Fecal glucocorticoid metabolites are correlated with urbanization but not body condition in eastern chipmunks (*Tamias striatus*)

Raven Ouellette[1] · Gabriela Mastromonaco[2] · Colin J. Garroway[3] · Albrecht I Schulte-Hostedde[1]

Abstract

Urban spaces offer both benefits (elevated food resources from human food sources and fewer predators) and energetic costs (the physiological stress response related to human activity) to wildlife. We investigated whether chipmunks (*Tamias striatus*) experienced variation in fecal glucocorticoid metabolites (FGMs) and body condition across an urbanization gradient. We predicted that chipmunks in more urban environments would have lower levels of FGMs and be in better body condition, compared to chipmunks living in more natural areas resulting from the novel environments found in urban spaces, and increased food resources. Fecal samples and body measurements were collected from chipmunks across 20 locations throughout Sudbury, Ontario, Canada to determine FGM concentrations and body condition, respectively. Each location was surveyed over a three-day period to determine the level of human activity to generate urbanization gradient scores. Our findings show a positive effect of an urbanization gradient on FGMs, suggesting chipmunks in more urban habitats exhibit higher levels of FGMs compared to conspecifics in less urban habitats. We also found that body condition was not related to urbanization, which may be because food is readily available within city limits. These findings highlight the importance of using an urbanization gradient to consider the effects of urbanization on stress-related metrics and consuming a human food waste diet on a small mammal species.

Keywords Cortisol · Fecal glucocorticoid metabolites (FGMs) · Urbanization gradient · *Tamias striatus* · Food waste · Body condition

Introduction

Urbanization – the process of altering a landscape for human settlement – is a global phenomenon (Shochat et al. 2010; Faeth *ert al*., 2011; Saari et al. 2016) that has forced many species to respond to rapid environmental change (McKinney 2002; Partecke et al. 2006). The growth of cities often forces a species to try to survive in new urban habitats (Palmer 2003). For example, the American mink (*Neovion vison*) has low colonization rates near urban areas

because urban growth has decreased the amount of suitable habitat (Wolff et al. 2015; Ahlers et al. 2016). Moreover, many mammalian carnivores, such as bobcats (*Lynx rufus*), gray foxes (*Urocyon cinereoargenteus*), and mountain lions (*Puma concolor*) are becoming locally extinct in urban areas because of increased human disturbance and habitat loss (Crooks 2002; Ordeñana et al. 2010). However, some species are able to acclimatize and physiologically adjust and respond to changes associated with urbanization (Kart et al. 2007). Scavengers and herbivores can take advantage of new anthropogenic environments by consuming food waste from humans due to the widespread and consistent availability (McKinney 2002; Sih et al. 2010; Birnie-Gauvin et al. 2016). Over time, urban-living individuals may begin to phenotypically diverge from their counterparts living in natural habitats as they acclimatize to a resource-filled environment, such that urban-dwelling individuals may differ in body condition, colouration, or behaviour (McKinney 2002; Hasegawa et al. 2014; Lyons et al. 2017; Baxter-Gilbert et al. 2019).

✉ Albrecht I Schulte-Hostedde
aschultehostedde@laurentian.ca

[1] Department of Biology, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, Canada

[2] Reproductive Physiology, Toronto Zoo, 361A Old Finch Avenue, Toronto, ON M1B 5K7, Canada

[3] Department of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, ON R3T 2N2, Canada

Once an animal is introduced to an urban habitat, they encounter new environments associated with urbanization and human activity (Palmer 2003; Tarlow and Blumstein 2007), including automobile and human traffic, noise and air pollution, destruction or alterations in habitat, and changes in predation and available diet (Sauvajot 1998; Hamer and McDonnell 2010; Fischer et al. 2014; Giraudeau et al. 2014). These factors may result in the activation of the hypothalamic-pituitary-adrenal (HPA axis). The HPA axis is a negative feedback system that begins to work when the hypothalamus releases corticotropin-releasing hormone (CRH). This triggers the anterior pituitary to release adrenocorticotrophic hormone (ACTH), followed by the adrenal cortex releasing a glucocorticoid hormone, such as corticosterone or cortisol (Sapolsky et al. 2000; Sapolsky 2004; Kudielka and Kirschbaum 2005). Cortisol and corticosterone both increase the concentration of glucose in the bloodstream (therefore increasing energy availability) and promote the function of muscles or accelerate physiological processes that are required to cope with an immediate stressor (Sapolsky et al. 2000; Sapolsky 2004; Kudielka and Kirschbaum 2005). The HPA axis is a mechanism that allows organisms to cope with short-term stress and return the body to homeostasis; however, over the long term, it can have negative fitness consequences. Prolonged or chronic elevation of glucocorticoid hormones may decrease immunity, reproductive success, and overall well-being (Sapolsky et al. 2000; Romero and Wikelski 2001; Carlitz et al., 2016).

