of an area, measured by median household income, has been shown to influence heterogeneity of food resources available to wildlife as wealthier areas may provide a more stable and better food source nutritionally (Magle et al. 2016).

Raccoons (*Procyon lotor*) are a mesocarnivore that has adapted to live within a variety of habitat types across North America, including urban areas with access to anthropogenic food waste (Thornton et al. 2020). Their broadly omnivorous diet and opportunistic feeding strategies allows for a flexible diet consisting of small mammals, invertebrates, fish, nuts, and berries in natural habitats, and corn or corn-based garbage in urban areas (Bartoszewicz et al. 2008). These diets differ in terms of their macronutrient composition, where urban diets are likely higher in carbohydrates and lipids (Murray et al. 2015, 2018). Though a natural diet contains carbohydrates as well, the main distinction between the two is the type of carbohydrate present. It is speculated that an urban and natural diets contain more energy (e.g. refined starches, sugars) and roughage

(i.e. fiber) carbohydrates, respectively. Although previous studies have shown the ecological effects to a population when consuming anthropogenic food waste (Prange et al. 2003, 2004, Prange and Gehrt 2004), little is known on how this change in diet affects the health of raccoons. For example, previous work has shown that consuming anthropogenic food waste is associated with dental caries and poor periodontal health (Hungerford et al. 1999) and hyperglycemia (Schulte-Hostedde et al. 2018).

Urbanization can affect an animal's body condition, a measure of an individual's energetic state relative to structural size (Schulte-Hostedde et al. 2005), although no consistent patterns are evident across studies. Many species in urbanized habitats tend to be in better body condition than their natural counterparts because of the fitness benefits (i.e. greater fasting endurance, reproductive output, and survival) provided by increased energy reserves (Soto-Calderón et al. 2016, Lyons et al. 2017). In contrast, studies have also shown that urban wildlife can be in poorer body conditions because it is not essential to have increased energy reserves in areas with predictable food resources (Bókonyi et al. 2012, Meillère et al. 2015). These inconsistent findings demonstrate the need for a large-scale multi-regional and multi-year approach.

I examined changes in body condition relative to an urbanization gradient across years and geographic regions in the raccoon (*Procyon lotor*), a species that lives in a variety of habitat types across North America, including urban areas (Bateman and Fleming 2012, Gross et al. 2012). With a broadly omnivorous diet and opportunistic feeding strategies (Lotze and Anderson 1979), I predicted that raccoons will be in better body condition in more urbanized areas because of greater access to anthropogenic food waste (Demeny et al. 2019).

Materials and Methods

Study area and field sampling

Field work was conducted in collaboration with the Ontario Ministry of Natural Resources and Forestry (OMNRF) Rabies Unit and Dr. Fanie Pelletier with the Ministère des Forêts, Faune et Parcs du Québec (MFFPQ) and its partners. OMNRF samples were collected from southern Ontario in October 2018 and 2019 and those from MFFPQ were collected from southern Québec in October 2009 and 2010 (Figure 1.1).

In Ontario, raccoons were captured with live traps (Tomahawk Model 106/108, Hazelhurst, Wisconsin, USA) baited with canned sardines (*Sardina pilchardus*). In Québec, raccoons were also captured with live traps (Havahart® Products, Lititz, Pennsylvania, USA) but baited with fresh mackerel (*Scomber scombrus*) or canned sardines and canned corn. A hand-held global positioning system (GPS) was used to obtain locations of each animal trapped and traps were opened daily. Once captured, raccoons were transported to a processing station where they were weighed in their traps to the nearest 0.1 kg and anaesthetized using an intramuscular injection. The OMNRF used a mixture of Telazol (Telazol®, 100 mg/mL, Zoetis Canada Inc., Kirkland, Québec, Canada) and medetomidine (Cepetor™, 1 mg/mL, Veterinary Purchasing Company Limited, St. Marys, Ontario, Canada) at dosages of 1.7 mg/kg body weight and 0.07 mg/kg body weight, respectively. The MFFPQ used a mixture of ketamine (Vetalar®, 100 mg/ml, Bioniche Canada Inc., Belleville, Ontario, Canada) and medetomidine (Domitor®, 1 mg/ml, Pfizer Canada Inc., Kirkland, Québec, Canada) at starting dosages of 5 mg/kg body weight and 0.05 mg/kg body weight, respectively. However, dosages were increased to 5.5 mg/kg body weight of ketamine and 0.055 mg/kg of medetomidine

after reflexes were maintained during anaesthesia with previous dosages. Once fully immobilized, the raccoon was removed from the trap and body length (measured from snout to vent, recorded to the nearest 1.0 cm), sex (male or female), and age class (juvenile or adult established from animal size and dental health) were determined. In both provinces, raccoons were marked with two ear tags (OMNRF: National Band and Tag Company©, Newport, Kentucky, USA; MFFPQ: Dalton ID Systems Ltd.©, Henley-on-Thames, Oxon, England), with an additional pit tag (AVID Canada©, Calgary, Alberta, Canada) added by the MFFPQ. At the completion of sample collection, the raccoon was placed back in the trap and injected with Atipamezole (OMNRF: Revertor™, 5 mg/mL, Veterinary Purchasing Company Limited, St. Marys, Ontario, Canada; MFFPQ: Antisedan®, 5 mg/mL, Pfizer Canada Inc., Kirkland, Québec, Canada) at a dosages of 0.35 mg/kg body weight (OMNRF) and 0.375 mg/kg (MFFPQ) to reverse the effects of the immobilization drugs. At full consciousness, the raccoon was returned to its capture site for release.

Statistical Analysis

All statistical analysis was completed using R (Version 1.3.159). Model results were displayed using partial residual plots in the R package *visreg* (Version 2.7.0), which isolates a relationship in a multiple regression between the response variable and one predictor variable while keeping all other variables constant (Breheny and Burchett 2020).

Body Condition Indices and Urbanization Scores

Body condition indices were calculated as residuals, which compares the observed mass of the individual to its predicted mass at a specific length. Residuals were calculated from an ordinary least squares (OLS) regression of log-transformed body mass against log-transformed body length to remove the heteroscedastic nature of the data (Schulte-Hostedde et al. 2005). An ‘urbanization score’ was calculated for each capture site to represent the effects of urbanization on body condition (Liker et al. 2008). To quantify the degree of urbanization, the occurrence of major land-cover types were scored, including buildings, roads, agriculture, vegetation cover, water, and barren land (Table 1.1) (Liker et al. 2008, Agriculture and Agri-Food Canada 2015). GPS coordinates were plotted on the Government of Canada’s Land Use 2010 map in ArcGIS (Version 10.6). A 1km x 1km buffer polygon (reflecting the average home range of a raccoon (Prange et al. 2004)) was created around each point, and was determined to be large enough to provide significant information on the urbanization of the landscape (Liker et al. 2008). To quantify the occurrence of each land-cover type at each capture site, total percentage area of each land-cover type within each 1km x 1km buffer polygon was calculated using the sum of their respective polygon types in that area (Seress et al. 2014). Data for ‘population density per square kilometer’ and ‘median household total income in 2015’ for each capture site were extracted using the 2016 Census subdivision data from Statistics Canada (<https://www150.statcan.gc.ca/n1/en/type/data>). Total percentages of land-cover types within a 1km x 1km buffer polygon, population density per square kilometer and median household total income data were scaled and inputted into a Principal Component Analysis (PCA), where the PC1 and PC2 scores extracted represented the ‘urbanization score’ for each site (Seress et al. 2014).

Model building

To determine the effect of urbanization on body condition, linear mixed-effect models were fitted using the lme4 package (Version 1.1-17) (Bates et al. 2014). Model assumptions were tested using residuals vs. fitted values plots, residual QQ plots, and residual histograms. With body condition as the dependent variable, fixed effects included PC1 and PC2 (indices of land use), and sex. Interaction terms between each continuous and categorical variable were included in the full model ($PC1 \times sex$, $PC2 \times sex$), and were removed in a backward elimination based on p-values at a significance level of 0.05. The fixed effects included in the final model were PC1, PC2, sex, and $PC1 \times sex$. Random effects included year (2009, 2018, 2019) nested in region (Québec, Ontario) because each year was unique to one region. Data from Québec in 2010 were excluded because different body length measurements (snout to end of tail) were taken from the remainder of the dataset (snout to vent). Significance of the fixed effects was tested using Type 3 Wald chi-square tests.

Based on the results from this initial model, analyses were also conducted separately within each region. A linear mixed model was used to analyze data within Ontario, including year (2018, 2019) as a random effect. Fixed effects included PC1, PC2, sex, and an interaction term between each continuous and categorical variable ($PC1 \times sex$, $PC2 \times sex$). Interaction terms were removed in a backward elimination process based on p-values at a significance level of 0.05 calculated by Type 3 Wald chi-square tests. No interaction terms were included in the final model. Analysis of covariance (ANCOVA) was used to analyze data within Québec while including only one year of data (2009) due to different body length measurements that were taken in 2010. Variables

in the full model included PC1, PC2, sex, and an interaction term between each continuous and categorical variable ($PC1 \times sex$, $PC2 \times sex$). Again, interaction terms were removed in a backward elimination process based on p-values at a significance level of 0.05 calculated by a Type 3 sum of squares, with the final model including no interaction terms.

Results

Data Handling and Sample Sizes

Urbanization scores were calculated using the full dataset, which included the capture sites of 838 individuals located in both Ontario and Quebec in the years 2009, 2010, 2018, and 2019. In Ontario, 276 and 273 individual raccoons were captured in 2018 and 2019, respectively. In Québec, 189 and 100 individuals were captured in 2009 and 2010, respectively. To calculate body condition indices, I used data from only the adult individuals. The final dataset used for body condition analyses included 567 individuals, with 210 individuals from Ontario in 2018, 178 individuals from Ontario in 2019, and 179 individuals from Québec in 2009. Again, data from Québec in 2010 was excluded from body condition analyses due to differences in body length measurements between regions and years.

The spatial distribution of sampling differed between Ontario and Québec as well. As shown in Figure 1.1, sampling within Ontario was more spatially regular compared to sampling in Québec, which was more dispersed. Additionally, as demonstrated in Figure 1.2, the Ontario sites varied more along the PC1 axis whereas Quebec sites varied more along the PC2 axis.

Urbanization Scores

Total percentages of land-cover types, population density per square kilometre, and median household income in 2015 were input into a PCA. The first principal component (PC1) explained 53.49% of the variation, distinguishing between urban and non-urban landscapes (Table 1.2, Figure 1.2). Higher PC1 values were associated with variables describing urbanized landscapes, which included buildings, roads, population density, and income (Table 1.2, Figure 1.2). Lower PC1 values were related to non-urban landscape measures and included agriculture, vegetation cover, and barren land (Table 1.2, Figure 1.2). The second principal component (PC2) explained 14.84% of the variation and differentiated agricultural and barren landscapes from natural environments (Table 1.2, Figure 1.2). As for PC2, a higher value was associated with variables describing agriculture and barren land, while a lower value was related to natural landscapes such as vegetation cover and water (Table 1.2, Figure 1.2).

Body Condition Indices

When data from both regions (Québec, Ontario) and all years (2009, 2018, 2019) were included in the model, I found a significant interaction between the effects of PC1 and sex on raccoon body condition (Table 1.3, Figure 1.3). Male body condition increased with increasing PC1 (i.e., with increasing urbanization), while female body condition declined with increasing PC1 (Figure 1.3). However, it is evident two clusters exist in Figure 1.3, which may be due to regional differences as seen by the separation of regions in PC space in Figure 1.2. To further inspect the data, analyses were conducted within each region.

Within Ontario, there was no significant interaction between PCs and sex, and raccoon body condition was positively related to PC1 (Table 1.4). Body condition of Ontario raccoons also declined with increasing PC2 (i.e., with increasing agricultural and barren land relative to natural landcover) (Table 1.4, Figure 1.4). Finally, body condition of Ontario raccoons was significantly higher in males than females (Table 1.4). In contrast, raccoon body condition was not significantly related to PC1, PC2 or sex in Québec (Table 1.5).

