Behavioral Ecology

The official journal of the
ISBE
International Society for Behavioral Ecology

Behavioral Ecology (2017), 28(6), 1464–1471. doi:10.1093/beheco/arx109

Original Article

Fat and happy in the city: Eastern chipmunks in urban environments

Jeremy Lyons,[a] Gabriela Mastromonaco,[b] Darryl B. Edwards,[a] and Albrecht I. Schulte-Hostedde[a]
[a]Department of Biology, Laurentian University, 935 Ramsey Lake Road, Sudbury, Ontario, P3E 2C6, Canada, and [b]Reproductive Physiology, Toronto Zoo, 361A Old Finch Avenue, Toronto, Ontario, M1B 5K7, Canada

Received 19 March 2017; revised 1 June 2017; editorial decision 10 June 2017; accepted 25 July 2017; Advance Access publication 22 August 2017.

Cities are rapidly expanding, and wildlife may experience different selection pressures in urban environments when compared to natural habitats. Phenotypic differences between urban and natural populations may occur because of the altered urban environment. Behavior, the activity of the hypothalamic-pituitary-adrenal (HPA) axis and body condition can be expected to differ between urban and natural habitats. We used the eastern chipmunk (*Tamias striatus*) to test for differences in behavior assayed from an open field test, hair and fecal cortisol concentrations, and body condition (size-corrected body mass), predicting that urban chipmunks would exhibit more exploratory behavior, higher cortisol concentrations, and higher body condition, than their counterparts from natural habitats. We sampled eastern chipmunks in 2 urban areas paired with natural habitats and subjected adult chipmunks to an open field test, collected hair and fecal samples for the determination of cortisol concentrations, and measured body size and body mass to estimate body condition. Eastern chipmunks in urban habitats had significantly different behavior, tending toward reduced locomotion and grooming, and greater latency, than their counterparts from natural habitats. Urban chipmunks also had lower fecal cortisol concentrations than those from natural habitats, and female chipmunks were in better body condition when captured in urban habitats. These results suggest that urban habitats may be relatively benign for urban chipmunks, perhaps because of reduced need for exploration and the availability of anthropogenic food subsidies associated with urban environments.

Key words: **body condition, cortisol, exploratory behavior, food subsidies, mammal.**

INTRODUCTION

Cities and their associated infrastructure are rapidly expanding as human populations become increasingly urbanized (Grimm et al 2008). These urban landscapes are highly modified from their natural state, and offer novel habitats for wildlife (Shochat et al. 2006). Urban processes may differ dramatically from natural processes that have governed the ecology and evolution of species in natural habitats (Shochat et al. 2006). These anthropogenic alterations to a landscape can impose rapid evolutionary change and select for heritable traits that differ between natural and urban environments (Stockwell et al. 2003; Adams 2005). While it is well known that species distributions may vary based on the degree of urbanization of the landscape, less well understood are the sub-lethal consequences of urbanization on wildlife (Birnie-Gauvin et al. 2016).

Urban habitats present a host of altered conditions—differences in food availability, rates and sources of mortality, thermal costs, and the acoustic environment, all challenge wildlife as they react to circumstances to which they are not necessarily adapted (Shochat et al. 2006). These environmental differences can lead to phenotypic divergence between natural and urban populations either because of the different phenotypic traits of urban colonizers (e.g. more explorative individuals may be more likely to disperse) and/or because urban environments may lead to phenotypic, and ultimately evolutionary, changes that lead to divergence in phenotypes (Shochat et al. 2006; Ripmeester et al. 2010).

There is a growing body of literature indicating that behavioral differences exist between urban populations of wildlife, and their counterparts in natural habitats (reviewed in Lowry et al. 2013). Individual differences in behavior (personality—Réale et al. 2007) are associated with success in urban habitats, particularly boldness (Lowry et al. 2013). Boldness is the willingness to take more risks, and is associated with dispersal (Fraser et al. 2001, Dingemanse et al. 2003) as well as exploratory behavior (Montiglio et al. 2012a). Thus, those individuals willing to take on more risk should be more likely to disperse into urban environments and endure the high degree of disturbance that occurs in these habitats (Lowry et al. 2013). Indeed, studies of birds have found that urban birds are

Address correspondence to A.I. Schulte-Hostedde. E-mail: aschulte-hostedde@laurentian.ca. D.B.E. coauthor is now at Cambrian College, 1400 Barrydowne Rd, Sudbury, Ontario, P3A 3V8, Canada.

bolder than individuals in natural populations (Evans et al. 2010, Lowry et al. 2011), although it is yet unclear whether boldness/exploration specifically facilitates colonization of urban habitats (Lowry et al. 2011).

In animals, the hypothalamic-pituitary-adrenal (HPA) axis mediates production and release of glucocorticoids including cortisol and corticosterone, which are necessary for mediating responses to novel environments (Kenagy and Place 2000; Macbeth et al. 2010; Ashley et al. 2011; Bonier 2012). The use of glucocorticoids as a measure of physiological health for wildlife has expanded, particularly with respect to determining the effects of anthropogenic activities (e.g. Dantzer et al. 2014). Urban habitats provide a number of novel challenges that the HPA axis must respond to, including the presence of, and interactions with, humans (Marzluff et al. 2001; Møller 2009; Ashley et al. 2011; Powell et al. 2013), traffic, light, noise, and chemical pollution, as well as mortality risk from domesticated animals and road crossings (Ditchoff et al. 2006; McGregor et al. 2008; Evans et al. 2010; Powell et al. 2013). In birds, there has been no consistent pattern in baseline glucocorticoid levels between populations in urban and natural habitats (Bonier 2012), and it seems likely that the response of the HPA axis to the novelty of urban habitats is species specific (Birnie-Gauvin et al. 2016).