Many species exhibit differences in glucocorticoid levels between urban or natural habitats (Atwell et al. 2012; Bonier 2012; Brearley et al. 2012; Deng et al. 2014). For example, cortisol levels in urban populations of various species, such as eastern chipmunks (*Tamias striatus;* Lyons et al. 2017), dark-eyed juncos (*Junco hyemalis*; Atwell et al. 2012), and tree lizards (*Urosaurus ornatus*; French et al. 2008) are lower compared to their counterparts dwelling in their natural habitats. These populations may be adapting to urban habitats, emancipating them from the negative effects of chronic elevated cortisol levels (French et al. 2008; Atwell et al. 2012; Lyons et al. 2017). However, in the short-term, individuals that disperse from natural to urban habitats may experience elevated glucocorticoids because of urban stressors (Partecke et al. 2006; Atwell et al. 2012). As a consequence, individuals living in urban and natural habitats may differ in life history traits, such as producing more or fewer offspring or size at birth (Boal and Mannan 1999; Sprau et al., 2016).

Glucocorticoid levels have been historically quantified via blood samples (Goymann 2005; Montiglio et al. 2012), but feces and urine are also used to quantify glucocorticoids (Palme, 2005, Sheriff et al., 2011). Fecal glucocorticoid metabolites (FGMs) are useful because they are less invasive to acquire and reflect an integrated average measure of circulating baseline glucocorticoid levels in the medium-term (over several hours to days) as compared to short-term fluctuations found in blood (Goymann 2005; Touma and Palme, 2005; Poessel et al., 2011).

As a result of a long-term increase in glucocorticoids from baseline levels, an individual may experience a decrease in body condition (Cote et al. 2010). Body condition estimates an individual's energy reserves (Schulte-Hostedde et al. 2005). Individuals with chronically high HPA axis activity may experience a decrease in body condition because they are using available energy to maintain homeostasis and may be unable to replenish fat reserves quickly enough to maintain their condition (Sapolsky et al. 2000; MacBeth et al. 2012; Zwijacz-Kozica et al. 2013). These stress responses mediated by the HPA axis are energetically expensive because the body is using fat stores and undergoing gluconeogenesis to mobilize available energy to survive (Boissy 1995; Kitaysky et al. 1999; Marin et al. 2007). For example, Cabezas et al. (2007) found that wild rabbits (*Oryctolagus cuniculus*) experienced a decrease in body condition when experimentally exposed to long-term stressors because of an increase in glucocorticoid blood levels. In the context of urbanization, however, little is known about how body condition may change over an urbanization gradient, and if this change may be correlated with cortisol levels. It is also important to consider that body condition may change across a gradient in response to anthropogenic food subsidies. Human food waste continues to be accessible to urban wildlife through waste receptacles, bird feeders, and direct feeding from humans to wildlife (Newsome and van Eeden 2017). Since urban and natural dwelling animals may have access to different amounts of food resources, changes in body condition or energetic reserves are expected. For example, Lyons et al. (2017) found that female eastern chipmunks from urban areas were in better body condition than those from natural areas, suggesting that females have more energy reserves, likely from consuming food through anthropogenic sources.