Discussion

My results varied with the geographic scale of analysis. When I analyzed all Ontario and Québec data together and accounted for regional and interannual variation, raccoon body condition was significantly related to an interaction between my urbanization index (PC1) and sex. When I analyzed Ontario data only, there was no significant interaction, and body condition exhibited a positive trend with PC1 and was significantly affected by PC2 and sex. While an effect of sex was not predicted in either model, these results did support the hypothesis that urbanization would have an effect on body condition. However, no significant effects were found when examining raccoon body condition in Québec. In any case, it should be noted that the statistically significant results presented have small effect sizes and interpretations should be made with caution due to the large sample size used (Sawilowsky 2009, Sullivan and Feinn 2012).

Effects of Urbanization and Sex

In a model incorporating both regional and yearly variation, body condition was significantly affected by the interaction between PC1 and sex, which did not support my prediction that both male and female raccoons would exhibit better body condition in

urban environments (Coon et al. 2019, Werner and Nunn 2020). Instead, as habitats changed from non-urban to urban environments, males were in better body condition and females were in poorer body condition. Regional analyses showed that males were in better body condition than females in all habitat types in Ontario, but not in Québec. Though an effect of sex was not predicted in a broad-scale or regional model, this could be associated with a few possible explanations.

Previous studies have shown that raccoons can invest into a second breeding season in the fall if the first one was unsuccessful (Gehrt and Fritzell 1996, Asano et al. 2003, Rosatte et al. 2006). My findings may suggest that reproductive strategies are sex-specific in areas containing predictable food resources, such as urban environments with anthropogenic food waste. As a result, males could be considered ‘capital’ breeders and rely on energy stores to facilitate reproduction while females may be ‘income’ breeders and consume food resources concurrently with the breeding season to ensure energy for reproductive demands, such as lactation (Stephens et al. 2014, Williams et al. 2017). My results could also suggest an effect of seasonality, as they were not consistent with a recent study that found raccoons exhibited a greater body mass with increased access to anthropogenic food waste in the summer (Schulte-Hostedde et al. 2018). Sampling for my study occurred in the fall, and previous studies have shown that raccoons may double their weight during this season in preparation for a body mass loss of up to 50% over winter (Pitt et al. 2008, McWilliams and Wilson 2015). In this case, male raccoons could be consuming enough food waste to support this mass loss in the winter (Pitt et al. 2008, McWilliams and Wilson 2015), while female raccoons could be investing into a second breeding season instead (Gehrt and Fritzell 1996, Asano et al. 2003, Rosatte et al. 2006).

Another factor that may influence sex-specific differences in body condition is home range size. Males may exhibit larger home ranges than females due to their polygynous mating behaviour and extensive movements in search of females (Gehrt and Frtzell 1997, Kamler and Gipson 2003). My study was based on data collected outside of the mating season for raccoons, and it is more likely that females are restricted in their movements while protecting their offspring in their dens as rearing is done alone (Gehrt and Frtzell 1997, Kamler and Gipson 2003). With larger home ranges, males are likely foraging more often and consuming a greater abundance of anthropogenic food waste in urban areas, leading to a better body condition than females. This result is similar to one found in a recent study showing that male urban lizards are more successful at foraging due to greater distances travelled, compared to female urban lizards, leading to males exhibiting a better body condition than females (Lazić et al. 2017). Overall, this result provides evidence that urbanization does impact body conditions of wildlife species on a broad scale.

When conducting finer-scale analyses in Ontario, a marginally non-significant effect of PC1 on body condition was found, where raccoons from urban environments tended to be in better body condition than those from non-urban environments. This supports the prediction that raccoons from urban environments would be in better body condition because the food sources are in greater abundance and contain higher amounts of carbohydrates and lipids compared to natural foods consumed by their non-urban counterparts (Santini et al. 2019, Coon et al. 2019). A recent study from Midwestern North America reported similar results, where raccoons exhibited higher body condition indices in urban environments due to their reliance on anthropogenic food waste

(Demeny et al. 2019). Additional resources provided by urban environments have also been shown to benefit the opossum (*Didelphis virginiana*), which were found to have a 34% greater body mass in urban areas than rural areas of Virginia (Wright et al. 2012). Although greater body condition indices and mass are generally considered a benefit to wildlife in terms of survival and reproduction, extremely high body condition indices and mass can have negative health consequences (Birnie-Gauvin et al. 2017, Demeny et al. 2019), as demonstrated by a recent study in Canada that showed increased access to anthropogenic food waste led to hyperglycemia in raccoons (Schulte-Hostedde et al. 2018).

The effects of anthropogenic landscape alterations on wildlife appear to depend on the nature of this change. I found that raccoons from areas with agriculture and barren land were in poorer body condition than those from areas with natural vegetation cover and water. Given that raccoons are opportunistic omnivorous species, I assumed they would be consuming corn in agricultural fields and barren land in close proximity to these fields, plus additional anthropogenic food waste from barren land in the cities, as previous studies have demonstrated (Schulte-Hostedde et al. 2018, Demeny et al. 2019, Osaki et al. 2019). These food resources found in human-modified landscapes differ from natural food sources in both quantity (i.e. greater abundance) and quality (i.e. carbohydrate, lipid, and protein composition), leading to the prediction that raccoons would be in better body condition in these same environments (Birnie-Gauvin et al. 2017). Though increased energy reserves are beneficial for survival, reproduction, and act as a buffer during times of food shortages, it is also associated with metabolic and locomotive costs (Schulte-Hostedde et al. 2005, Meillère et al. 2015). Therefore, this

finding does support the hypothesis that raccoons from areas with a predictable food source would be in poorer body condition to avoid unnecessary stresses (Schulte-Hostedde et al. 2005, Meillère et al. 2015). Similar results were reported in an urban exploiter study of house sparrows (*Passer domesticus*) that were found to have reduced body size and body mass in urban areas, compared to rural environments (Meillère et al. 2015). However, other indices of sparrow body condition were not found to be different between urban and rural habitat types (Meillère et al. 2015). These inconsistent results demonstrate the need for numerous body condition measurements and physiological biomarkers to be accounted for in future studies to fully understand the impacts of urbanization on wildlife.

No Effect of Urbanization

Further regional analyses showed that urbanization did not have the same impact on raccoons within Québec, as there were no significant effects found. This may be due to the limited variation in the sites selected, as the distribution along the urbanization gradient (PC1) showed that the majority of sites were non-urban and did not include samples from major urban centres or residential areas, compared to samples collected from Ontario (Figure 1.2). Alternatively, or in addition, dissimilarities between Ontario and Québec municipal regulations, such as garbage collection and disposal, may lead to regional differences in availability of anthropogenic food waste and in turn, body condition patterns of urban wildlife between the two provinces.

Conclusion

My study results suggest that sex-specific changes occur in body condition with urbanization and can be used as a preliminary model for researchers globally to understand the effects of urbanization in various species. Future research can aim to understand the health risks associated with land-use changes in wildlife, as this could have important implications for conservation (Bruskotter et al. 2017). For example, an animal that is in extremely good body condition when consuming anthropogenic food waste in urban environments may also be experiencing negative health consequences such as obesity (Birnie-Gauvin et al. 2017, Demeny et al. 2019) or hyperglycemia (Schulte-Hostedde et al. 2018). Alternatively, animals may be adapting to the consumption of additional carbohydrates and lipids from anthropogenic food waste in human-modified landscapes (Harris and Munshi-South 2017). Recent research has shown that white-footed mice (*Peromyscus leucopus*) found in urban areas have genetically adapted to the consumption of excessive lipids and carbohydrates (Harris and Munshi-South 2017), which may be the case in raccoons as well.

From a management standpoint, the interactions between humans and wildlife can be improved by developing more effective waste management policies. For example, it has been shown that reducing the amount of garbage and ornamental fruits have reduced the number of coyotes living in an area (Larson et al. 2020). If similar results such as these are found in other wildlife species such as raccoons, implementing better waste management policies could benefit the animal in terms of health, while also reducing human-animal conflict.

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Table 1.1: Description of each land-cover type from the 2010 Land Use Map of Canada
(Agriculture and Agri-Food Canada 2015).

Land Use Type	Description
Buildings	Settlement
Roads	Primary, secondary, and tertiary
Water	Natural and man-made (e.g., reservoirs)
Agriculture	Cropland Pastureland (Livestock grazing fields)
Vegetation Cover	Forest Wetland Areas covered in grasses and shrubs for no apparent use
Barren Land	Unused land in agricultural and residential areas Industrial yards Natural rock outcrops (e.g., shield, alvars)

Table 1.2: PC factor loadings for PC1 and PC2 from the PCA of land-cover types, population density, and income variables within a 1-km² buffer of each raccoon capture site (n = 838).

Variable	PC1	PC2
Buildings	0.463	0.110
Roads	0.442	0.153
Agriculture	-0.371	0.410
Vegetation Cover	-0.283	-0.557
Water	-0.072	-0.381
Barren Land	-0.167	0.567
Population Density/km ²	0.433	-0.004
Median Household Total Income	0.390	-0.140
% Variation Explained	53.49	14.84

Table 1.3: Summary of fixed effects for linear mixed model (package ‘lme4’) of raccoon body condition (residual mass at length) as a function of various predictor variables.

Model was fitted using data collected in southern Québec in 2009 and southern Ontario in 2018 and 2019 (n = 567). Principal components of landscape features (PC1, PC2) are described in Table 1.2. Random effects in model (not shown) included region (Québec, Ontario) and year (2009, 2018, 2019) nested within region. Type 3 Wald chi-square tests were used to test for significance (* $p < 0.05$).

Variable	Estimate	SE	X²	df	<i>p</i>
PC1	-0.001	0.006	0.010	1	0.920
PC2	-0.009	0.008	1.342	1	0.247
Sex	-0.007	0.007	1.077	1	0.299
PC1*Sex	-0.009	0.003	6.464	1	0.011*

Table 1.4: Summary of fixed effects for linear mixed model (package ‘lme4’) of raccoon body condition (residual mass at length) as a function of various predictor variables.

Principal components of landscape features (PC1, PC2) are described in Table 1.2.

Model was fitted using data collected from southern Ontario in 2018 and 2019 (n = 389), with year included as a random effect. Type 3 Wald chi-square tests were used to test for significance of fixed effects (* $p < 0.05$).

Variable	Estimate	SE	X²	df	<i>p</i>
PC1	0.016	0.009	3.350	1	0.067
PC2	-0.044	0.018	6.014	1	0.014*
Sex	-0.021	0.009	5.585	1	0.018*

Table 1.5: Summary of a one-way ANCOVA for raccoon body condition (residual mass at length) as a function of various predictor variables. Principal components of landscape features (PC1, PC2) are described in Table 1.2. Model was fitted using data collected from southern Québec in 2009 (n = 179). Significance tested using Type 3 sums of squares (* $p < 0.05$).