It has been increasingly recognized that predictable anthropogenic food subsidies can have important effects on wildlife populations (Oro et al. 2013), and that anthropogenic food sources in cities may have consequences for urban wildlife (e.g. Bozek et al. 2007; Rodewald et al. 2011). Having access to predictable and abundant food resources may affect the individual energetic state of an animal, thus affecting relative body mass (body condition) (Schulte-Hostedde et al. 2005). The predictability of food resources may make carrying fat or protein unnecessary resulting in reduced body condition, whereas the fitness benefits of having more energy reserves may lead animals in urban habitats to having enhanced body condition relative to their counterparts in natural habitats. The quality of any anthropogenic food resources, and any associated toxins can also impact the body condition of urban animals (Shochat 2004; Birnie-Gauvin et al. 2016).

Here, we examine phenotypic differences in urban and natural populations of the eastern chipmunk (*Tamias striatus*), a small rodent native to much of eastern and central Canada and the United States. In many ways, this species is ideal to test hypotheses in urban ecology because of its relative abundance in both natural and urban habitats, as well as past work on behavior (e.g. Martin and Réale 2008a, 2008b; Patterson and Schulte-Hostedde 2011) and baseline cortisol levels (Mastromonaco et al. 2014). There has been substantial research on how urban environments affect behavior, glucocorticoids and energy reserves in birds (e.g. Partecke et al. 2006; Møller 2009; Bonier 2012; Atwell et al. 2012), but there is very little known about other taxa, including mammals. We predicted that chipmunks from urban habitats would have higher levels of exploratory behavior than those from natural habitats. The novel nature of urban habitats also led us to predict that baseline cortisol levels from both hair and feces would be higher in chipmunks from urban habitats. Finally, given the generally expected increase in available food resources in towns and cities resulting from anthropogenic food subsidies, we predicted that body condition would be higher in urban chipmunks than those from natural habitats.

METHODS

Study site

Chipmunks were collected and sampled from two different habitats defined as urban and natural. Urban sites were chosen from the Town of Huntsville (45°20' N 79°13W) and the City of Greater Sudbury (46°29' N, 81°00' W), Canada. These urban sites were paired with natural areas in Algonquin Provincial Park (45.8°N 78.7°W), and outside Sudbury, respectively. Huntsville is a town outside of Algonquin Park (population approximately 19 000) that swells in size significantly over the tourist (summer) season. Sudbury is the largest city in northern Ontario (population approximately 160 000) and is surrounded by natural areas.

A list of sites used can be found in Supplementary Table 1. Urban sites were chosen based on proximity to roads and walking trails, and their proximity to anthropogenic infrastructure (i.e. roads, fences, city structures), but where animals would come into contact with human activities and infrastructure. Natural habitats were selected in areas away from major human settlements and in forested areas where animals could escape human interaction and were not noticeably isolated from other chipmunk populations by anthropogenic barriers.

Trapping

Sampling was conducted between May and August of both 2012 and 2013. Chipmunks were trapped alternating weeks between urban and natural sites using Longworth live traps baited with sunflower seeds soaked in water overnight. Traps were set late morning and checked approximately 2 h later. Typically 60 traps were used per trapping session. Juveniles were not used in the analysis as they show consistently different reactions to stressful stimuli compared with adults (Boissy 1995; Bonier 2012). Captured individuals were transferred to a handling bag and were sexed and assessed for reproductive condition (breeding or non-breeding). Males were classified as in breeding condition if they had an enlarged scrotum, and females were classified as in breeding condition if they were lactating. Individuals were weighed with a Pesola scale, and classified as juvenile if their weight was below 80 g at initial capture and they were non-reproductive (Patterson and Schulte-Hostedde 2011). Individuals were marked with one or two metal ear tags to identify them with unique numerical codes. Juveniles and other non-target species were immediately released. Calipers were used to measure skull length (length of back of skull to tip of nose ±1 mm), skull width (length of skull between each eye ±1 mm), and hind foot length (length of heel to the tip of the longest toe ±1 mm).

Behavior

An open field test is an effective method to measure an animal's activity, in particular the distance traveled in a novel environment (Montiglio et al. 2010). The test was performed in an opaque plastic arena (76 cm × 42 cm at the bottom and 90 cm × 53 cm at the top; height = 42 cm) with a clear, acrylic lid and 10 equally spaced holes (5 cm diameter) cut into the bottom (see (Patterson and Schulte-Hostedde 2011). A grid of squares (10 cm × 10 cm) was taped to the underside of the Plexiglass floor (Semenova et al. 2001). A hollow tube with cover was placed in the box to allow animals to enter and be removed from the box. Once the chipmunk entered the enclosure its behavior was recorded via video camera (HD, 1920 × 1080) for 3 min (Montiglio et al. 2010) by an observer who remained silent and out of sight for the duration of the test.

The videos were scored by a single observer based on time (s) spent grooming, time spent jumping to top of the container or remaining hanging from top of container or entrance (climbing), time spent head scanning, time spent running around the field box (locomotion), time spent investigating each hole in the trap (head dipping), and time spent rearing up on its hind legs viewing its surroundings (rearing), time spent standing still, and time spent inside the hollow tube (latency). Chipmunks show a degree of habituation to repeated open field trails (Martin and Réale 2008b); therefore, only videos of the initial trials were used in our analysis.

Hair/fecal cortisol analysis

Hair accumulates glucocorticoids over the period of active hair growth since the animal's last moult, which allows the measurement of hormone levels without the risk of spiking glucocorticoid levels in plasma due to the stress caused by handling (Macbeth et al. 2010, Ashley et al. 2011). Hair samples were collected by shaving a small 1 × 1 cm patch of hair from the right hind leg of each animal with a battery powered razor (ConAir Beard and Moustache Trimmer model GMT100RQCS, Stamford, CT) during the initial capture. Hair is a relatively stable medium noted to maintain levels of blood based hormones for periods of weeks to months (Macbeth et al. 2010; Ashley et al. 2011). Hair samples were kept in 1.5 mL Eppendorf tubes at room temperature.