The effects of urbanization on wildlife have generally been characterized by defining urbanization categorically. Populations are defined as originating from either natural or urban habitats (Sears 1989; Iglesias-Carrasco et al. 2017; Lyons et al. 2017; Shimamoto et al. 2019; but see Meillère et al. 2016; Price et al. 2018; Szulkin et al. 2020). While classifying habitat as either "urban" or "natural" is common in urban ecology, alternative approaches can be more nuanced. Urbanization can be described as a gradient, ranging from urban parks to the concrete and asphalt of buildings and roads. For example, Price et al. (2018) generated an urbanization gradient (referred to as the rural-urban score) to examine cortisol concentrations in yellow-bellied marmots

(*Marmota flaviventris*) and measured various anthropogenic variables to determine how "urban" or "natural" their study locations were. Defining and measuring a series of variables that can distinguish a more urban area from a more natural habitat could provide more accurate interpretations related to the impacts an urban environment may have on a given species.

Human food waste should be more prevalent in habitats that are more urban along an urbanization gradient, specifically areas that produce the most food waste, such as areas with grocery stores and restaurants or residential neighbourhoods. We investigated FGM concentrations and body condition of eastern chipmunks (*Tamias striatus*) in relation to an urbanization gradient within a city. We predicted that chipmunks in more urban habitats would exhibit lower FGM levels and greater energy reserves (reflected by body condition) compared to chipmunks living in more natural habitats. In more urban habitats, chipmunks may be consuming readily available and abundant anthropogenic food waste and therefore may have more energy to invest into their body condition, whereas chipmunks in more natural habitats have to exert greater amounts of energy to forage for food and may experience higher levels of FGMs since they must forage greater distances for natural food sources (Lyons et al. 2017).

Methods

Study sites

Adult eastern chipmunks were sampled from early April until late August of 2019 throughout Sudbury, Ontario, Canada (46.4917ºN, 80.9930ºW). A total of 20 study sites were sampled including four locations within a conservation area (Lake Laurentian), a single location on an extensive trail system (Kelly Lake Trail), three locations within a public park (Bell Park), four locations at Laurentian University, and eight backyards located throughout Sudbury (Fig. 1). Sherman traps (H.B. Sherman Inc., Tallahassee, Florida) were set at all 20 locations, near areas with dense vegetation cover, and in areas where chipmunk burrows were found. Approximately 80–100 traps (10 traps per site, on average 2 m apart from each other) were baited with peanut butter and oats every day at 07:00 and checked every two hours until 16:00. Traps were set at each location twice a week.

Trapping and sample collection

Upon capture, chipmunks were transferred into a handling bag and the reproductive state and age were assessed. Males were classified as non-reproductive or reproductive (scrotal), while females were classified as non-reproductive or reproductive (either lactating or pregnant). Age was determined by mass, such that a chipmunk under 80 g at the time of the initial capturewas considered a juvenile, while over 80 g was considered an adult (Patterson and Schulte-Hostedde 2011). Each chipmunk was weighed using a Pesola® scale ($\pm$ 0.1 g), and morphometric measurements were obtained using a ruler ($\pm$ 1 mm) and caliper ($\pm$ 0.1 mm). The morphometric data that was collected included skull length and width, hindfoot length, and body mass (Schulte-Hostedde and Millar 2000; Lyons et al. 2017).

At initial capture, each of the 59 chipmunks was given a metal ear tag with a unique number sequence (National Band and Tag Company, Newport, Kentucky), allowing individual identification. Fecal samples were collected by removing feces from traps or collecting them directly from the individual during handling. A single fecal sample was collected and analyzed for each individual chipmunk, even if the chipmunk was recaptured. Following the protocol of Palme et al. (2005), the fecal samples were placed in 3 mL centrifuge tubes with 1 mL of 80% methanol per 0.1 g of fresh feces to preserve the sample for FGM analysis. The samples were refrigerated at -20ºC until they were used for analysis. Our methods followed the guidelines promoted by the Canadian Council on Animal Care and were accepted by the Animal Care Committee at Laurentian University (AUP 2019-04-24).

Hormone extraction

The stored fecal samples (n = 59) were transferred from 2 ml pre-weighed polypropylene tubes into glass tubes. One millilitre of 100% methanol was used to rinse the polypropylene tube and the remainder was pipetted into the glass tube. All samples were left to evaporate under a fume hood for approximately 48 h. Glass tubes were re-weighed to determine fecal weights, and 80% methanol in water was added at a ratio of 0.05 g/ml. Samples were vortexed and mixed overnight on a plate shaker. Supernatants from extracted samples were stored at -20ºC until analysis.