Variable	Estimate	SE	Partial-F	df	<i>p</i>
PC1	0.000	0.018	0.000	1, 175	0.996
PC2	-0.003	0.008	0.120	1, 175	0.729
Sex	0.010	0.011	0.813	1, 175	0.368

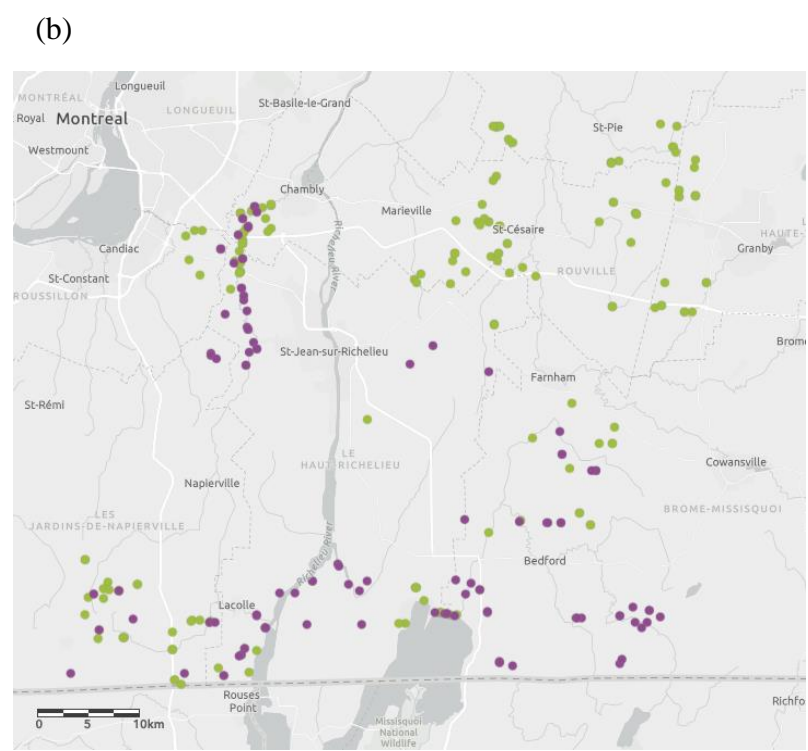
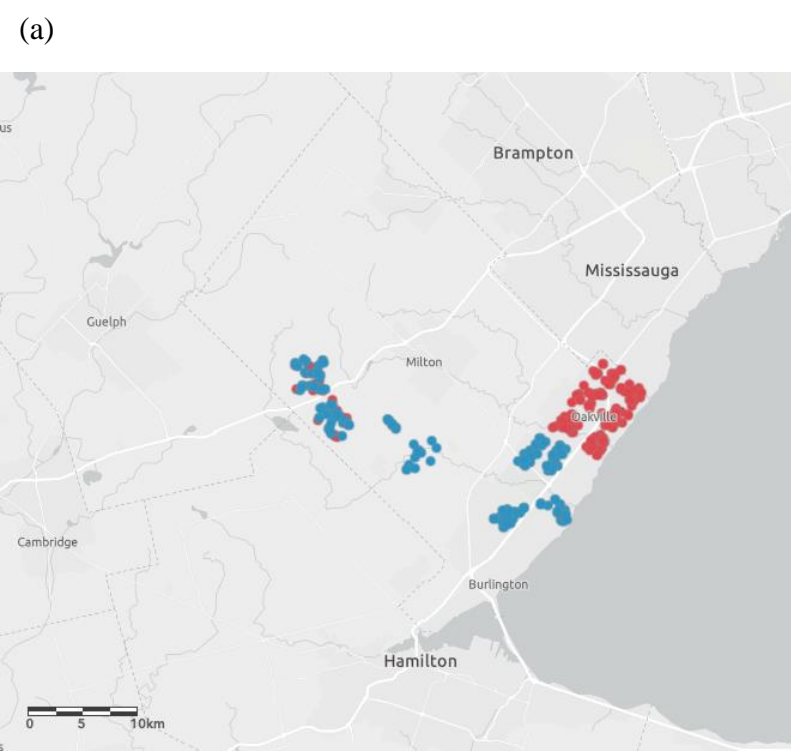


Figure 1.1: Map showing raccoon capture sites within study regions of (a) southern Ontario for 2018 (n = 276, ●) and 2019 (n = 273, ●) and (b) southern Québec for 2009 (n = 189, ●) and 2010 (n = 100, ●).

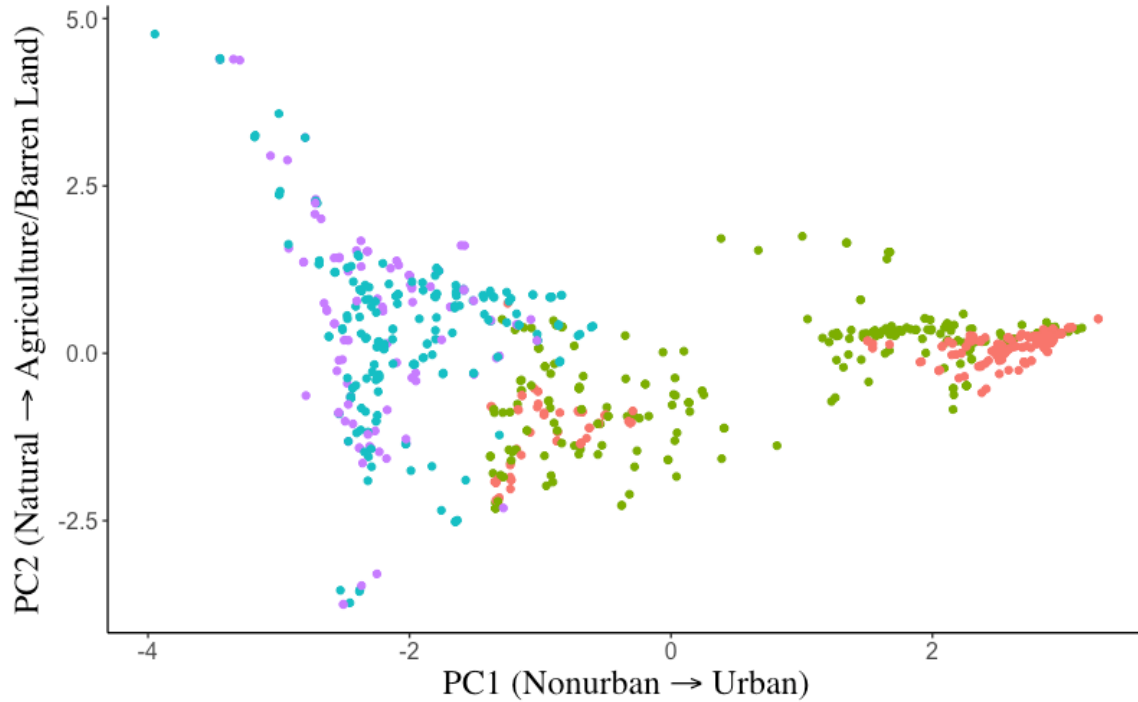


Figure 1.2: Distribution of urbanization scores at each raccoon capture site, representing non-urban to urban (PC1) and natural to agricultural/barren land (PC2) environments.

Principal components of landscape features (PC1, PC2) are described in Table 1.2.

Individuals were captured in southern Ontario in 2018 (n = 276, ●) and 2019 (n = 273, ●) and in southern Québec in 2009 (n = 189, ●) and 2010 (n = 100, ●).

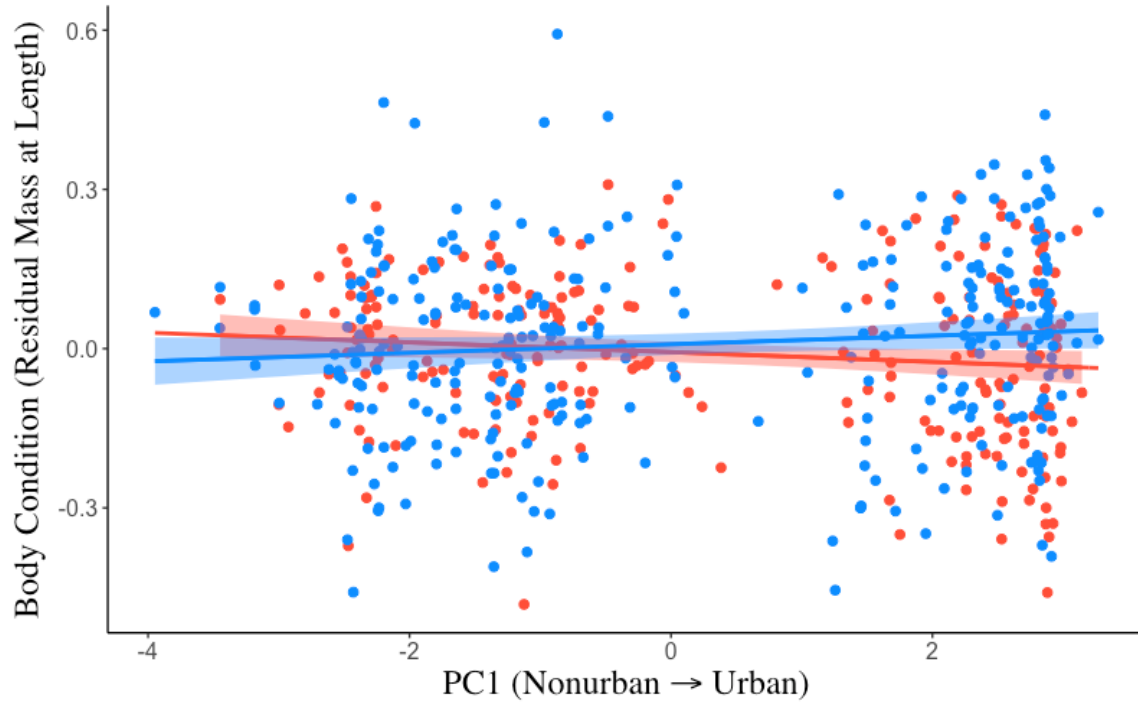


Figure 1.3: Partial residual plot of raccoon body condition (residual mass at length) vs. landscape PC1 score for adult males (blue) and females (red) sampled in Québec in 2009 and Ontario in 2018 and 2019 ($n = 567$, $p = 0.011$). Lines are fitted OLS regressions and shaded areas are 95% confidence intervals. Slopes differed significantly between sexes (Table 1.3).

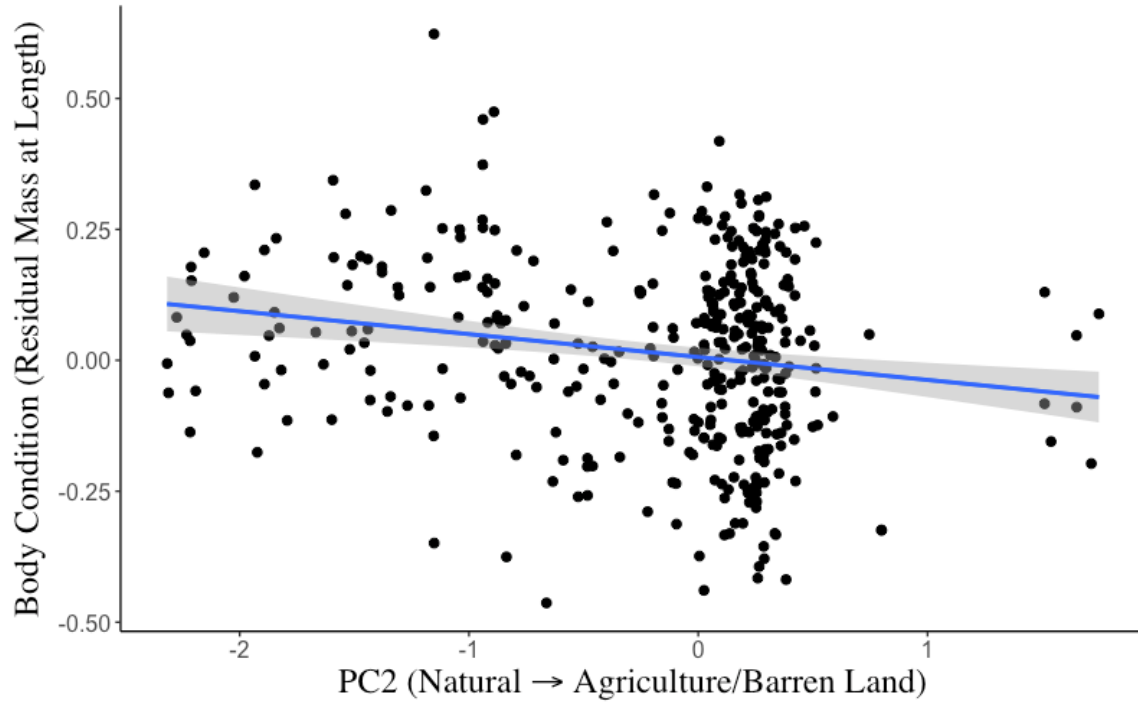


Figure 1.4: Partial residual plot of raccoon body condition (residual mass at length) vs. landscape PC2 score for individuals (sexes combined) sampled in Ontario in 2018 and 2019 ($n = 389$, $p = 0.014$). Line is fitted OLS regression and shaded area is 95% confidence interval.