Fecal samples represent an accumulation of cortisol over a period during the animal's digestion, typically hours or days, though less when the animal is exposed to an immediate stressor such as handling (Ashley et al. 2011). Fecal samples were collected from a pillowcase placed underneath the animal during each capture whenever possible. Fecal samples were weighed ±0.001 g and put into 1.5 mL Eppendorf tubes to which 80% methanol was added at a ratio of approximately 0.1 g feces per 1 mL methanol. Immersed fecal samples were kept refrigerated.

All hair and fecal samples were sent to the Endocrinology Laboratory at the Toronto Zoo to undergo hormone extraction and enzyme immunoassay (EIA) to determine cortisol concentrations. Procedures for extraction and measurement of cortisol and metabolites from hair and feces were previously validated and described in Mastromonaco et al. (2014) and Montiglio et al. (2012b), respectively.

Hair

Hair samples were cut into 5 mm pieces, placed into 7 mL glass scintillation vials and weighed using a Mettler AB54-S balance. Hair samples were washed with 100% methanol by vortexing for 10 s and pipetting off the methanol immediately after. Extraction of cortisol was done by adding 80% methanol at a ratio of 0.005 g hair to 1 mL methanol, vortexing for 5–10 s and rotating 24 h on a Barstead Lab-Line Multi-Purpose Rotator. Samples were then centrifuged for 10 min at 2400 × *g* and the supernatants (methanol extracts) were transferred to new 7 mL glass scintillation vials and evaporated in a fume hood. The dried extracts were stored at −20 °C until analysis.

Feces

Immediately prior to extraction, the fecal pellets within each Eppendorf tube were broken up with a clean spatula and mixed with the methanol. Extraction of cortisol metabolites was done by adding 80% methanol at a ratio of 0.5 g feces to 1 mL methanol, briefly vortexing and rotating samples overnight in a Barstead Lab-Line Multi-Purpose Rotator. Samples were then centrifuged for 10 min at 2400 × g and the supernants (methanol extracts) were transferred to new 7 mL glass scintillation vials. The extracts were stored at −20 °C until analysis.

Cortisol enzyme immunoassay

Samples were removed from the freezer and brought to room temperature prior to analysis. Dried hair extracts were reconstituted in assay buffer (0.1 mM sodium phosphate buffer, pH 7.0, containing 9 g of NaCl and 1g of bovine serum albumin per liter) and sonicated for 20 s followed by vortexing for 10 s. Final volumes of assay buffer added resulted in hair extracts being run at 3× concentration. Fecal extracts were diluted 1:50 in assay buffer and vortexed for 10 s.

Hair cortisol and fecal cortisol metabolites were quantified using a method modified from Munro and Lasley (1988). Cortisol antiserum (R4972; C. Munro, University of California, Davis, CA, USA) was diluted in coating buffer (50 mM bicarbonate buffer, pH 9.6) at 1:12 000. The cross-reactivities of the cortisol antiserum were: cortisol, 100%: prednisolone, 9.9%: prednisone, 6.3%: cortisone, 5%: corticosterone, 0.7%: 21-deoxycortisone, 0.5%: deoxycorticosterone, 0.3%: other, <0.3%. Cortisol-horseradish peroxidase conjugate (C. Munro, University of California, Davis, CA, USA), was diluted in assay buffer at 1:60 000. The standard used was cortisol (Steraloids Inc., Newport, RI, USA: cat # Sigma H-0135: 0.078–20 ng/mL = 78–20 000 pg/mL). Controls consisted of laboratory stocks of pooled fecal extracts obtained from female spotted necked otters (*Hydrictis maculicollis*) and run at 25% and 65% binding. Assay sensitivity was 41.8 pg/mL. Inter-assay CV's were 9.8% and 14.9% at 25% and 65% binding, respectively, and intra-assay CV was 3.6%.

Microtiter plates (Nunc Maxisop, VWR, Mississauga, ON, Canada) were coated with 50μL of cortisol antibody diluted in coating buffer and incubated overnight at 4 °C. Unbound antiserum was washed three times from coated plates with 0.02% Tween 20 solution using a microplate washer (Bio-Tek Instruments, Winooski, VT, USA). Following the wash, 50 μL of hair or fecal samples, standards, and controls diluted in assay buffer were added to wells in duplicate, followed by 50 μL of cortisol-horseradish peroxidase conjugate diluted in assay buffer. Plates were incubated for 2 h at room temperature. Following incubation, the plates were washed three times and 100 μL of substrate solution (50 mM citrate, 1.6 mM hydrogen peroxide, and 0.4 mM 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, pH 4.0) was added. The intensity or absorbance of the yellow color in each well on the microtiter was measured at 405 nm using a spectrophotometer (MRX microplate reader, Dynex Technologies, Chantilly, VA, USA) 30–45 min after the substrate was added. Cortisol levels are presented as ng of cortisol g-1 of hair or wet feces.

All experimental procedures were in accordance with guidelines from the Canadian Council on Animal Care and were approved by the Animal Care Committee at Laurentian University (AUP 2012-01-04).

Statistical analysis

Analyses were carried out in the R environment (R 2.15.1; R Core Team 2012) and plots constructed using ggplot2 (Wickham 2009). To determine the effect of source habitat (natural vs. urban) on fecal and hair cortisol levels as well as body condition (dependent variables), we used linear mixed-effects models (lme4; Bates et al.

2013). Along with habitat, models included sex, reproductive status (breeding or not), and year of study as independent main effects. We also incorporated into models region of study (i.e. Huntsville/Algonquin Park, Sudbury) as a blocking factor with two levels as an independent main effect, and study site as a random effect. We tested for select interactions involving habitat (habitat × sex and habitat × reproductive status). The contribution of an interaction term to the final model was determined using a likelihood ratio test (LRT) comparing the model with the interaction term to the nested model without. The interaction term was excluded and the smaller, more parsimonious model retained if the explanatory power of the model was not improved by the inclusion of the interaction, based on the LRT (Pinheiro and Bates 2000). Alongside coefficients from linear mixed models, we present coefficients from Wald F tests and P values adjusted using Kenward–Roger Approximation technique (car package; Fox and Weisberg 2011) in addition to bootstrapped likelihood confidence intervals.