The use of FGM has been validated in eastern chipmunks (Montiglio et al. 2012). The samples were analyzed for FGM concentrations in duplicates using the methods following Dulude-de Broin et al. (2019). This method is used by the Toronto Zoo Endocrinology Laboratory across species to determine FGM concentrations. However, the FGM antiserum and horseradish peroxidase conjugate in our analysis was diluted to 1:10 000 and 1:33 500, respectively.

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

Fig. 1 Map of the 20 study sites visited and the respective land cover types found at each study site for eastern chipmunks (*Tamias striatus*) generated from the 2015 Land Cover of Canada map (Latifovic et al. 2017; Latifovic 2019). Study sites included 8 backyards (1, 3–5, 17–20), Bell Park (14–16), Laurentian University campus (6–9), Lake Laurentian Conservation Area (10–13), and Kelly Lake Trail (2). (See Supplemental Table 1 for land cover types for each study site)
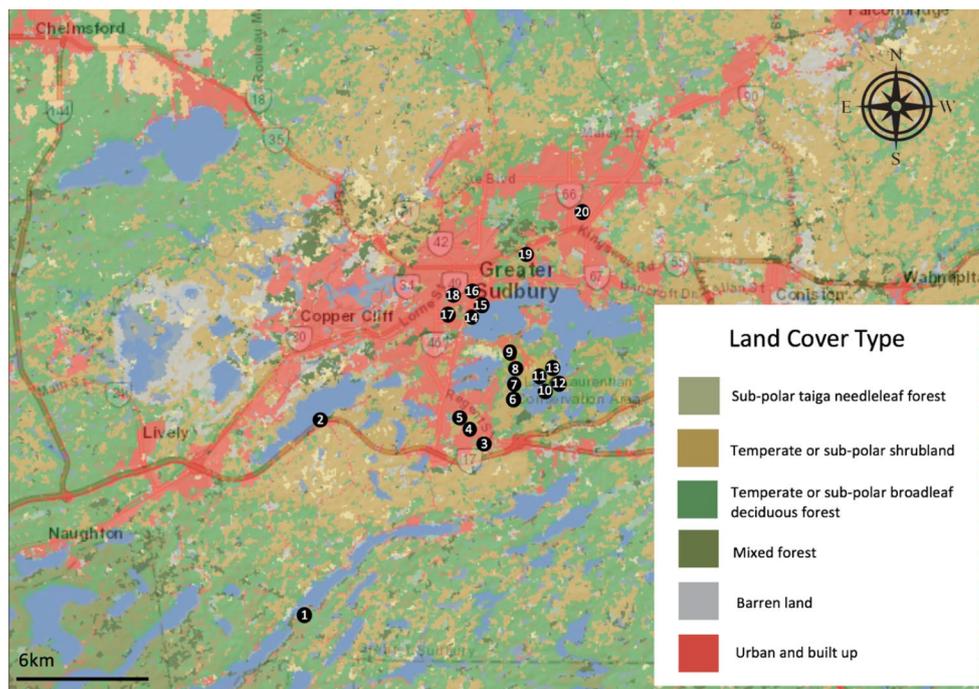
left to sit for two hours at room temperature and 100 µl substrate solution was subsequently added. Using a spectrophotometer (MRX microplate reader, Dynex Technologies, Chantilly, VA), absorbance was measured at 405 nm.

To assess repeatability of results, calculation of intra- and inter-assay coefficients of variation (CV) was evaluated. Intra-assay CVs were consistently monitored on each plate in real time by examining the CV of each duplicate run on the plate. Only values from duplicates with <10% CV were recorded as data. Inter-assay CVs were evaluated using fecal extract controls (23% and 60% binding) loaded in duplicate on each plate. Serial dilutions of pooled fecal extract showed parallel displacement with the cortisol standard curve (t=0.24, p=0.81, df=8). The recovery of known concentrations of cortisol was 90.4% ± 5.8 (mean ± SE). The measured hormone concentrations in the spiked samples correlated with the expected concentrations of cortisol (r=0.99, p>0.001; Fig #). Inter-assay CVs were 5.3% (23% binding) and 7.5% (60% binding) (Standard curve provided as Supplemental Fig. 3).