Chapter 2: Effects of anthropogenic food subsidies on dietary patterns, glucose metabolism, and fat metabolism in raccoons
(Procyon lotor)

Abstract

Urbanization is expanding globally with humans modifying natural landscapes that wildlife species have previously adapted to and altering resources and habitat use. Although some species do not survive, many thrive in these urbanized environments with a flexible diet, which includes the consumption of anthropogenic food waste that may have associated physiological consequences. This research investigates the effects of consuming human food subsidies on dietary patterns, glucose metabolism, and fat metabolism in raccoons (*Procyon lotor*) across an urbanization gradient in Ontario. Dietary patterns were interpreted from carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios that reflect the original sources of dietary carbon and animal protein consumption, respectively. Glucose metabolism was measured using glycated serum proteins (GSP) to attain average glucose levels over a 2-3 week period. Fat metabolism was determined using leptin, a hormone released by adipose cells to regulate the storage of fats. Results showed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were greatest in areas of agriculture and barren landscapes, meaning that raccoons likely consumed C_4 plants (e.g. corn) and animal protein during the fall sampling period. GSP levels were also elevated in areas of agriculture and barren land, though leptin was not. This research can provide information in the field of conservation and a preliminary model for researchers globally to understand the effects of urbanization on wildlife. It can also play a role in the development of protocols to prevent crop damage from raccoons, both reducing conflict between humans and wildlife in human-modified landscapes while improving animal health.

Keywords: urban ecology, anthropogenic food waste, stable isotope, glycated serum protein, leptin

Introduction

The process by which natural landscapes of vegetation, soil, and water are modified by humans to include infrastructure and roads is called ‘urbanization’ (Haverland and Veech 2017). Cities are expanding globally to accommodate the growing human population and consequently causing rapid changes to natural environments that many wildlife species have previously adapted to survive within (Reichert et al. 2017, Stark et al. 2020). Some species may not be able to adapt to these drastic changes, and recent studies have been conducted to answer questions related to the evolutionary consequences associated with human-modified landscapes (Shochat et al. 2006, Gibbs et al. 2019).

As previous studies have shown, urban landscapes impact ecological factors such as predator-prey relationships (Eötvös et al. 2018, Jokimäki et al. 2020), spread of pests and diseases (Gordon et al. 2016, Soulsbury and White 2016), and spatial distribution of wildlife populations (Ehlers Smith et al. 2018, Zungu et al. 2020). Since some species cannot adapt to the changing conditions, urbanization has been shown to have a negative impact on biodiversity as populations of sensitive species decline (Łopucki and Kitowski 2017, Schmidt et al. 2020). However, other species thrive in urban environments, such as mesocarnivores, because of their opportunistic and omnivorous feeding habits that allow them to exploit associated novel food resources (Newsome et al. 2015, Larson et al. 2020).

Urbanization can impact the abundance and availability of food resources in an area (Bateman and Fleming 2012, Murray et al. 2015, Magioli et al. 2019). However, a common misconception is that landscapes can be described categorically, such as

‘urban’, ‘agricultural’, or ‘natural’, when actually a gradient of urbanization and disturbance provides a more realistic interpretation of habitat conditions (Seress et al. 2014, Suarez-Rubio and Krenn 2018). One method to develop a representative urbanization gradient is by including information on landscape characteristics and demographics, which should reflect the quality (i.e. carbohydrate, lipid, and protein composition) and quantity of food in an area (Seress et al. 2014, Suarez-Rubio and Krenn 2018). Land use characteristics include buildings, roads, agriculture, vegetation, water, and barren land. In addition to land use patterns, food resources may change based on human population density and socioeconomic status (Mckinney 2001, Luck 2007, Magle et al. 2016). Human population density of an area can serve as a measure of food resource accessibility because greater amounts of food waste are typically found in areas occupied by more people (Mckinney 2001, Luck 2007). Socioeconomic status can also provide insight into available food resources as waste generation patterns follow income levels, where wealthier areas generally provide a more stable food source that is nutritionally similar to food from natural environments in terms of carbohydrates, lipids, and proteins (Magle et al. 2016).

Raccoons (*Procyon lotor*) are considered mesocarnivores that have adapted to live in many different habitat types due to their opportunistic nature and omnivorous diet. Their flexible diet allows them to successfully feed on anthropogenic food waste in human-modified landscapes (Thornton et al. 2020), or small mammals, berries, invertebrates, fish and nuts from more natural habitats (Bartoszewicz et al. 2008). Composition of macronutrients is likely different depending on the diet the animal is consuming, where an urban diet is higher in carbohydrates and lipids (Murray et al. 2015,

2018). Though a high proportion of carbohydrates are present in a natural diet as well, it is speculated that the type of carbohydrates present in each diet is different. Specifically, roughage carbohydrates (i.e. fiber) are thought to be present mainly in a natural diet and energy carbohydrates (e.g. refined starches, sugars) within an urban diet. Research has examined how urbanization impacts raccoons on a population level (Prange et al. 2003, 2004, Prange and Gehrt 2004), but little is known about how human-modified environments with food resources of different nutritional quality (i.e. carbohydrate, lipid, and protein composition) and quantity impact the health of individual raccoons. Evidence thus far has shown that raccoons consuming food resources within an urbanized environment may suffer from dental caries and reduced periodontal health (Hungerford et al. 1999), and may also exhibit effects of hyperglycemia (Schulte-Hostedde et al. 2018).

Urbanization can influence an animal's dietary patterns, which can be assessed using stable isotope analysis. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios are commonly used to provide insights into an animal's long-term feeding strategies (DeNiro and Epstein 1978, 1981, Boecklen et al. 2011, Layman et al. 2012) and can be used to quantify access to, or consumption of, anthropogenic food waste. Specifically, ratios of heavy to light isotopes for carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$), denoted by delta (δ), are used to determine an animal's sources of primary production and trophic position, respectively (DeNiro and Epstein 1978, 1981). Isotopic signatures provide time-integrated measures of assimilated diet when the tissue tested was metabolically active (Miller et al. 2008, Kays and Feranec 2011). For example, hair corresponds to the period of hair growth while bone collagen is an average reflection over a longer time period, such as 20 years (Miller et al. 2008, Boecklen et al. 2011, Kays and Feranec 2011).

Carbon stable isotope ratios ($\delta^{13}\text{C}$) are used to provide insight on the ultimate sources of dietary carbon, and can be applied to distinguish plant materials found in natural and urban environments (Magioli et al. 2019). Natural environments provide foods derived primarily from C_3 plants (e.g., woody trees) that provide more negative $\delta^{13}\text{C}$ values (i.e., more ^{13}C -depleted), while urban environments may include relatively more foods derived from agricultural C_4 plants (e.g., corn) that exhibit more positive $\delta^{13}\text{C}$ signatures (DeNiro and Epstein 1978, Tieszen et al. 1983). Since many human foods are rich in corn (e.g. snack foods, sweeteners, cereals, flour-based products, meat and dairy feed), isotopic signatures should reflect these differences if animals are consuming these products (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019).

Nitrogen stable isotope ratios ($\delta^{15}\text{N}$) are used to determine an organism's trophic position ranging from primary producer to carnivore, or the amount of animal protein consumed (DeNiro and Epstein 1981, Minagawa and Wada 1984). Selective retention of ^{15}N as ^{14}N is excreted, leads to an enriched $\delta^{15}\text{N}$ signature with successive trophic transfers, specifically by 3-4 ‰ for each trophic level (Ambrose 1991, Kelly 2000). These differences in isotopic signatures will appear if animals from urban environments are consuming protein from higher trophic levels (e.g. animal feed, garbage, human food waste) than their counterparts in natural environments (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019).

Glucose metabolism may also be influenced by urbanization. Changes in glucose can be demonstrated by glycated serum proteins (GSP), formed through a nonenzymatic reaction between a monosaccharide (most commonly glucose) and the amino group of a protein (Welsh et al. 2016). GSP can provide insight into an animal's glycemic control as

measurements reflect average blood glucose concentrations over a 2-3 week period, compared to glucose measurements that can vary hourly depending on the recent food consumed (Welsh et al. 2016). Previous studies have shown that animals from areas of increasing urbanization with easier access to simple sugars (i.e. glucose) from anthropogenic food waste exhibit greater GSP concentrations, demonstrating differences in glycemic control with various diets (McCain et al. 2008, Schulte-Hostedde et al. 2018).

Leptin is associated with adiposity in various species (Banks et al. 2001, Shibata et al. 2005, Spady et al. 2009), and is also expected to be higher in animals living in more urbanized environments due to greater access to anthropogenic food waste that contains more fats compared to natural food sources (Schulte-Hostedde et al. 2018). Leptin is a hormone released by adipose cells to regulate feeding behaviour by interacting with the central nervous system through the blood-brain barrier (Zhang et al. 1994, Friedman and Halaas 1998). Leptin resistance occurs at chronically elevated levels of leptin, and results in the onset of diet-induced obesity as interactions with the brain are inhibited (Münzberg et al. 2005, Myers et al. 2012).

This research investigated dietary patterns, glucose metabolism, and fat metabolism in raccoons (*Procyon lotor*) across a gradient of natural and anthropogenically modified landscapes. The raccoon is an opportunistic and omnivorous mesocarnivore that is widespread and common across many habitat types in North America (Thornton et al. 2020). I predicted that raccoons in more urban habitats would exhibit higher $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, GSP, and leptin because of their greater reliance on human-produced food sources.

Materials and Methods

Study Area and Field Sampling

Field work was conducted in collaboration with the Ontario Ministry of Natural Resources and Forestry (OMNRF) Rabies Unit and Dr. Fanie Pelletier with the Ministère des Forêts, Faune et Parcs du Québec (MFFPQ) and its partners. OMNRF samples were collected from southern Ontario in October 2018 and 2019 and those from MFFPQ were collected from southern Québec in October 2009 and 2010 (Figure 1.1).

In Ontario, raccoons were captured with live traps (Tomahawk Model 106/108, Hazelhurst, Wisconsin, USA) baited with canned sardines (*Sardina pilchardus*). In Québec, raccoons were also captured with live traps (Havahart® Products, Lititz, Pennsylvania, USA) but baited with fresh mackerel (*Scomber scombrus*) or canned sardines and canned corn. A hand-held global positioning system (GPS) was used to obtain locations of each animal trapped and traps were opened daily. Once captured, raccoons were transported to a processing station where they were weighed in their traps to the nearest 0.1 kg and anaesthetized using an intramuscular injection. The OMNRF used a mixture of Telazol (Telazol®, 100 mg/mL, Zoetis Canada Inc., Kirkland, Québec, Canada) and medetomidine (Cepetor™, 1 mg/mL, Veterinary Purchasing Company Limited, St. Marys, Ontario, Canada) at dosages of 1.7 mg/kg body weight and 0.07 mg/kg body weight, respectively. The MFFPQ used a mixture of ketamine (Vetalar®, 100 mg/ml, Bioniche Canada Inc., Belleville, Ontario, Canada) and medetomidine (Domitor®, 1 mg/ml, Pfizer Canada Inc., Kirkland, Québec, Canada) at starting dosages of 5 mg/kg body weight and 0.05 mg/kg body weight, respectively. However, dosages were increased to 5.5 mg/kg body weight of ketamine and 0.055 mg/kg of medetomidine

after reflexes were maintained during anaesthesia with previous dosages. Once fully immobilized, the raccoon was removed from the trap and body length (measured from snout to vent, recorded to the nearest 1.0 cm), sex (male or female), and age class (juvenile or adult established from animal size and dental health) were determined. In both provinces, raccoons were marked with two ear tags (OMNRF: National Band and Tag Company©, Newport, Kentucky, USA; MFFPQ: Dalton ID Systems Ltd.©, Henley-on-Thames, Oxon, England), with an additional pit tag (AVID Canada©, Calgary, Alberta, Canada) added by the MFFPQ. In Ontario, guard hair samples were collected from the animal for stable isotope analysis. Blood samples of 3-5 mL were taken from the sub-clavian vein using a vacutainer needle complex, stored at 4°C overnight, and centrifuged at 1000 x g at 4°C for 12 minutes to attain serum samples used for GSP and leptin laboratory analysis. Blood was collected with the MFFPQ as well, but was not used in this study due to improper storage techniques. Serum was not extracted prior to storing, causing the samples to become hemolyzed. Therefore, the plate reader was unable to detect levels of GSP and leptin in the assay. Regardless, at the completion of sample collection, the raccoon was placed back in the trap and injected with Atipamezole (OMNRF: Revertor™, 5 mg/mL, Veterinary Purchasing Company Limited, St. Marys, Ontario, Canada; MFFPQ: Antisedan®, 5 mg/ml, Pfizer Canada Inc., Kirkland, Québec, Canada) at a drug dosage of 0.35 mg/kg body weight at OMNRF and 0.375 mg/kg at MFFPQ to reverse the effects of the immobilization drugs. At full consciousness, the raccoon was returned to its capture site for release.