Fecal and hair cortisol values were transformed using the natural logarithm to satisfy assumptions for linear models. General model assumptions were checked using diagnostic plots and histograms for individual variables. Body condition was taken as the residuals from the regression of body mass on body size. To achieve this, we measured skull length, skull width and hind foot length of each individual and entered these into a Principal Components Analysis. The first principal component axis was taken to represent body size, and was used as the independent variable in the regression with body mass to calculate residual mass and determine body condition (Schulte-Hostedde et al. 2005).

We used a multivariate approach to determine whether behavioral performance in the open field test was related to habitat. Behavioral variables were continuous integers, and most variables were strongly right-skewed, therefore we fit these data to a multivariate Generalized Linear model with a negative binomial distribution using the mvabund package (Wang et al. 2013). We checked the suitability of using a negative binomial distribution by plotting the residuals of the model versus the fitted values. Our model included the eight behavioral variables as the dependent variables and condition measure (hair cortisol, fecal cortisol, residual mass), habitat (natural and urban), sex and reproductive status as main effects. We also tested for the contribution of interactions between each respective condition measure and sex and reproductive condition, as well as the interaction between residual mass and habitat. The analysis returns Wald test statistics and calculates P values based on a resampling procedure. As above, we tested for the inclusion interactions using a LRT. The mvabund package does not supply a deconstruction of the multivariate response variable. Because of this, to help illustrate the contribution of individual behavioral variables within the multivariate response to significant main effects we also report results from univariate tests corrected for multiple comparisons.

RESULTS

A total of 140 individuals were sampled throughout the study—79 between May and August of 2012 (30 from urban habitat, 49 from natural habitat), and 51 individuals between May and August of 2013 (30 from urban habitat and 31 from natural habitat). Fifty-one individuals from natural habitat were female (31 lactating/20 non-breeding), and 29 were male (14 scrotal and 15 non–non-breeding). Thirty-eight individuals from the urban habitat were female (15 lactating and 23 non-breeding) and 22 were male (11 scrotal/11 non-breeding). Not all individuals had all variables measured.

The first principal component explained 49% of the variance in body size measures with skull length (0.659), skull width (0.402) and hind foot length (0.636) all contributing positively on that axis.

Hair/fecal cortisol

Mean hair cortisol was 162.31 ng/g ± 187.74 (SD), and mean fecal cortisol was 273.75 ng/g ± 226.76 (SD). There was no effect of habitat (urban/natural) on hair cortisol ($P = 0.41$), although there were significant effects of Sex (males > females), Year (2012 > 2013) and Region (Huntsville/Algonquin > Sudbury) (Table 1). There was a significant effect of habitat, however, on fecal cortisol concentration, with chipmunks in urban habitats having significantly lower fecal cortisol than chipmunks from natural habitats (Figure 1, $P = 0.03$). Fecal cortisol was also significantly higher in individuals that were reproductively active and in the Huntsville/Algonquin region (Table 1).

Table 1

Fixed effects estimates from linear mixed-effects models of environmental factors on hair and fecal cortisol levels and body condition

Parameter	Effect	Estimate	SEM	*F*	*P*	Lower CI	Upper CI
Hair cortisol	Habitat	0.159	0.17	0.77	0.41	−0.108	0.501
n = 138	Sex	0.281	0.124	4.92	**0.03**	**0.067**	**0.545**
	Reproductive status	0.103	0.122	0.69	0.41	−0.136	0.334
	Year	0.617	0.163	12.74	**<0.001**	**0.342**	**0.965**
	Region	−0.520	0.170	7.05	**0.02**	**−0.844**	**−0.196**
Fecal cortisol	Habitat	−0.497	0.141	9.96	**0.03**	**−0.768**	**−0.226**
n = 98	Sex	0.073	0.145	0.24	0.63	−0.205	0.349
	Reproductive status	0.518	0.141	12.83	**<0.001**	**0.248**	**0.788**
	Year	−0.192	0.159	1.21	0.28	−0.497	0.112
	Region	−0.575	0.159	11.21	**0.01**	**−0.879**	**−0.271**
Body condition	Habitat	6.591	2.043	**9.30**	**0.04**	**2.649**	**10.533**
n = 135	Sex	0.364	2.063	0.02	0.89	−3.617	4.345
	Reproductive status	11.209	2.029	**34.41**	**<0.001**	**7.295**	**15.125**
	Year	6.873	2.798	**7.54**	**0.009**	**2.132**	**11.613**
	Region	3.888	2.463	0.93	0.36	−0.864	8.640
	Habitat × Sex	−11.282	4.149	**7.09**	**0.009**	**−19.257**	**−3.306**

Bold reflects statistical significance ($P < 0.05$).

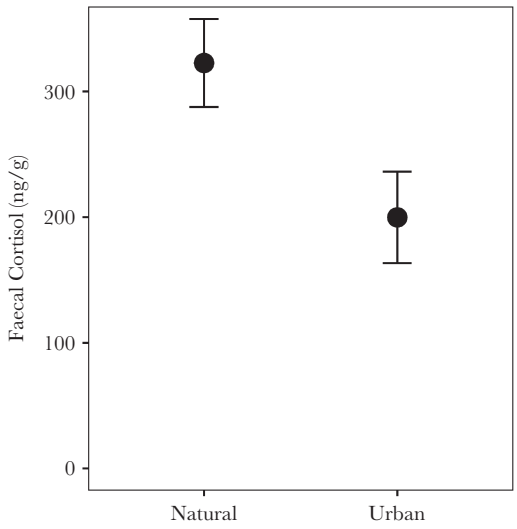

Figure 1
Chipmunks caught in urban environments had lower fecal cortisol levels than those caught in natural settings (see Table 1). Bars represent standard error.