Natural-urban score (NUS) data collection

Using methods outlined in Price et al. (2018), we completed surveys and used land cover data to generate a score that represented how urban or natural each study site was (the natural-urban score; NUS). While conducting surveys, we monitored the number of pedestrians, dogs, cars, and bikes that passed by all study sites in an hour, the number of trash pieces found within a 100 m radius of the trapline,

the number of trash cans found within a 100 m radius of the trapline, and the number of bird feeders found within a 100 m radius of the trapline. Trash was defined as any item on the ground that could be held in a trash can, ranging from wrappers and recyclables to peanut shells. Trash cans were defined as any bin that could hold food waste, such as typical garbage bins or compost bins. In lieu of counting the number of cars per hour on busy major roads and highways, such as roads near Bell Park and Kelly Lake Trail, an average number of cars per hour was determined from traffic data supplied from the Ontario Ministry of Transportation to the City of Greater Sudbury (Greater Sudbury Statistics, 2017). All variables were collected from 07:00–13:00 over a three-day period at each study site. After the three-day observation period concluded, averages of all measured variables were calculated, which generated a single number for each variable measured per location (Price et al. 2018).

We determined the type of land cover found at all study sites within a 100 m radius using the 2015 Land Cover of Canada map (Latifovic et al. 2017; Price et al. 2018; Latifovic 2019). A 100 m radius ensured that land cover was consistently and equally measured across study sites, allowing us to extract numerical data for vegetation cover within the radius across locations. The categories of land cover found at various study sites included: sub-polar taiga needleleaf forest, temperate or sub-polar shrubland, temperate or sub-polar broadleaf deciduous forest, mixed forest, barren land, and urban sites (See Supplementary Table 1).

Statistical analysis of NUS

All statistical analyses were performed using R (version 3.6.2, R Core Team, 2019). The land cover data, as well as surveyed observations were summarized with a principal component analysis (PCA) that generated a natural-urban score for each site (Fig. 2; Table 1).

Population size and density

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

Statistical analysis of FGM concentrations, body condition, and NUS

We used the average skull length, skull width, and hindfoot length (log-transformed) of each individual to determine body size, (Schulte-Hostedde et al. 2005; Lyons et al. 2017). A PCA was performed to calculate a measure of overall size, and then the PC1 scores were extracted. Log-transformed values for body mass and PC1 scores were inputted into a simple linear regression and the residuals were calculated to represent the body condition score of each individual
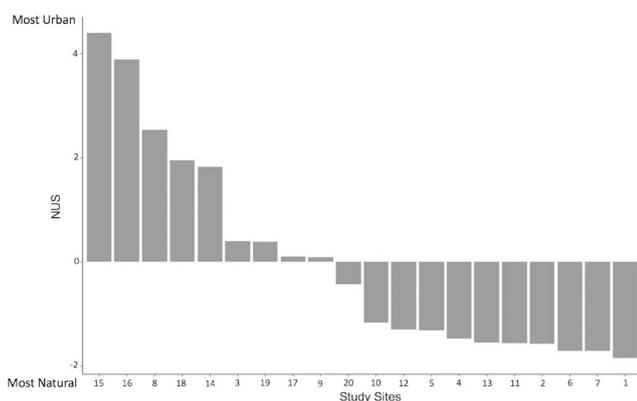
Fig. 2 Bar graph of NUS (natural-urban scores) from all study sites 1 to 20. On the y axis, positive values indicate more urban areas and negative values indicate more natural areas

(Schulte-Hostedde et al. 2005; Lyons et al. 2017). Lastly, FGM concentrations were log-transformed to meet normality assumptions.

We tested our hypotheses with two generalized linear models. Our first model examined the effect of urbanization on body condition while statistically controlling for sex, reproductive condition, FGM concentrations, and Julian date. Our second model tested for effects of urbanization on FGM concentrations while statistically controlling for sex, reproductive condition, Julian date, and body condition. For each model we first tested the interactions between FGM concentrations and NUS, NUS and anthropogenic food sources, population density and NUS, NUS and body condition, and FGM concentration and sex. If interactions were not significant, we reran the models without the interaction term.