Laboratory Analysis

Carbon and Nitrogen Stable Isotope Analysis

For $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses, 0.5–1.0 mg of hair material was combusted online using a Eurovector 3000 (Milan, Italy – www.eurovector.it) elemental analyzer. The resulting CO_2 and N_2 was separated by gas chromatography (GC) and introduced into a Nu Horizon (Nu Instruments, Wrexham, UK – www.nu-ins.com) triple-collector isotope-ratio mass-spectrometer via an open split and compared to a pure CO_2 or N_2 reference gas. Stable nitrogen ($^{15}\text{N}/^{14}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$) isotope ratios were expressed in δ notation, as parts per thousand (‰) deviation from the primary standards, atmospheric AIR and Vienna Pee Dee Belemnite (VPDB), respectively. Using previously calibrated internal laboratory standards (powdered keratin [BWB II: $\delta^{13}\text{C} = -20.0\text{‰}$, $\delta^{15}\text{N} = -14.1\text{‰}$ and gelatin: $\delta^{13}\text{C} = -13.6\text{‰}$, $\delta^{15}\text{N} = -4.7\text{‰}$]) within run ($n = 5$), precision for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ measurements were $\sim \pm 0.15\text{‰}$.

Glycated Serum Protein and Leptin Analysis

A commercial fructosamine assay kit (Abcam©, Toronto, Ontario, Canada) and a commercial canine leptin enzyme immunosorbent assay (Millipore Sigma, Oakville, Ontario, Canada) were used to determine the serum concentrations of glycated serum protein and leptin, respectively. Each analysis was performed in duplicate.

To determine GSP concentrations, a microplate-based calorimetric assay with wells containing samples, background, and fructosamine calibrators was used to measure fructosamine concentrations. Concentrations were based on the ability of fructosamine to reduce nitroblue tetrazolium (NBT) to a purple end-product under alkaline conditions. The formation of formazan, the end-product of NBT reduction, is proportional to

fructosamine concentration in the sample and its absorbance at 530nm is measured using a spectrophotometer at 5 and 15 minutes. The difference in absorbance's between these times was used in further calculations to attain fructosamine concentrations.

Leptin concentrations were measured using a direct Sandwich ELISA as these have been previously validated for use in raccoons (Shibata et al. 2005). The microplate was coated with Goat anti-Canine leptin antibody, where the following reagents were added for binding in sequential order: serum samples/standards, detection antibody (biotinylated Goat anti-Canine leptin antibody), and enzyme solution (streptavidin-horseradish peroxidase). A wash to remove unbound materials was completed after addition. Then, a substrate solution (3,3',5,5'-tetramethylbenzidine) is added to develop a blue colour, with its intensity being proportional to the concentration of leptin in the sample. At optimal colour development, a stop solution (0.3 HCl) is added to terminate further reactions, and samples should turn yellow after acidification. At this point, absorbance is measured at 450nm and 590nm using a spectrophotometer and leptin concentrations in the sample are interpolated from a reference curve using the known leptin concentrations of the standards in the assay.

Statistical Analysis

All statistical analysis was completed using R (Version 1.3.959). Results of each model are visualized using partial residual plots in the R package *visreg* (Version 2.7.0) (Breheny and Burchett 2020). Each plot isolates a relationship between the response variable and one explanatory variable, while holding other explanatory variables at a constant (Breheny and Burchett 2020).

Body Condition Indices and Urbanization Scores

Body condition was calculated to include as an independent variable in each model to determine its effect on the response variables. Only adult data were used in this calculation. Data from Québec in 2010 was excluded because different body length measurements (snout to end of tail) were taken from the remainder of the dataset (snout to vent). The final dataset included 567 individuals, with 210 individuals from Ontario in 2018, 178 individuals from Ontario in 2019, and 179 individuals from Québec in 2009. Though individuals from Québec were not included in analyses of stable isotopes, GSP, or leptin, the area was accounted for when calculating body condition indices to attain a larger sample size and ultimately, a more accurate representation of body condition changes both regionally and yearly. As per Chapter 1, body condition indices were calculated as mass-size residuals, which compares the observed mass of the individual to its predicted mass at a specific length. Residuals were calculated from an ordinary least squares (OLS) regression using log-transformed body mass and log-transformed body length (Schulte-Hostedde et al. 2005).

Urbanization scores were calculated using the full dataset, which included 838 individuals. From Ontario I collected 276 individuals in 2018 and 273 individuals in 2019, while from Québec I collected 189 individuals in 2009 and 100 individuals in 2010. Though individuals from Québec were not included in analyses of stable isotopes, GSP, or leptin, the area was accounted for when calculating urbanization scores to attain a more global representation of landscape variation. As per Chapter 1, an ‘urbanization score’ was calculated for each capture site to represent the effects of urbanization on body condition (Liker et al. 2008). To quantify the degree of urbanization, the occurrence

of major land-cover types were scored, including buildings, roads, agriculture, vegetation cover, water, and barren land (Table 1.1) (Liker et al. 2008, Agriculture and Agri-Food Canada 2015). GPS coordinates were plotted on the Government of Canada's Land Use 2010 map in ArcGIS (Version 10.6). A 1km x 1km buffer polygon (reflecting the average home range of a raccoon (Prange et al. 2004)) was created around each point, and was determined to be large enough to provide significant information on the urbanization of the landscape (Liker et al. 2008). To quantify the occurrence of each land-cover type at each capture site, total percentage area of each land-cover type within each 1km x 1km buffer polygon was calculated using the sum of their respective polygon types in that area (Seress et al. 2014). Data for 'population density per square kilometer' and 'median household total income in 2015' for each capture site were extracted using the 2016 Census subdivision data from Statistics Canada (<https://www150.statcan.gc.ca/n1/en/type/data>). Total percentages of land-cover types within a 1km x 1km buffer polygon, population density per square kilometer and median household total income data were scaled and inputted into a Principal Component Analysis (PCA), where the PC1 and PC2 scores extracted represented the 'urbanization score' for each site (Seress et al. 2014).

Carbon and Nitrogen Stable Isotope Analysis

Analyses for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were based on a subset of 64 adult individuals sampled from Ontario in 2018. An analysis of covariance (ANCOVA) was used to determine how each of these ratios changed with urbanization. Model assumptions were tested using residuals vs. predicted plots, residual QQ plots, and residual histograms. For each dependent variable, the full model included PC1, PC2, body condition, sex, and an

interaction between each continuous and categorical variable ($PC1 \times sex$, $PC2 \times sex$, $body\ condition \times sex$). Interaction terms were removed in a backward elimination process based on p-values at a significance level of 0.05 calculated by a Type 3 sum of squares, with the final model including no interaction terms.

Glycated Serum Protein (GSP) Analysis

A total of 88 adults were used for GSP analyses from Ontario in 2018 ($n = 43$) and 2019 ($n = 45$). A linear mixed-effects model in the lme4 package (Version 1.1-17) (Bates et al. 2014) was used to determine how GSP varies with landscape conditions. Model assumptions were tested using residuals vs. predicted plots, residual QQ plots, and residual histograms. Potential pipetting errors during laboratory work were identified as outliers through visual examination of residuals plots and were removed. With GSP as the dependent variable, model fixed effects included PC1, PC2, body condition, sex, and interaction terms for each $sex \times covariate$ combination ($PC1 \times sex$, $PC2 \times sex$, $body\ condition \times sex$). The random effect included in the model was year (2018, 2019). Interaction terms were removed in a backward elimination process based on p-values at a significance level of 0.05, with the final model including no interaction terms. Significance of the fixed effects was tested using Type 3 Wald chi-square tests.

Leptin Analysis

A total of 65 adults were used for leptin analyses from Ontario in 2018 ($n = 40$) and 2019 ($n = 25$). A generalized linear mixed-effects model using a gamma distribution with a log link function in the lme4 package (Version 1.1-17) (Bates et al. 2014) was used to determine how leptin varies with landscape conditions. Model was fitted with a

gamma distribution to account for non-normal data, determined when testing model assumptions using a residual vs. fitted values plot, residual QQ plot, and residual histogram. With leptin as the dependent variable, model fixed effects included PC1, PC2, body condition, sex, and interaction terms for each sex \times covariate combination (PC1 \times sex, PC2 \times sex, body condition \times sex). The random effect included in the model was year (2018, 2019). Interaction terms were removed in a backward elimination process based on p-values at a significance level of 0.05, with the final model including no interaction terms. Significance of the fixed effects was tested using Type 3 Wald chi-square tests.

Results

Urbanization Scores

Results are the same as those presented in Chapter 1. Total percentages of land-cover types, population density per square kilometre, and median household income in 2015 were input into a PCA. The first principal component (PC1) explained 53.49% of the variation, distinguishing between urban and non-urban landscapes whose metrics were associated with positive and negative factor loadings, respectively (Table 1.2, Figure 1.2). Higher PC1 values were associated with metrics describing urbanized landscapes, which included buildings, roads, population density, and income (Table 1.2, Figure 1.2). Lower PC1 values were related to non-urban landscape measures and included agriculture, vegetation cover, and barren land (Table 1.2, Figure 1.2). The second principal component (PC2) explained 14.84% of the variation and differentiated agricultural and barren landscapes from natural environments whose metrics were associated with positive and negative factors loadings, respectively (Table 1.2, Figure 1.2). As for PC2, a higher value was associated with metrics describing agriculture and

barren land, while a lower value was related to natural landscapes such as vegetation cover and water (Table 1.2, Figure 1.2).

Carbon and Nitrogen Stable Isotope Analysis

I found a significant negative relationship between raccoon hair $\delta^{13}\text{C}$ and PC1, indicating that raccoons consumed a more ^{13}C -depleted diet in more urban environments (Table 2.1, Figure 2.1). In addition, $\delta^{13}\text{C}$ increased significantly with PC2 where raccoons from environments of agriculture or barren land had higher $\delta^{13}\text{C}$ signatures than those from natural environments (Table 2.1, Figure 2.2). Finally, $\delta^{13}\text{C}$ increased significantly with body condition (Table 2.1). In contrast to $\delta^{13}\text{C}$, I found that $\delta^{15}\text{N}$ was only significantly related to PC2; the $\delta^{15}\text{N}$ of raccoon hair increased with PC2 indicating higher trophic position in areas of agriculture and barren land compared to natural environments (Table 2.2, Figure 2.3).

Glycated Serum Protein (GSP)

GSP was significantly negatively related to PC1, where raccoons from more urban environments had lower GSP concentrations than those from less urban environments (Table 2.3, Figure 2.4). In addition, GSP increased significantly with PC2, indicating that raccoons from areas of agriculture and barren land exhibited higher concentrations of GSP than those from more natural environments (Table 2.3, Figure 2.5). Finally, GSP concentrations varied significantly between sexes, where males had higher GSP concentrations than females (Table 2.3).

Leptin

Leptin was significantly related to body condition, where raccoons that were in better body condition generally had higher leptin levels (Table 2.4). However, leptin levels were not significantly related to either PC1 or PC2 (Table 2.4).

Discussion

My prediction that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of raccoon hair, and GSP of raccoon blood serum, would be higher in more urban areas was not supported by this analysis. However, my results did indicate that $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and GSP were all related to landscape characteristics and greatest in areas of agriculture and barren land. In addition, my analysis demonstrated that $\delta^{13}\text{C}$ and leptin increased with body condition, as expected. To my surprise, GSP also differed between sexes, with males having significantly greater GSP concentrations than females. Urbanization was found to have no effect on raccoon serum leptin concentrations, which was not predicted.