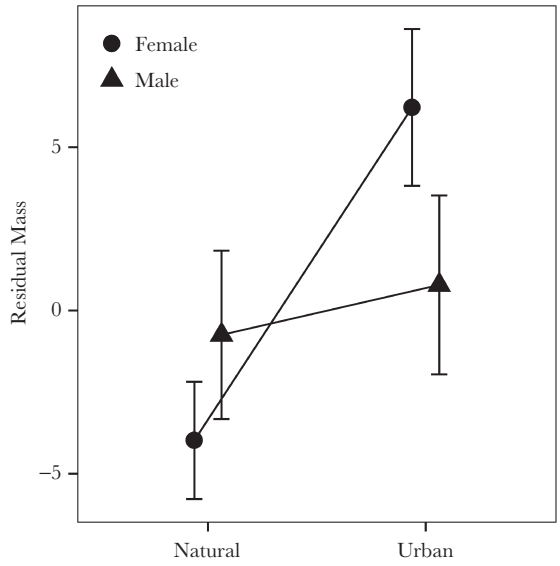

Figure 2
Chipmunks tended to have higher residual mass in urban than natural environments but this relationship was largely due to a much stronger effect in females than males. Bars represent standard error.

Body condition

There was a significant interaction effect of Habitat × Sex ($P = 0.009$) on body condition (Table 1). Additionally, there was a main effect of Habitat (urban/natural) on body condition. The interaction effect was driven by female chipmunks that were much heavier in urban habitats than natural habitats (Figure 2). Other main effects that indicated significant effects on body condition included Reproductive Status (reproductive > non-reproductive), and Year (2013 > 2012) (Table 1).

Behavior

The analysis of the open field test indicated a significant effect of habitat (urban/natural) on a multivariate model of behavioral traits when fecal cortisol was included as a main effect (Table 1). Because the multivariate result is a measure of the combined effects of behavioral variables, we examined the univariate analyses to infer the likely behavioral variables that drove the significant difference between urban and natural habitats. Of the eight behavioral variables, locomotion, grooming, and latency were the closest to exhibiting a significant difference (see Table 2). Urban chipmunks tended to spend less time undergoing locomotion, less time grooming, and more time latent than their counterparts from natural habitats (Figure 3).

DISCUSSION

The general pattern of our results suggests that urban habitats may be relatively benign compared to natural habitats for individual chipmunks. Urban chipmunks were less active, had reduced cortisol, and females were in better body condition than their counterparts from natural areas.

The behavioral data indicated a significant difference in behavior between chipmunks from urban and natural areas but conformed to a negative binomial distribution. The multivariate approach we adopted to analyze these data did not permit us to deconstruct the multivariate response variable. Thus we interpreted this result using univariate analyses. As such, it appeared that urban chipmunks were less active (reduced locomotion), groomed less and were more latent than chipmunks from natural habitats. These behavioral traits can be interpreted in the context of personality. For example, reduced locomotion is associated with reduced exploratory behavior, and grooming is positively associated with reactions to a stressor (Martin and Réale 2008b). Latency is considered a measure of the emotional reaction to a stimulus (Walsh and Cummins 1976). Taken together, the results of the open field test suggest that urban chipmunks are less active and perhaps less reactive to a stressor than chipmunks in a natural habitat. The reduced exploratory behavior in our study is in contrast with other studies of urban wildlife. For example, eastern chipmunks that occupied territories near where humans frequented also were more explorative (Martin and Réale 2008a). Urban populations of the anole lizard *Anolis sagrei* exhibited more exploratory behavior when confronted with a novel environment (Lapiedra et al. 2017). It is intuitive that individuals that are found in a novel environment should have behavioral traits that facilitate dispersal such as explorative behavior, but alternative hypotheses may explain the pattern we found. Sol et al. (2013) articulated several stages of colonization associated with the establishment of urban populations. The arrival stage is associated with dispersal and habitat selection, and can be expected to be associated with behavioral types that include exploration, boldness and aggression (Sol et al. 2013). However, subsequent stages endure significant selection, and so new behavioral phenotypes can increase in frequency. A behavioral phenotype that reflects reduced exploration and reactions to stress may be favored in the context of an environment where exploratory behavior can be costly. Urban environments may have large and predictable anthropogenic food subsidies, for example, that might mitigate the foraging costs associated with natural habitats (Lowry et al. 2013). Predation risk may be lessened in urban environments because of the prevalence of anthropogenic food subsidies, (Fischer et al. 2012), reducing selection on stress responses and exploratory behavior. Finally, it is possible that urban chipmunks did not disperse during urban development, and thus chipmunks with exploratory behavior left the urban habitat, leaving behind those that were not explorative.

The conclusion that urban chipmunks are less reactive to a stressor based on reduced grooming behavior in the open field test is supported by our analysis of fecal cortisol. While there was no effect of habitat on hair cortisol, fecal cortisol was significantly lower in urban chipmunks—chipmunks captured in natural habitats had on average cortisol values that were ≈ 65% higher than urban chipmunks. Most of the literature on endocrine ecology of urban populations is related to birds (see Bonier 2012). The limited evidence from mammals is consistent with that found in birds—that the endocrine responses to urban habitats are species-specific (Bonier 2012). Squirrel gliders (*Petaurus norfolcensis*) had higher hair cortisol concentrations when adjacent to major roads and lowest in interior habitats (Brearley et al. 2012). Brazilian free-tailed bats (*Tadarida brasiliensis*) roosting under bridges have lower blood cortisol levels than those roosting in caves (Allen et al. 2011). Our results also suggest that anthropogenic infrastructure does not negatively impact the stress response of eastern chipmunks, unlike the stress response that appears to be prevalent with other human activities in other species (Dantzer et al. 2014).