Results

Sample size

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

Natural-urban score (NUS) PCA

PC1 explained 44.9% of the variation within the surveying data and land cover map data (Table 1), while PC2 explained 20.3% of the variation (Table 1). PC1 tended to distinguish the degree of urbanity among locations (e.g. a more positive factor represents a variable associated with a more urban area, while a more negative factor represents a variable associated with a natural area), while PC2 tended to discriminate food resources from non-food resources (Table 1). The average number of pedestrians per hour was the factor that had a strong positive relationship to PC1 (0.423; Table 1), while vegetation cover had a strong negative relationship to PC1 (-0.297; Table 1). The average number of trash cans found within a 100 m radius of the trapline had a strong positive relationship to PC2 (0.521, Table 1), while the average number of bikes per hour was found to be the opposite (-0.478; Table 1). Because PC1 had the greatest variation and distinguished the degree of urbanization between variables, it was used as our estimate NUS for each site. A more positive NUS value indicates a more urban area, while a more negative NUS value indicates a less urban area (Fig. 2). PC2 was indicative of anthropogenic food sources.

Table 1 A PCA analysis was conducted to create the scores for the urbanization gradient based on the habitats of eastern chipmunks (*Tamias striatus*). A summary of PC1 and PC2 loadings consisting of all variables surveyed at each study site and land cover data in Sudbury, Ontario. PC1 explains 44.9% of the variation in the dataset, while PC2 explains 20.3%

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

Table 2 A global generalized linear model examining body condition for eastern chipmunks (*Tamias striatus*; n = 59) in response to log(FMG), population density, NUS, anthropogenic food sources, sex, reproductive condition, and Julian date in Sudbury, Ontario

Variable	Estimate	Standard Error	t-value	p-value	Adjusted R2
Intercept	-0.12	0.07	-1.69	0.09	0.02
Log (FMG)	0.01	0.01	0.83	0.40	
Population density	1.12	0.74	1.50	0.13	
NUS*	< 0.01	< 0.01	-1.23	0.22	
Anthropogenic food sources	< 0.01	< 0.01	0.26	0.70	
Sex	< 0.01	0.01	-0.12	0.90	
Reproductive Condition	0.02	0.01	1.55	0.12	
Julian date	< 0.01	< 0.01	1.21	0.22	

*NUS, natural urban score representing the values used to generate the urbanization

Table 3 A global generalized linear model examining FMG concentrations for eastern chipmunks (*Tamias striatus*; n = 59) in response to population density, NUS, anthropogenic food sources, sex, reproductive condition, Julian date, and body condition in Sudbury, Ontario

Variable	Estimate	Standard Error	t-value	p-value	Adjusted R2
Intercept	2.72	0.41	6.58	**< 0.01**	0.02
Population density	-6.68	5.54	-1.20	0.23	
NUS*	0.07	0.03	2.13	**0.02**	
Anthropogenic food sources	< 0.01	0.04	0.14	0.88	
Sex	-0.03	0.13	-0.28	0.77	
Reproductive condition	0.13	0.14	0.92	0.35	
Julian date	< 0.01	< 0.01	-0.43	0.66	
Body condition	0.85	1.02	0.83	0.40	

*NUS, natural urban score representing the values used to generate the urbanization

Body condition residuals

PC1 explained 44.7% in the body condition variation, and all loadings were positive (skull length = 0.653, skull width = 0.674, and hindfood length 0.345). The linear regression of log-transformed body mass and PC1 scores displayed a positive relationship, thus all residuals could be extracted to represent body size to describe PC1 in subsequent analyses (See Supplementary Fig. 1).

Body condition generalized linear model

Body condition was not related to FGMs (t = 0.83, p = 0.40, Table 2) or NUS (t = -1.23, p = 0.22, Table 2). Additionally, body condition was not related to population density (t = 1.50, p = 0.13, Table 2), anthropogenic food sources (t = 0.26, p = 0.70, Table 2), sex (t = -0.12 p = 0.90, Table 2), reproductive condition (t = 1.55, p = 0.12, Table 2), or Julian date (t = 1.21, p = 0.22, Table 2).