Effect of Urbanization

Analysis of dietary patterns revealed that raccoons from areas of agricultural disturbance showed the greatest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, exhibiting a diet high in C_4 plant materials and animal protein, respectively. In regards to $\delta^{13}\text{C}$ specifically, I predicted that raccoon diet would be more dependent on C_4 plant sources, and thus raccoon tissues would have higher $\delta^{13}\text{C}$, in human-altered landscapes, as reported in previous studies (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019). For example, a study found that San Joaquin kit foxes (*Vulpes macrotis mutica*) from urban areas had higher $\delta^{13}\text{C}$ values than those from nonurban areas (Newsome et al. 2010). These urban kit foxes were found to have similar signatures to human residents of the area as well, providing evidence of a shared food resource (Newsome et al. 2010). In

contrast, my results were mixed. The observed negative relationship between raccoon $\delta^{13}\text{C}$ and my index of urbanization (PC1) runs counter to my prediction. However, the observed positive relationship between $\delta^{13}\text{C}$ and my index of agricultural disturbance (PC2) does support this prediction. With corn being a more readily available food source during the fall sampling period, raccoons may be preferentially consuming this resource, as they are an opportunistic and omnivorous species (Demeny et al. 2019). Additionally, their annual molt occurs simultaneously to this diet change and the hair sampled is likely reflecting this food intake (Demeny et al. 2019). As for $\delta^{15}\text{N}$, I found that raccoon $\delta^{15}\text{N}$ increased significantly with PC2, where raccoons from areas of agriculture and barren land had enriched $\delta^{15}\text{N}$, compared to those from natural environments. This does not follow the prediction that $\delta^{15}\text{N}$ would increase along an urbanization gradient (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019). One possible explanation is that raccoons are consuming protein of a higher trophic level in areas of agriculture and barren land, such as meat, dairy, or egg products (Aoki et al. 2017). For example, a recent study in Japan found that raccoon dogs (*Nyctereutes procyonoides*) consumed $\delta^{15}\text{N}$ -rich prey (e.g. salamanders, frogs, snakes) in areas of forest core and forest periphery, but those from forest periphery exhibited greater $\delta^{15}\text{N}$ signatures due to the consumption of garbage, anthropogenic food waste, and corn as well (Osaki et al. 2019). Another suggestion for this finding is that raccoons are consuming animal protein in greater abundances (e.g. small vertebrates, invertebrates) in areas of agriculture and barren land, as fruit is not as highly available compared to natural landscapes (Warsen et al. 2014, Magioli et al. 2019). A previous study supports this explanation, as the findings revealed that omnivores increase their intake of animal matter in human-modified landscapes to

replace the diminished availability of fruits, increasing their $\delta^{15}\text{N}$ signatures (Magioli et al. 2019). By understanding the dietary patterns associated with various human disturbances, this study researched further into how these impacted physiological parameters, specifically body condition, GSP, and leptin.

I also found that higher body $\delta^{13}\text{C}$, presumably from increased consumption of C_4 plants, was associated with higher body condition in raccoons, following what was found from a previous study by Demeny et al., (2019). Leptin and body condition also exhibited a positive relationship, providing evidence that variations in body condition are likely associated with fat mass (Shibata et al. 2005). Though higher body condition is usually viewed as a benefit for wildlife, extremely high body condition could indicate an excess of fat that is a health concern (Birnie-Gauvin et al. 2017). Raccoons with greater access to anthropogenic food waste have been shown to have higher body mass and exhibit evidence of hyperglycemia (Schulte-Hostedde et al. 2018). My results also demonstrate this finding, as GSP was significantly impacted by PC1 and PC2 as well and highest in areas of agriculture and barren land. But, this finding again does not follow the prediction that GSP concentrations would be highest in raccoons from urban environments due to the consumption of anthropogenic food waste that contain high amounts of carbohydrates (McCain et al. 2008, Schulte-Hostedde et al. 2018). Though these results of GSP contradict findings of previous studies such as Newsome et al., (2010) and Schulte-Hostedde et al., (2018), it does suggest an effect of seasonality, as this study sampled raccoons during the fall, instead of the summer as was done by Schulte-Hostedde et al., (2018). In turn, elevated GSP concentrations are reflected in raccoons that are experiencing a shift in diet to fall resources that are more readily available, such as those

from agricultural and barren landscapes (Welsh et al. 2016). Future research should examine these variables over multiple seasons to truly understand the effect that season has on raccoons.

No Effect of Urbanization

Given a previous study in urban baboons has indicated leptin levels increase with access to garbage (Banks et al. 2003), it was predicted leptin would be greater in raccoons from urban environments with intake of high-fat food resources, such as anthropogenic food waste. But, urbanization was not found to impact leptin concentrations in raccoons, as demonstrated by the non-significant PC1 and PC2 results. This is consistent with a recent study conducted on raccoons in Canada, which also found no relationship of leptin with increased access to anthropogenic food waste (Schulte-Hostedde et al. 2018). One possible explanation is the fall sampling period, as raccoons from all habitat types generally gain weight during this time to account for the limited food resources available during the winter months and thus, differences in adiposity would likely not be evident (Pitt et al. 2008, McWilliams and Wilson 2015). Alternatively, raccoons may be adapting to a diet high in lipids, similar to results reported in a previous study on white-footed mice (*Peromyscus leucopus*) (Harris and Munshi-South 2017). Though there is no evidence of obesity, raccoons are experiencing other physiological consequences associated with a change in diet, such as elevated GSP concentrations.

Effect of Sex

I found that male raccoons had higher GSP concentrations than females. Although sex differences were not predicted, there could be multiple hypotheses for this finding. Firstly, this finding could suggest that reproductive tactics are sex-specific in areas with a predictable food source, such as human-disturbed landscapes, and may use different resources to facilitate reproduction, as females invest into a second breeding season in the fall if the first one was unsuccessful (Gehrt and Fritzell 1996, Asano et al. 2003, Rosatte et al. 2006). Resources used are dependent on the activity that is being executed, such as its intensity and length (Soulsbury 2019). Males exhibit a polygynous mating behaviour over an extended period of time (Law and Mehta 2018), and therefore likely use lipids as the main source of fuel, as lipids can function as an energy reserve and store greater amounts of energy for long-term use, compared to carbohydrates (Soulsbury 2019). But, carbohydrates do provide a more readily available source of energy that can be used during short and intense periods of activity (Soulsbury 2019). Females allocate resources towards offspring production and may rely on resources for fuel in the form of lipids, carbohydrates, or simultaneous usage of both (Soulsbury 2019). In this case however, females are likely using carbohydrates as their main source of fuel, which drive the differences seen in GSP concentrations between males and females. Ultimately, this hypothesis suggests that males and females utilize different fuels to facilitate reproduction.

Secondly, an effect of sex on GSP concentrations may be a result of home range sizes instead, as females are likely restricted to their den site to care for their offspring while males are able to expand their movements in search of food as rearing of offspring is done alone (Gehrt and Fritzell 1997, Kamler and Gipson 2003). In this case, males may

be consuming different food resources that contain greater amounts of carbohydrates than females and ultimately leading to sex differences in GSP concentrations. For example, a study completed with urban lizards found that males have increased body condition than females because they consume greater abundances of food due to a greater distance travelled (Lazić et al. 2017). Overall, this provides evidence that blood glucose of raccoons may be affected by both urbanization and sex.

Conclusion

This study suggests that anthropogenic alteration of landscapes influence an animal's dietary patterns and glucose metabolism, and may have associated health risks. Although generally considered a benefit to wildlife, extremely high body condition can indicate negative health consequences, such as obesity (Birnie-Gauvin et al. 2017, Demeny et al. 2019) or hyperglycemia (Schulte-Hostedde et al. 2018). Otherwise, animals may be adapting to diets high in carbohydrates and lipids as recent research with white-footed mice (*Peromyscus leucopus*) has shown (Harris and Munshi-South 2017). Future research should collect data over multiple seasons to determine the effect of seasonality on dietary patterns, fat metabolism, and glucose metabolism and the health risks associated with human-modified landscapes, as it can have important conservation implications (Bruskotter et al. 2017).

This research demonstrates the need to improve policies regarding crop cultivation and waste management to prevent raccoons from consuming food resources from agricultural landscapes and barren land. This is shown in a previous study where the number of coyotes visiting an area decreased when the amount and accessibility of garbage was reduced as well (Larson et al. 2020). Since this study showed that a raccoons

diet in the fall primarily consists of food resources from agricultural and barren landscapes, policies regarding wildlife management in these areas should be improved. Reconsideration of land sharing (i.e. integrating conservation and farming) and land sparing (i.e. separating conservation and farming) policies in agricultural land management should be completed in collaboration with ecologists to ensure the programs are effective for the conservation of certain wildlife species (Phalan et al. 2011). To develop a program that is most beneficial, current crop yields and species population densities of the area should be taken into consideration (Phalan et al. 2011). With the development of updated policies, the conflict between humans and wildlife should decrease, while also improving the health of urban species.

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Table 2.1: Summary of a one-way ANCOVA for $\delta^{13}\text{C}$ as a function of various predictor variables. Principal components of landscape features (PC1, PC2) are described in Table

1.2. Model was fitted using data collected from southern Ontario in 2018 ($n = 64$).

Significance tested using Type 3 sum of squares (* $p < 0.05$).

Variable	Estimate	SE	Partial-F	df	<i>p</i>
PC1	-0.689	0.185	13.858	1, 59	<0.001***
PC2	1.329	0.394	11.409	1, 59	0.001**
Body Condition	2.237	1.083	4.271	1, 59	0.043*
Sex	-0.109	0.184	0.355	1, 59	0.554

Table 2.2: Summary of a one-way ANCOVA for $\delta^{15}\text{N}$ as a function of various predictor variables. Principal components of landscape features (PC1, PC2) are described in Table

1.2. Model was fitted using data collected from southern Ontario in 2018 ($n = 64$).

Significance tested using Type 3 sum of squares (* $p < 0.05$).

Variable	Estimate	SE	Partial-F	df	<i>p</i>
PC1	-0.085	0.109	0.612	1, 59	0.437
PC2	1.164	0.232	25.146	1, 59	<0.001***
Body Condition	0.296	0.638	0.215	1, 59	0.644
Sex	0.032	0.108	0.088	1, 59	0.768

Table 2.3: Summary of fixed effects for linear mixed model (package ‘lme4’) of GSP as a function of various predictor variables. Model was fitted using data collected in southern Ontario in 2018 and 2019 (n = 88). Principal components of landscape features (PC1, PC2) are described in Table 1.2. Random effects in model (not shown) included year (2018, 2019). Type 3 Wald chi-square tests were used to test for significance (* $p < 0.05$).

Variable	Estimate	SE	X²	df	<i>p</i>
PC1	-15.378	7.686	4.003	1	0.045*
PC2	34.260	15.271	5.033	1	0.025*
Body Condition	-67.515	46.687	2.091	1	0.148
Sex	-30.619	8.285	13.660	1	<0.001***

Table 2.4: Summary of fixed effects for generalized linear mixed model (package ‘lme4’) of leptin as a function of various predictor variables. Model was fitted using data collected in southern Ontario in 2018 and 2019 (n = 65). Principal components of landscape features (PC1, PC2) are described in Table 1.2. Random effects in model (not shown) included year (2018, 2019). Type 3 Wald chi-square tests were used to test for significance (* $p < 0.05$).