Table 2

Results of the multivariate negative binomial generalized linear models

Test	Effect	Wald	*P*
Multivariate	*Hair cortisol*	3.34	0.22
	Habitat	3.79	0.10
	Sex	2.52	0.58
	Reproductive condition	3.42	0.18
	Fecal cortisol	2.69	0.53
	Habitat	**4.47**	**0.03**
	Sex	2.15	0.75
	Reproductive condition	2.66	0.51
Univariate	Locomotion	1.78	0.37
	Head Dipping	1.14	0.71
	Scanning	0.75	0.78
	Grooming	2.26	0.22
	Climbing	1.19	0.71
	Rearing	0.36	0.85
	Stillness	0.43	0.85
	Latency	2.06	0.26
Multivariate	*Residual mass*	3.91	0.09
	Habitat	3.90	0.08
	Sex	2.40	0.65
	Reproductive condition	3.73	0.11
	Residual mass × Habitat	4.33	0.06

Our multivariate dependent variable was comprised of the 7 behavioral variables outlined in the Methods. We followed the multivariate fecal cortisol model with univariate models corrected for multiple tests of each of the individual behavioral variables to help resolve the contribution of the behaviors to the significant effect of Habitat (natural vs. urban) run in the fecal cortisol model.

The reduced baseline fecal cortisol in urban chipmunks may result from desensitization to stressors due to down-regulation of the HPA axis. This limits the negative effects of long-term exposure to stressors (Partecke et al. 2006). This can occur when an animal is repeatedly exposed to the stressor (Fokidis et al. 2009). Down-regulation of HPA activity reduces glucocorticoid production in urban birds exposed to frequent stressors (Fokidis et al. 2009). This physiological mechanism may allow chipmunks to successfully colonize urban habitats. Species that cannot down-regulate the stress response might not be able to tolerate, and thus would avoid, the disturbances associated with cities. On the other hand, species (including perhaps eastern chipmunks) that can down-regulate the stress response may be capable of successful reproduction in urban habitats with a high degree of anthropogenic disturbance.

We found different patterns of cortisol between urban and natural habitats depending on the substrate—hair or feces. These results may reflect differences in the time scale each substrate represents. Cortisol is deposited in hair over a period of time that occurs during the molt and represents the response to stress over the longer term on the scale of weeks and months, whereas fecal cortisol metabolites provide a profile of cortisol secretion that represents the previous hours or days (Sheriff et al. 2011). There are 2 possibilities that explain the lack of habitat (urban vs. natural) effect on hair cortisol. First, hair cortisol may not be variable enough to detect differences between urban and natural habitats. Second, the distribution of urban and natural habitats may be such that, over the moulting period, eastern chipmunks encounter about equal proportions of urban and natural habitat, and so at the time scale reflected in hair cortisol there are no differences. A similar pattern was found in eastern chipmunks in logged and undisturbed habitats (Mastromonaco et al. 2014). While there is some evidence that eastern chipmunks will avoid roads (Ford and Fahrig 2008), they also

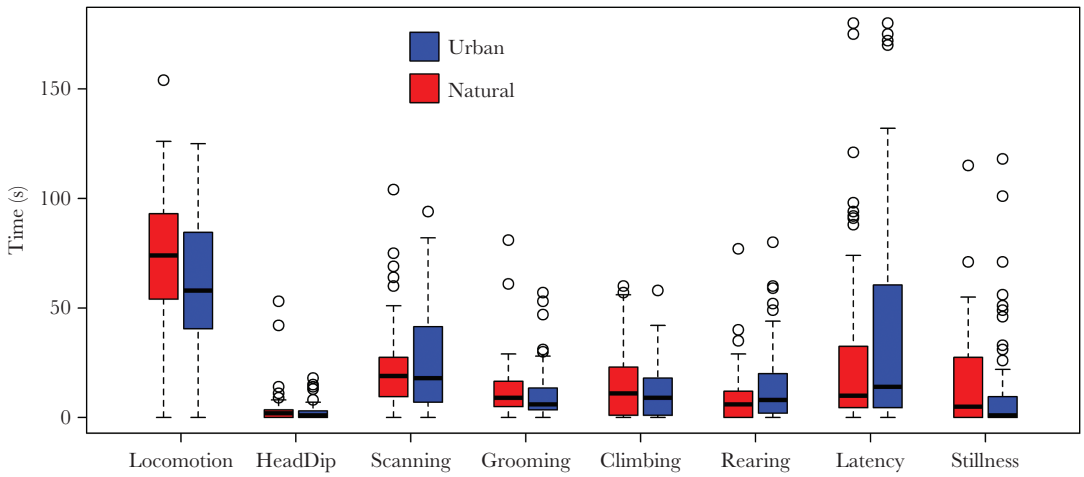

Figure 3

Representation of the univariate behavioral differences between urban and rural chipmunks from the multivariate model including fecal cortisol (see Table 2). Plotted are medians, quartiles, and ranges.

are known to cross large gaps in fragmented landscapes that consist of hardwood forest and short grass pasture (Bowman and Fahrig 2002). Thus, there may be some opportunity for movement of eastern chipmunks between urban and natural habitats.

Body condition, as measured by residual mass, reflects the energy reserves carried by an individual in the form of fat and protein (Schulte-Hostedde et al. 2001). Our results therefore indicate that female eastern chipmunks are carrying more energy reserves than their counterparts in natural habitats. The presence of predictable food subsidies through the presence of human refuse can affect the ecology and evolution of urban wildlife (Oro et al. 2013). In particular, the availability of food resources in urban environments can mitigate the impacts of seasonal variation on wildlife (Shochat et al. 2006). Specifically, omnivores may be beneficiaries of human subsidies (Fedriani et al. 2001). Eastern chipmunks have a broad diet—eating nuts, seeds, invertebrates, fungi, frogs, snakes, and even other small mammals (Sutton 1982) and so may benefit from anthropogenic food subsidies in urban environments.

The fact that female chipmunks were in better body condition in urban habitats, and not males, may be related to the energetic requirements for lactation, and the predictable access to food resources. Lactation is the most energetically demanding stage of reproduction for female mammals (Millar 1978). If access to food resources is enhanced in urban habitats relative to natural habits because of anthropogenic food subsidies, female chipmunks may be better able to fuel lactation. Body mass can be an important determinant of female reproduction in chipmunks (Svendson and White 1997) and other rodents (Ribble 1992). Thus female reproductive success should be higher in urban habitats than in natural habitats.