FGM generalized linear model

FGMs were found to be significantly associated with NUS (t = 2.13, p = 0.02, Table 3), and was therefore presented as a bivariate model (Fig. 3). Additionally, FGM concentrations were not related to population density (t = -1.20, p = 0.23, Table 3), anthropogenic food sources (t = 0.14, p = 0.88, Table 3), sex (t = -0.28, p = 0.77, Table 3), reproductive condition (t = 0.92, p = 0.35, Table 3), Julian date (t = -0.43, p = 0.66, Table 3, Supplemental Fig. 2), or body condition (t = 0.83, p = 0.40, Table 3).

Discussion

We found a significant effect of the urbanization gradient on FGM concentrations from chipmunks, such that chipmunks from the most urban areas exhibited the highest levels of FGMs. However, we observed no significant effect of the urbanization gradient on the body condition of chipmunks.

Fig. 3 Linear regression of partial residuals of fecal glucocorticoid metabolite (FMG) concentrations from eastern chipmunks (*Tamias striatus*) over the natural-urban score (NUS; p = 0.02, r^2 = 0.06, n = 59). On the x axis, positive values indicate more urban areas and negative values indicate more natural areas. The shaded area denotes a 95% confidence interval
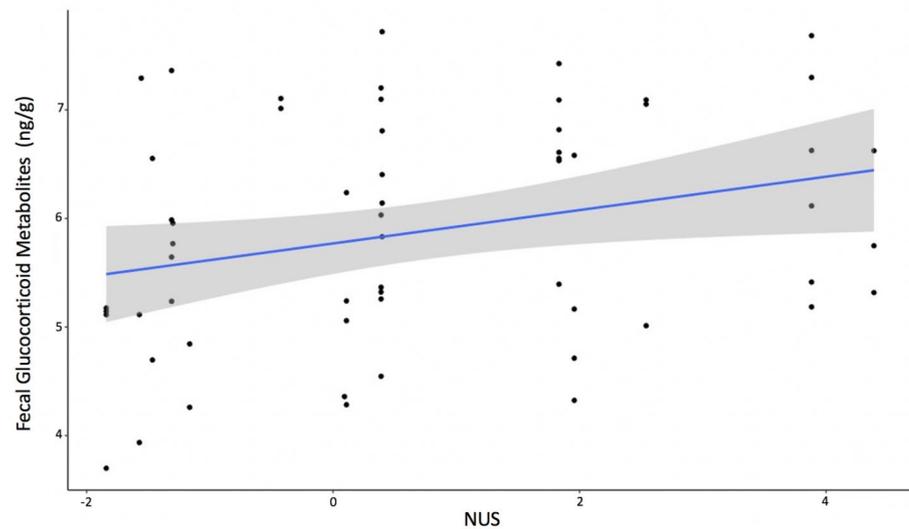
These results partially support our initial predictions but the lack of effect of urbanization on body condition was surprising.

It is possible that chipmunks in urban habitats may have access to enough food resources to maintain body condition, regardless of FGM concentrations, especially because all of our study sites fell within Sudbury city limits (Kitaysky et al. 1999; Bonier et al. 2007). For example, a study examining the diet of herring gulls (*Larus argentatus*) found that those gulls feeding on human food waste were consuming a diet that had a higher caloric value, protein content, and fat content than a natural diet (Pierotti and Annett 1990, 1991). Although we did not examine diet composition or quality, it is possible, like herring gulls, that chipmunks may be consuming a diet that allows them to maintain body condition, despite experiencing higher levels of FGMs than their less urban counterparts. Chipmunks in more natural areas may still have access to anthropogenic food resources from travelling to more urban areas within their home range, or from humans directly providing food, for example, hiking on trail systems. In other words, the trade-off that should exist between body condition and elevated cortisol levels may be mitigated by the access to high-energy anthropogenic food resources.

The results from this study appear to contradict Lyons et al. (2017), where eastern chipmunks in urban habitats had lower FGM levels than those in natural habitats, and in which females had elevated body condition in urban habitats. We quantified the level of urbanization at each study site, while Lyons et al. (2017) categorically defined an urban versus natural habitat. Our study also accounted for the degree of urbanization by quantifying human activity and possible food sources (e.g. trash pieces and garbage bins). In addition, our study was exclusively within a city,

whereas Lyons et al. (2017) used natural sites that were not within the city. Thus, although our results appear to contradict Lyons et al. (2017), it clear that our methodology was more specific to an urban context.