Variable	Estimate	SE	X²	df	<i>p</i>
PC1	-0.122	0.102	1.431	1	0.232
PC2	0.279	0.197	2.003	1	0.157
Body Condition	1.510	0.632	5.707	1	0.017*
Sex	0.025	0.098	0.065	1	0.800

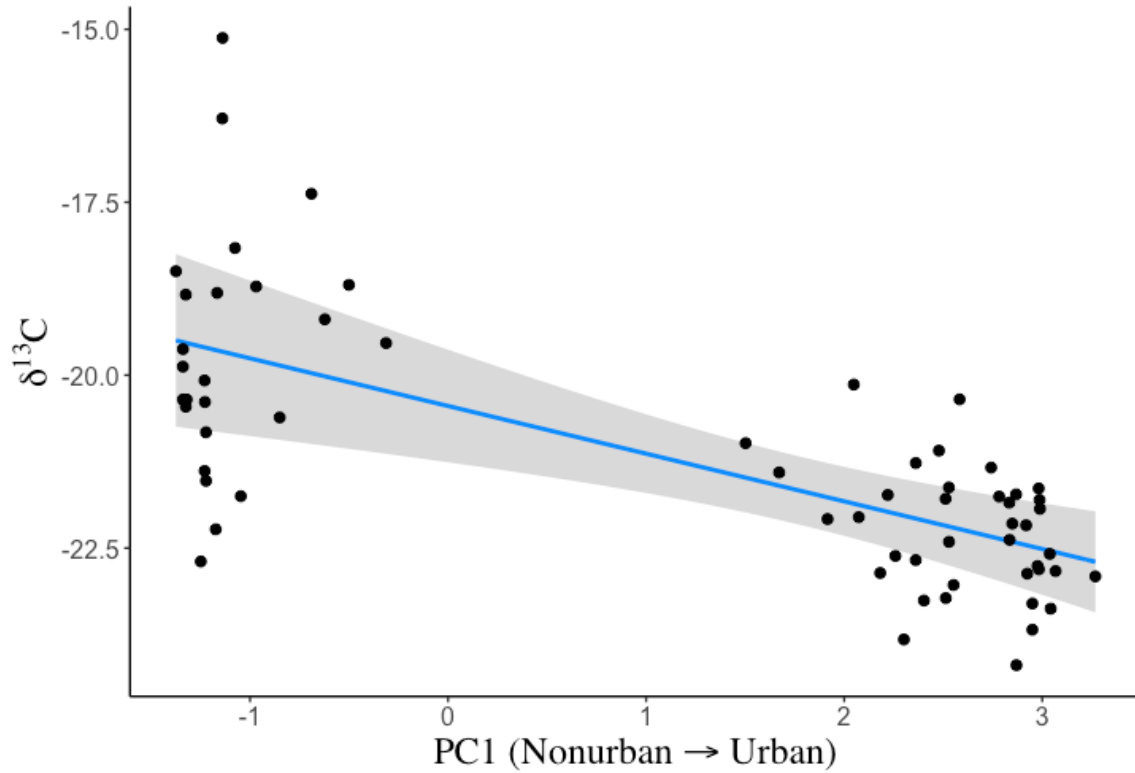


Figure 2.1: Partial residual plot of $\delta^{13}\text{C}$ vs. landscape PC1 score for individuals (sexes combined) sampled in Ontario in 2018 ($n = 64$, $p = <0.001$). Line is fitted OLS regression and shaded area is 95% confidence interval.

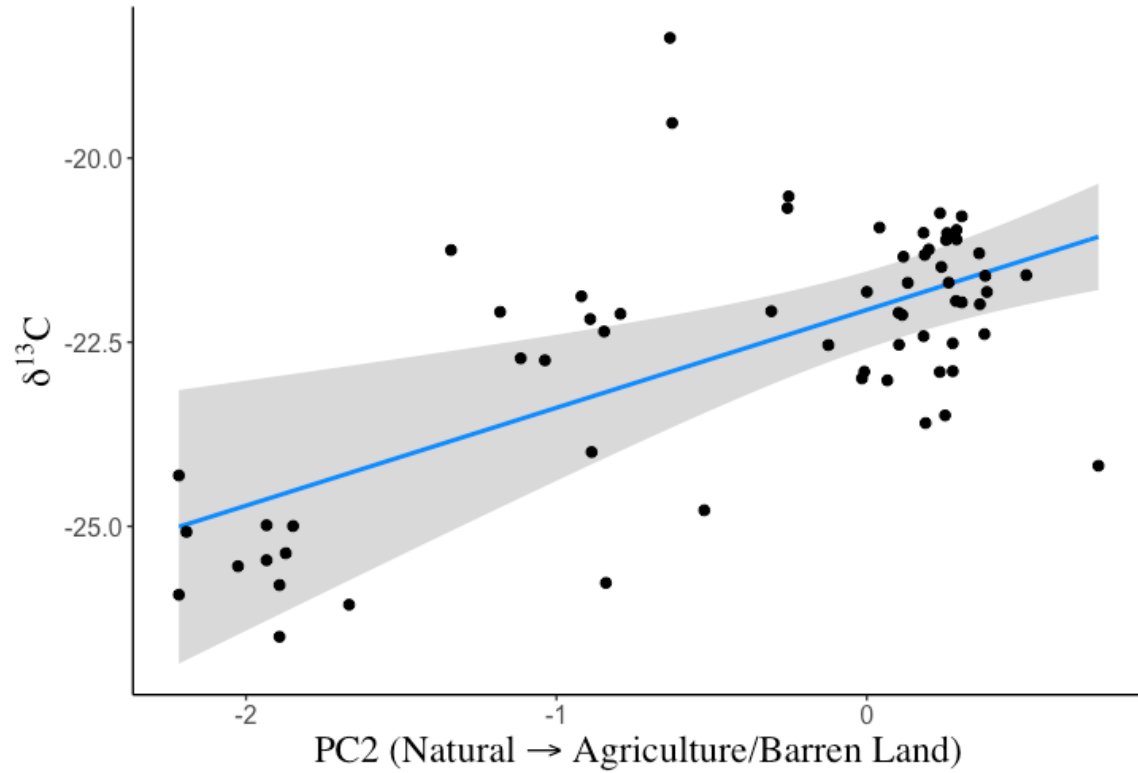


Figure 2.2: Partial residual plot of $\delta^{13}\text{C}$ vs. landscape PC2 score for individuals (sexes combined) sampled in Ontario in 2018 ($n = 64$, $p = 0.001$). Line is fitted OLS regression and shaded area is 95% confidence interval.

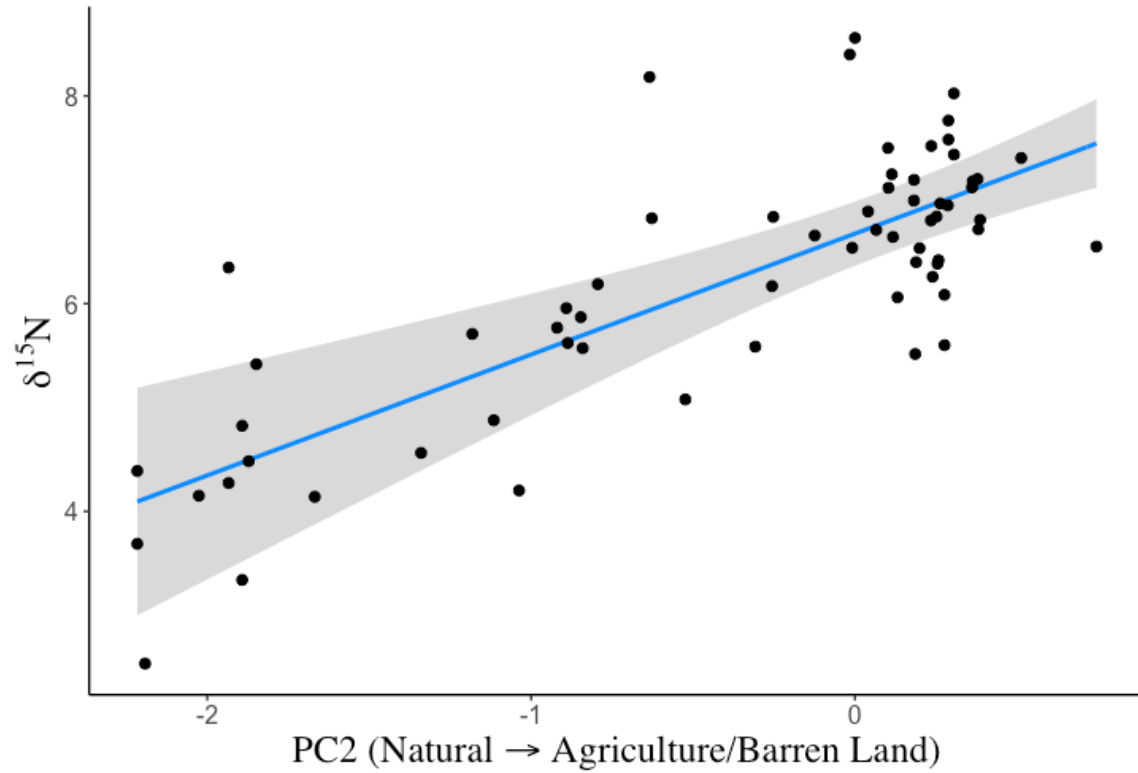


Figure 2.3: Partial residual plot of $\delta^{15}\text{N}$ vs. landscape PC2 score for individuals (sexes combined) sampled in Ontario in 2018 ($n = 64$, $p = <0.001$). Line is fitted OLS regression and shaded area is 95% confidence interval.

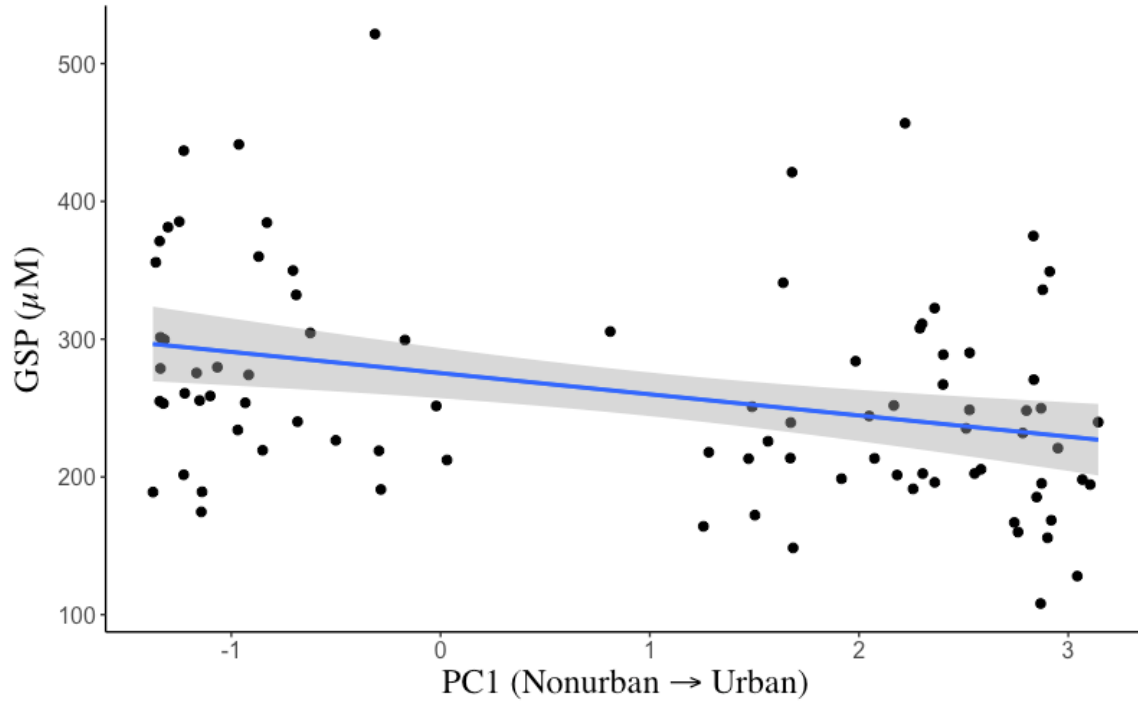


Figure 2.4: Partial residual plot of GSP vs. landscape PC1 score for individuals (sexes combined) sampled in Ontario in 2018 and 2019 ($n = 88$, $p = 0.045$). Line is fitted OLS regression and shaded area is 95% confidence interval.

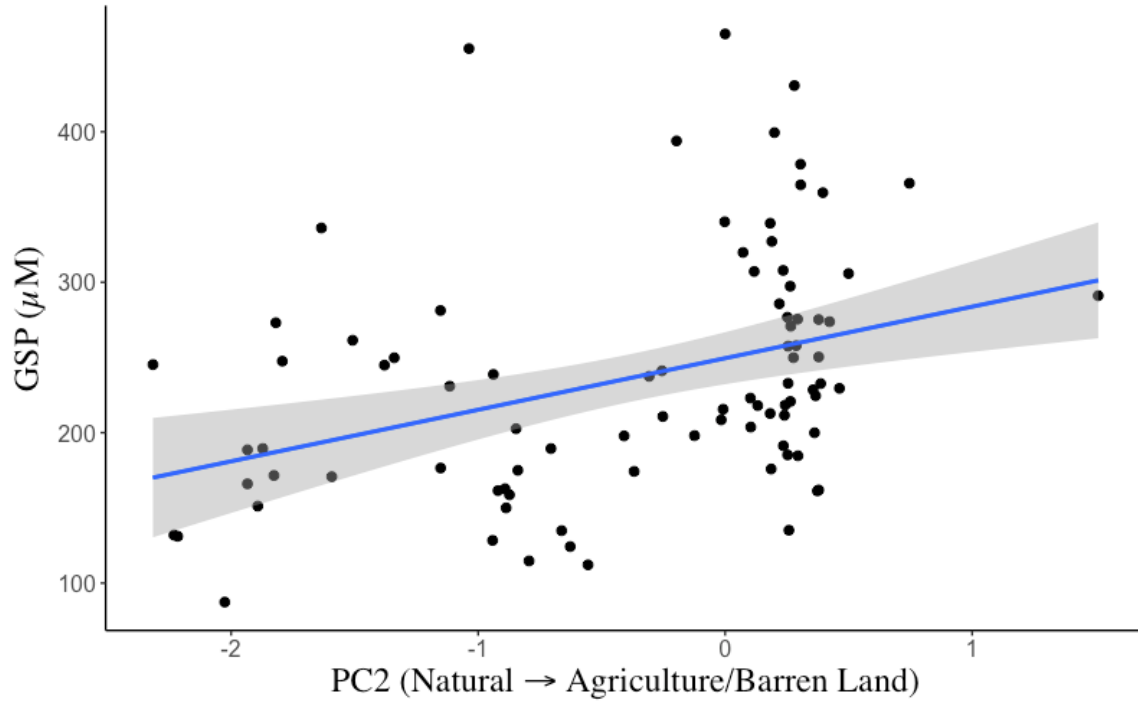


Figure 2.5: Partial residual plot of GSP vs. landscape PC2 score for individuals (sexes combined) sampled in Ontario in 2018 and 2019 ($n = 88$, $p = 0.025$). Line is fitted OLS regression and shaded area is 95% confidence interval.