Our study describes patterns of phenotypic divergence between natural and urban habitats, and the ecological and evolutionary consequences of this divergence remain to be investigated. For example, given the pattern of behavior, cortisol, and energy reserves, differences in metabolic rate may also be evident. Chipmunks in urban environments may have lower metabolic rates than their counterparts in natural habitats because of reduced activity and lower levels of cortisol (Burton et al. 2011). In addition, assessing genetic structure and patterns of gene flow among urban and natural populations will allow for the assessment of genetic divergence.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

FUNDING

This work was supported by the Canada Research Chair in Applied Evolutionary Ecology (230840), the Natural Sciences and Engineering Research Council (NSERC) of Canada (Discovery Grant RGPIN-2014–05073) and the Canadian Foundation for Innovation (Project # 9924, 220224).

We thank Algonquin Provincial Park, the Algonquin Wildlife Research Station, the Town of Huntsville, Laurentian University, private property owners and others who gave us permission to sample chipmunks on their property. Two anonymous reviewers provided valuable suggestions.

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Lyons et al. (2017).

Handling editor: Bob Wong

REFERENCES

Adams LW. 2005. Urban wildlife ecology and conservation: a brief history of the discipline. Urban Ecosyst. 8:139–156.

Allen LC, Turmelle AS, Widmaier EP, Hristov NI, McCracken GF, Kunz TH. 2011. Variation in physiological stress between bridge- and cave-roosting Brazilian free-tailed bats. Conserv Biol. 25:374–381.

Ashley NT, Barboza PS, Macbeth BJ, Janz DM, Cattet MR, Booth RK, Wasser SK. 2011. Glucocorticosteroid concentrations in feces and hair of captive caribou and reindeer following adrenocorticotropic hormone challenge. Gen Comp Endocrinol. 172:382–391.

Atwell JW, Cardosa GC, Whittaker DJ, Campbell-Nelson S, Robertson KW Ketterson ED. 2012. Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. Behav. Ecol. 23:960–969.

Bates D, Maechler M, Bolker B, Walker S. 2013. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0–4 [cited 2016 June 10]. Available from: http://CRAN.R-project.org/package=lme4.

Birnie-Gauvin K, Peiman KS, Gallagher AJ, de Bruijn R, Cooke SJ. 2016. Sub-lethal consequences of urban life for wild vertebrates. Environ Rev. 24:416–425.

Boissy A. 1995. Fear and fearfulness in animals. Q Rev Biol. 70:165–191.

Bonier F. 2012. Hormones in the city: endocrine ecology of urban birds. Horm Behav. 61:763–772.

Bowman J, Fahrig L. 2002. Gap crossing by chipmunks: an experimental test of landscape connectivity. Can J Zool. 80: 1556–1561.

Bozek CK, Prange S, Gehrt SD. 2007. The influence of anthropogenic resources on multi-scale habitat selection by raccoons. Urban Ecosyst. 10:413–425.

Brearley G, McAlpine C, Bell S, Bradley A. 2012. Influence of urban edges on stress in an arboreal mammal: a case study of squirrel gliders in south-east Queensland, Australia. Conserv Biol. 27:1407–1419.

Burton T, Killen SS, Armstrong JD, Metcalfe NB. 2011. What causes intra-specific variation in resting metabolic rate and what are its ecological consequences? Proc Biol Sci. 278:3465–3473.

Dantzer B, Fletcher QE, Boonstra R, Sheriff MJ. 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conserv Physiol. 2:cou023.

Dingemanse NJ, Both C, van Noordwijk AJ, Rutten AL, Drent PJ. 2003. Natal dispersal and personalities in great tits (*Parus major*). Proc Biol Sci. 270:741–747.

Ditchoff SS, Saalfeld ST, Gibson CJ. 2006. Animal behavior in urban ecosystems: Modifications due to human stress. Urban Ecosyst. 9:5–12.

Evans J, Boudreau K, Hyman J. 2010. Behavioral syndromes in urban and rural populations of Song Sparrows. Ethology. 116:588–595.

Fedriani JM, Fuller TK, Sauvajot RM. 2001. Does availability of anthropogenic food enhance densities of omnivorous mammals? An example with coyotes in southern California. Ecography. 24:325–331.

Fischer JD, Cleeton SH, Lyons TP, Miller JR. 2012. Urbanization and the predation paradox: the role of trophic dynamics in structuring vertebrate communities. BioScience. 62:809–818.

Fokidis HB, Orchinik M, Deviche P. 2009. Corticosterone and corticosteroid binding globulin in birds: relation to urbanization in a desert city. Gen Comp Endocrinol. 160:259–270.

Ford AT, Fahrig L. 2008. Movement patterns of eastern chipmunks (Tamias striatus) near roads. J. Mamm. 89: 895–903.

Fraser DF, Gilliam JF, Daley MJ, Le AN, Skalski GT. 2001. Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. Am Nat. 158:124–135.

Fox J, Weisberg S. 2011. An {R} companion to applied regression analysis, 2nd ed. Thousand Oaks (CA): Sage

Grimm NB, Faeth SH, Golubiewski NE, Redman CL, Wu J, Bai X, Briggs JM. 2008. Global change and the ecology of cities. Science. 319:756–760.

Kenagy GJ, Place NJ. 2000. Seasonal changes in plasma glucocorticosteroids of free-living female yellow-pine chipmunks: effects of reproduction and capture and handling. Gen Comp Endocrinol. 117:189–199.

Lapiedra O, Chejanovski Z, Kolbe JJ. 2017. Urbanization and biological invasion shape animal personalities. Glob Chang Biol. 23:592–603.

Lowry H, Lill A, Wong BBM, 2011. Tolerance of auditory disturbance by an avian urban adapter, the noisy miner. Ethology. 117:490–497.