Chipmunks exhibit higher levels of FGMs in urban sites; therefore, it is possible that urban habitats may be considered "stressful" by chipmunks, compared to their natural habitats. The natural-urban score was correlated with variables related to human activity, including the presence of pedestrians, cars, bikes, and dogs. These types of activities are known to increase measures of stress in many species, such as wildcats (*Felis silvestris*; Pinerio et al. 2012), swamp rats (*Scapteromys aquaticus*) and Azara's grass mice (*Akodon azarae*; Gomez-Villafane et al. 2012) as well as woodland caribou (*Rangifer tarandus*; Ewacha et al. 2017). Young marmots may perceive dogs as predators in urban habitats based on their fecal glucocorticoid results (Price et al. 2018). For this reason, it is not surprising that chipmunks experience an increase in FGM concentrations near the most urban area within the gradient, as these areas had the most human and dog presence. These findings also explain why we did not find a significant interaction between FGMs and body condition as predicted. The energetic cost of elevated HPA axis activity may be mitigated by the increased availability of food resources found in more urban sites. In other words, while elevated FGMs may be stimulated by the activities of humans and dogs, the energetic costs of this may be offset by the presence of food subsidies via human food waste in an urban setting.

High concentrations of FGMs are not in of themselves "bad" – that is, activation of the HPA axis and the secretion of glucocorticoids is an adaptation meant to mobilize energy in the face of one or more stressors. However, given that the stressors associated with urbanization are inherent

to this novel environment, we assume that elevated levels of FMGs represent chronically increased activity of the HPA axis which could lead to a decrease in energy investment towards immunity and reproduction (Sapolsky et al. 2000; Romero and Wikelski 2001; Mbora and McPeak, 2009; Carlitz et al., 2016). Reduced investment in immunity may lead to greater susceptibility to pathogens and parasites, as has been found in some urban-dwelling species (Bradley et al. 2008; Liccioli et al. 2012; Lehrer et al., 2010). A reduction in reproductive investment could have consequences for reproductive success, whether by reducing litter sizes or producing underweight offspring (Ruiz et al. 2002; Liker et al., 2008).

The presumed increase in available food resources and shelter in urban habitats relative to natural habitats could increase local population densities which can have effects on FGMs related to social interactions and the transmission of parasites/pathogens (Reperant et al. 2009; Morand et al. 2015; Haigh et al. 2017). The increased population density can facilitate social contacts if home ranges are more likely to overlap, leading to greater horizontal transmission of.

of parasites and pathogens (Bradley and Altizer 2006; Himsworth et al. 2013). As noted above, transmission of parasites and pathogens may be facilitated by an immune system challenged by chronically elevated glucocorticoids. Thus, future work should examine the dynamics of parasites and pathogens in an urban context.

Additionally, higher levels of social interactions amongst rodents have been linked to.

increased glucocorticoids (Wolff and Sherman 2007; Schoepf and Schradin 2013; Haigh et al. 2017). Haigh et al. (2017) found that human traffic was not linked to increased levels of FGMs in European red squirrels (*Sciurus vulagris*) as predicted; rather the interactions amongst squirrels themselves were responsible for the increase in FGMs. Nonetheless, we found no effect of population density on FGMs using our study design. Because all of our sites were within the city, the variation in density may not be as large than if we had included natural sites outside the city, suggesting that further investigation is warranted. Finally, it is important that we determine rates of dispersal from natural areas to the city in order to assess whether chipmunks are "naïve" to the urban environment or are established residents in order to accurately predict the effects of the urban environment on HPA axis activity.

Conclusions

We detected higher levels of FGMs in eastern chipmunks inhabiting areas with a high urban score than those chipmunks sampled at more natural sites. Chipmunks in urban habitats may experience higher levels of glucocorticoids because of increased human disturbance. Our study shows that despite the higher levels of FGM concentrations in chipmunks from more urban areas, they do not differ in body condition along the urbanization gradient, which may be related to the abundance of food resources within city limits. These findings highlight the importance of considering the level of urbanization in a particular area to better understand how animals are responding to the presence of anthropogenic elements within their habitats, since urbanization is impacting wildlife globally.

Declarations

Data policy statement Upon acceptance of our manuscript, our data will be uploaded to DRYAD.

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