General Discussion

The process in which land is transformed from its natural state to an area containing manmade structures is called ‘urbanization’, a phenomenon that is increasing globally as the human population grows (Grimm et al. 2008, Liu et al. 2014). This results in rapid and drastic changes to natural habitats that some species cannot adapt to (Shochat et al. 2006, Gibbs et al. 2019), such as alterations in ecological processes like predator-prey relationships (Eötvös et al. 2018, Gallo et al. 2019), spatial distribution (Ehlers Smith et al. 2018, Zungu et al. 2020), and the spread of pests and diseases (Gordon et al. 2016, Soulsbury and White 2016). As some species experience population declines (Łopucki and Kitowski 2017, Schmidt et al. 2020), others thrive in urban environments due to the consumption of anthropogenic food waste (Červinka et al. 2014, Stark et al. 2020), a food resource that is in greater abundance and of different nutritional quality (i.e. carbohydrate, lipid, and protein composition) than natural food resources (Seress et al. 2014, Suarez-Rubio and Krenn 2018). Studies regarding the impacts of access to anthropogenic food waste on individual health are limited and as such, require further investigation to provide insight on the true effects of urbanization on wildlife.

This study aimed to determine the effects of an urban diet on raccoons (*Procyon lotor*), a species that has adapted to live in a variety of habitat types across North America, including urban areas, due to their broadly omnivorous and opportunistic diet (Bartoszewicz et al. 2008, Thornton et al. 2020). Specifically, the study examined the dietary patterns of raccoons (measured by C and N stable isotopes) and its associated physiological consequences seen across an urbanization gradient. Physiological

parameters assessed were body condition in Ontario and Québec, in addition to glycosylated serum protein (GSP) and leptin in Ontario only.

Examination of dietary patterns showed an effect of urbanization, where raccoons from areas of agriculture and barren land exhibited the greatest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, revealing a diet high in C_4 plant material, likely corn, and animal protein, respectively (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019). Given that this result contradicts previous studies that sampled in the summer in which urban raccoons exhibited the highest $\delta^{13}\text{C}$ due to the consumption of anthropogenic food waste (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019), it suggests a seasonality effect on diet, where raccoons may shift their diet depending on the most readily available food resource at the time. In regards to $\delta^{13}\text{C}$ for example, a study found that urban San Joaquin kit foxes (*Vulpes macrotis mutica*) had higher $\delta^{13}\text{C}$ signatures than nonurban kit foxes (Newsome et al. 2010). $\delta^{13}\text{C}$ signatures were also found to be similar between urban kit foxes and human residents from the area, further demonstrating the shared food resource between humans and wildlife (Newsome et al. 2010). However, with corn being a more readily available food source during the fall, raccoons may preferentially consume corn and corn-based products around this time instead (Demeny et al. 2019). With the annual molt coinciding with this diet change, hair sampled would likely capture this resource and increase $\delta^{13}\text{C}$ signature (Demeny et al. 2019). As for $\delta^{15}\text{N}$, signatures were found to be greatest in areas of agricultural and barren landscapes, compared to natural environments, and does not follow the prediction that urban raccoons would have the highest $\delta^{15}\text{N}$ due to the consumption of anthropogenic food waste (Newsome et al. 2010, Osaki et al. 2019, Handler et al. 2019). This is likely because the

consumption of animal protein is greater as a result of reduced availability of C₃ plants, such as berries (Warsen et al. 2014, Magioli et al. 2019). However, the source of animal protein may differ between agricultural and barren landscapes, compared to natural environments, which can drive differences in $\delta^{15}\text{N}$ signatures as well. For example, raccoons from agricultural and barren landscapes have access to protein-rich food resources such as animal feed, chicken eggs, and possibly chickens themselves (Aoki et al. 2017) that would increase $\delta^{15}\text{N}$ signatures. As a previous study with raccoon dogs (*Nyctereutes procyonoides*) has shown, those from the forest periphery exhibited higher $\delta^{15}\text{N}$ signatures than those from the forest core because of the consumption of garbage, anthropogenic food waste, and corn, in addition to $\delta^{15}\text{N}$ -rich prey (i.e. frogs, snakes, salamanders) found in both areas (Osaki et al. 2019). With evidence of a shift in diet between seasons, this research examined further the physiological consequences associated with consumption of such food resources in terms of body condition, GSP, and leptin.

A positive relationship was found between body condition and $\delta^{13}\text{C}$, suggesting that raccoons are in better body condition with the consumption of corn or corn-based products that are in greater abundance and of different nutritional quality (i.e. carbohydrate, lipid, and protein composition) than natural resources (Demeny et al. 2019). Body condition has also shown to have a positive relationship with leptin, providing evidence that changes in body condition are likely associated with fat mass and attributed to changes in diet (Shibata et al. 2005). Though a good body condition is generally considered a benefit for wildlife as fat reserves increase fitness (i.e. survival and reproductive success), extremely good body conditions can be associated with

negative health consequences (Birnie-Gauvin et al. 2017). For example, a recent study has shown that the consumption of anthropogenic food waste in urban raccoons increases body mass and blood glucose concentrations, providing evidence for hyperglycemia (Schulte-Hostedde et al. 2018). Similarly, this study reveals that raccoons shifting their diets to consume abundant high-sugar, high-fat resources from agricultural and barren landscapes exhibit elevated GSP concentrations as well, but no difference in leptin. Given that urban baboons have shown increases in leptin levels with easier access to garbage, it was expected raccoons would exhibit similar trends in human-modified landscapes (Banks et al. 2003). However, urbanization was found to have no effect on leptin concentrations in raccoons, which could be reasoned by two possible explanations. Firstly, since raccoons were sampled during the fall, this finding could be a result of the raccoons consuming greater amounts of food in both urbanized and non-urbanized environments to account for limited food resources in the winter (Pitt et al. 2008, McWilliams and Wilson 2015). Secondly, raccoons may be adapting to a diet high in lipids, as recent research with white-footed mice (*Peromyscus leucopus*) has shown (Harris and Munshi-South 2017). Though there is no indication of obesity, these results demonstrate other consequences associated with increased body condition indices, such as evidence of hyperglycemia (Schulte-Hostedde et al. 2018).

Though an effect of sex was not expected, broad scale analyses (Ontario and Québec) showed that urban environments had a sex-specific effect on body condition, where males were in better body conditions than females, which could be associated with a few possible explanations. Firstly, this finding could suggest different reproductive tactics between the sexes in areas of a predictable food source since female raccoons have

shown to invest into a second breeding season in the fall provided the first one is unsuccessful (Gehrt and Fritzell 1996, Asano et al. 2003, Rosatte et al. 2006). In this case, males could invest into a ‘capital’ breeding strategy and consume food opportunistically, while females invest into an ‘income’ breeding tactic and consume food when needed, saving energy for reproductive demands like lactation (Stephens et al. 2014, Williams et al. 2017). Secondly, an effect of sex could be due to differences in home range sizes, as females could be restricted to their den site when rearing offspring alone while males travel further to forage more often (Gehrt and Fritzell 1997, Kamler and Gipson 2003). This result is consistent with a study conducted on urban lizards, which found males who travelled further distances were more successful at feeding themselves, and consequently exhibited greater body conditions than females (Lazić et al. 2017). Thirdly, raccoons are likely consuming additional food resources during the fall sampling period to account for a mass loss of up to 50% in the winter (Pitt et al. 2008, McWilliams and Wilson 2015). However, females are likely investing into a second breeding season instead, and do not show the same weight gain as males (Gehrt and Fritzell 1996, Asano et al. 2003, Rosatte et al. 2006).

Finer scale analyses (Ontario) revealed that raccoons within agricultural and barren landscapes were in poorer body condition than those from natural environments suggesting that raccoons are avoiding the metabolic and locomotive stress associated with increased fat reserves (Bókony et al. 2012, Maclagan et al. 2018). This result is supported by another study on urban house sparrows that were found to have reduced body size and mass in urban environments, compared to rural areas (Meillère et al. 2015). However, other body condition indices showed no effect, and provided evidence that this

species was not nutritionally suffering with urbanization (Meillère et al. 2015). With raccoons exhibiting elevated GSP concentrations, as demonstrated above, raccoons are also nutritionally sufficient despite having poorer body conditions in agricultural and barren landscapes.

Unexpectedly, similar results were not found when a finer scale analysis within Québec was conducted, as urbanization did not have an effect on raccoon body condition, the only parameter examined in the region. This is likely a result of uneven sampling within Québec, as majority of the sites were considered non-urban and did not include residential areas or major urban centres. Another possible explanation could be differences in municipal waste disposal policies, where raccoons from Québec and Ontario may have different opportunities to access anthropogenic food waste, resulting in different body condition patterns across the two regions.

Finally, an effect of sex was found with GSP as well, where males are shown to have elevated GSP concentrations compared to females. Though not predicted, this could be explained with multiple hypotheses. Firstly, this finding could suggest that raccoons have sex-specific breeding tactics in areas of a predictable food source and may use different resources to facilitate reproduction depending on intensity and length of the activity (Soulsbury 2019). This means that males who exhibit a polygynous mating behaviour over a long duration likely use lipids as a fuel source, as lipids function as an energy reserve and contain greater amounts of energy than carbohydrates (Soulsbury 2019). But, carbohydrates provide an energy source that is more readily available, which could be used by females for offspring production (Soulsbury 2019). Female reproductive tactics may involve the use of carbohydrates, lipids, or a combination of the two

depending on the activity being performed (Soulsbury 2019). In this case however, it is most likely that the female is facilitating reproduction using readily available carbohydrates as differences in GSP concentrations between sexes were found.

Ultimately, this hypothesis states that resource use towards reproduction may change depending on the sex and activity being performed. Secondly, GSP concentrations may be effected by sex due to the differences in distances travelled for food, or home range size (Lazić et al. 2017). For example, males who travel further may be consuming foods that contain greater amounts of carbohydrates than females, who are restricted in their movements as they rear their offspring by their den site and cannot access these same food resources (Gehrt and Frtzell 1997, Kamler and Gipson 2003). Overall, this finding reports that raccoon physiology may be affected by both urbanization and sex.

This study demonstrates the effect of season on diet for raccoons, where food resources change from anthropogenic food waste during the summer to corn and corn-based products throughout the fall, a more readily available food source at the time. Future research should account for samples across multiple seasons to truly understand its effect on various physiological parameters, such as body condition, GSP, and leptin that was examined in this study. For example, it would be beneficial from a conservation perspective to understand if this change in diet throughout the year is associated with any negative health consequences, such that was previously seen with obesity (Birnie-Gauvin et al. 2017, Demeny et al. 2019) and hyperglycemia (Schulte-Hostedde et al. 2018). Otherwise, wildlife may be adapting to the consumption of an urban diet and its high components of carbohydrates and lipids, as seen recently in research with white-footed mice (*Peromyscus leucopus*) (Harris and Munshi-South 2017).

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