Lowry H, Lill A, Wong BB. 2013. Behavioural responses of wildlife to urban environments. Biol Rev Camb Philos Soc. 88:537–549.

Lyons J, Mastromonaco G, Edwards D, Schulte-Hostedde A. 2017. Data from: fat and happy in the city: eastern chipmunks in urban environments. *Dryad Digital Repository*. http://dx.doi.org/10.5061/dryad.0gg8n.

Macbeth BJ, Cattet MRL, Stenhouse GB, Gibeau ML, Janz DM. 2010. Hair cortisol concentration as a noninvasive measure of long-term stress in free-ranging grizzly bears (*Ursus arctos*): considerations with implications for other wildlife. Can J Zool. 88:935–949.

Martin JGA, Réale D. 2008a. Animal temperament and human disturbance: implications for the response of wildlife to tourism. Behav Process. 77:66–72.

Martin JGA, Réale D. 2008b. Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*. Anim Behav. 75: 309–318.

Marzluff JM, Bowman R, Donnelly RE. 2001. Avian conservation and ecology in an urbanizing world. New York: Kluwer.

Mastromonaco GF, Gunn K, McCurdy-Adams H, Edwards DB, Schulte-Hostedde AI. 2014. Validation and use of hair cortisol as a measure of chronic stress in eastern chipmunks (*Tamias striatus*). Conserv Physiol. 2:cou055.

McGregor RL, Bender DJ, Fahrig L. 2008 Do small mammals avoid roads because of the traffic? J Appl Ecol. 45:117–123.

Millar JS. 1978. Energetics of reproduction in *Peromyscus leucopus*: The cost of lactation. Ecology. 59:1055–1061.

Møller AP. 2009. Successful city dwellers: a comparative study of the ecological characteristics of urban birds in the Western Palearctic. Oecologia. 159:849–858.

Montiglio P-O, Garant D, Thomas D, Réale D. 2010. Individual variation in temporal activity patterns in open-field tests. Anim Behav. 80:905–912.

Montiglio, P-O, Garant D, Pelletier F, Reale D. 2012a. Personality differences are related to long-term stress reactivity in a population of wild eastern chipmunks, *Tamias striatus*. Anim Behav. 84:1071–1079.

Montiglio P-O, Pelletier F, Palme R, Garant D, Réale D, Boonstra R. 2012b. Non-invasive monitoring of fecal cortisol metabolites in the eastern chipmunk (*Tamias striatus*): validation and comparison of two enzyme immunoassays. Physiol Biochem Zool. 85:183–193

Munro CJ, Lasley BL. 1988. Non-radiometric methods for immunoassay of steroid hormones. *Prog Clin Biol Res*. 285:289–329.

Oro D, Genovart M, Tavecchia G, Fowler MS. 2013. Ecological and evolutionary implications of food subsidies from humans. Ecol Lett. 16:1501–1514.

Partecke J, Schwabl I, Gwinner E. 2006. Stress and the city: urbanization and its effects on the stress physiology in European blackbirds. Ecology. 87:1945–1952.

Patterson LD, Schulte-Hostedde AI. 2011. Behavioral correlates of parasitism and reproductive success in male eastern chipmunks, Tamias striatus. Anim Behav. 81:1129–1137.

Pinheiro JC, Bates DM. 2000. Mixed-effects Models in S and S-Plus. New York: Springer-Verlag.

Powell C, Lill A, Johnstone CP. 2013. Body condition and chronic stress in urban and rural Noisy Miners. Open Ornithol J. 6:25–31.

R Core Team. 2012. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: http://www.R-project.org/.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev Camb Philos Soc. 82:291–318.

Ribble DO. 1992. Lifetime reproductive success and its correlates in the monogamous rodent, *Peromyscus californicus*. J Anim Ecol. 61:457–468.

Ripmeester EA, Mulder M, Slabbekoorn H. 2010. Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird. Behav Ecol. 21:876–883.

Rodewald AD, Kearns LJ, Shustack DP. 2011. Anthropogenic resource subsidies decouple predator-prey relationships. Ecol Appl. 21:936–943.

Schulte-Hostedde AI, Millar JS, Hickling GJ. 2001. Evaluating body condition in small mammals. Can J Zool. 79:1021–1029.

Schulte-Hostedde AI, Zinner B, Millar JS, Hickling GJ. 2005. Restitution of mass-size residuals: validating body condition indices. Ecology. 86:155–163.

Sheriff MJ, Dantzer B, Delehanty B, Palme R, Boonstra R. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia. 166:869–887.

Semenova TP, Anoshkina IA, Khomut BM, Kolaeva SG. 2001. Seasonal peculiarities of behavior of ground squirrel *Citellus undulatus* in holeboard and open field tests. Behav Processes. 56:195–200.

Shochat E, Warren PS, Faeth SH, McIntyre NE, Hope D. 2006. From patterns to emerging processes in mechanistic urban ecology. Trends Ecol Evol. 21:186–191.

Sol D, Lapiedra O, Gonzalez-Lagos C. 2013. Behavioural adjustments for a life in the city. Anim Behav. 85:1101–1112.

Stockwell CA, Hendry AP, Kinnison MT. 2003. Contemporary evolution meets conservation biology. Trends Ecol. Evol. 18:94–101.

Sutton DP. 1982. *Tamias striatus*. Mamm. Sp. 168:1–8.

Svendson GE, White MM. 1997. Body mass and first-time reproduction in female chipmunks (*Tamias striatus*). Can. J. Zool. 75:1891–1895.

Walsh RN, Cummins RA. 1976. The Open-Field Test: a critical review. Psychol Bull. 83:482–504.

Wang Y, Naumann U, Wright S, Warton D. 2013. mvabund: statistical methods for analysing multivariate abundance data. R package version 3.8.4 [cited 2016 June 10]. Available from: http://CRAN.R-project.org/package=mvabund.

Wickham H. 2009. ggplot2: elegant graphics for data analysis. New York: Springer.