Behavior, Physiology, and Reproduction of Urban and Rural Song Sparrows (*Melospiza Melodia*)

Sarah L. Foltz

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Biological Sciences

Ignacio T. Moore
Dana M. Hawley
William A. Hopkins
Jeffrey R. Walters

April 22nd, 2015
Blacksburg, Virginia

Keywords: urban, aggression, reproductive success, corticosterone, song sparrow

Behavior, Physiology, and Reproduction of Urban and Rural Song Sparrows (*Melospiza Melodia*)

Sarah L. Foltz

ABSTRACT

Urban areas are a unique and growing habitat type. Animals living in this novel habitat are faced with new challenges, but may also encounter novel opportunities. Though urban animals have been observed to differ from their rural counterparts in a variety of behavioral and physiological traits, little is known about the specific features of urban areas that drive these differences and whether they are adaptive. Understanding this process is important from a conservation perspective and also to gain insight into how animals colonize novel habitats more generally. Using song sparrows (*Melospiza melodia)*, a native songbird commonly found in urban areas, I explored responses to urbanization and the drivers and consequences of these responses with an eye toward understanding whether song sparrows had successfully adapted to urban habitats (Chapter I). I began by comparing body condition and levels of corticosterone, a hormone associated with energy management and the stress response in birds, between urban and rural populations (Chapter II). There was more variation across years than between habitats, suggesting that a variable environmental factor common to both habitats is the primary driver of these traits. I then compared territorial aggression levels and tested the effect of food availability on aggression (Chapter III). Fed rural birds and all urban birds had higher aggression levels than unfed rural birds, indicating that territorial aggression is related to resource availability in this species and that urban habitats may be perceived as more desirable. Finally, I looked for differences in reproductive timing and success and for relationships between reproductive success and aggression (Chapter IV). Higher reproductive success in urban populations, coupled with differences in the timing of successful nests between habitats, suggest differences in predation risk and predator community structure between habitats. In Chapter V, I synthesize my major findings and suggest directions for future research building on these results. I conclude that urban song sparrows differ from rural birds, that these differences are influenced by resource availability, and that urban habitats can potentially support stable song sparrow populations, though more research is necessary to determine the fitness impacts of specific traits that change with urbanization.

DEDICATION

To the birds.

ACKNOWLEDGEMENTS

Ignacio Moore – for giving me a chance to do my own research, insisting that I do it well, and editing approximately a bazillion drafts of posters, presentations, grants, and papers

Members of my committee (Dana Hawley, Jeff Walters, Bill Hopkins) – for your time and advice along the way

Members of the Moore lab, past and present (Jonathan Moore, Julie Danner, Camilo Escallon, Laura Schoenle, Jenny Ouyang, Cas Eikenar, Adam Lendavai), and the McGlothlin and Sewall labs – for reading drafts, sitting through practice presentations, commenting on analyses and general support

Fran Bonier – for letting me tag along on her dissertation research and getting me hooked on urban ecology in the process, and for occasional emergency advice sessions throughout the course of this work

Jim Adelman – for advice on statistical analyses and much-needed R coaching

L.I.S.A. – for additional statistical advice

My family – for raising me to believe that I could do this

My husband, Jason Davis – for keeping me sane (mostly)

ATTRIBUTION

Dr. Ignacio T. Moore, Professor, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA. Dr. Moore was my advisor and advisory committee chair. He is a co-author on all publications and manuscripts (Chapters II, III, and IV) resulting from this dissertation.

Allen E. Ross, undergraduate student, Department of Mathematics, Virginia Tech, Blacksburg, VA 24061, USA. Mr. Ross is a co-author on Chapters II, III, and IV and assisted with data collection for this dissertation.

Brenton T. Laing, undergraduate student, Department of Psychology, Virginia Tech, Blacksburg, VA 24061, USA. Mr. Laing is currently a graduate student in the Department of Recreation and Leisure Studies, East Carolina University, Greenville, NC 27858. He is a co-author on Chapters II and III and assisted with data collection and analysis for this dissertation.

Kathryn E. Battle, undergraduate student, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA. Ms. Battle is currently a graduate student in the Department of Applied Ecology, North Carolina State University, Raleigh, NC 27695. She is a co-author on Chapters III and IV and assisted with data collection for this dissertation.

Dr. Jason E. Davis, Assistant Professor, Department of Biology, Radford University. Dr. Davis is a co-author on Chapter I, provided advice, helped with data collection, and provided research equipment.

Ryan P. Rock, undergraduate student, Department of Fisheries and Wildlife, Virginia Tech, Blacksburg, VA 24061, USA. Mr. Rock is a co-author on Chapter III and assisted with data collection for this dissertation.

Virginia W. Greene, undergraduate student, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA. Ms. Greene assisted with data collection for Chapter II of this dissertation.

James A. Tallant, undergraduate student, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA. Mr. Tallant is a co-author on Chapter II and assisted with data collection for this dissertation.

Rene C. Vega, undergraduate student, Department of Biology, Radford University, Radford, VA 24142. Mr. Vega is a co-author on Chapter II and assisted with data collection for this dissertation.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER II

CHAPTER III

CHAPTER IV

LIST OF TABLES

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER I: INTRODUCTION

Sarah L. Foltz

Urban Habitats

Urban areas are a rapidly expanding habitat. Globally, more than half of the human population already lives in cities, and the current urban population is expected to grow by 2.5 billion people by 2050 ("United Nations" 2014). Urban areas must grow to accommodate this influx of humans, and their growth necessarily comes at the expense of less-developed land. Wildlife near developing areas will need to adjust to urban living conditions or be forced into shrinking and increasingly fragmented rural areas.

Urban areas can be considered a novel type of habitat. Though many of the features of urban habitat that at first seem unique have analogues in the natural world, urban habitats concentrate and combine these features in ways not found elsewhere (Faeth et al. 2011). Thus, urban wildlife is presented with a variety of novel challenges and opportunities. Different foods (Fedriani et al. 2001; Marzluff and Neatherlin 2006; Heiss et al. 2009), noise and light pollution (Rheindt 2003), high densities of roads and buildings (Riley et al. 2006; Fahrig and Rytwinski 2009; Gelb and Delacretaz 2009), and changes in predator populations (Stracey and Robinson 2012) are just some of the many differences that animals moving into urban areas might experience. To live in urban areas, animals must cope with the challenges presented by these habitats. Not all species are capable of doing so.

In general, animal species richness tends to decline with increasing urbanization, though for some taxa, including birds, species diversity is highest at an intermediate point along the urbanization gradient (Chace and Walsh 2006; McKinney 2008; Faeth et al. 2011). A few species even seem to thrive in urban habitats. Avian abundance can actually increase with urbanization despite declines in species richness (Chace and Walsh 2006; Faeth et al. 2011), often due to the presence of non-native urban exploiters such as house sparrows (*Passer domesticus*), European starlings (*Sturnus vulgaris*), and rock pigeons (*Columba livia)* (Shochat et al. 2010). Studying those species that do inhabit urban areas may lead to insights about those that do not and reveal the features of urbanization that most impact urban wildlife.

Hormonal Response to Stress

The glucocorticoid stress response has been previously predicted to be a potentially important factor in the successful colonization of novel habitats (Wingfield and Kitaysky 2002). At baseline levels, glucocorticoids help to maintain energetic balance by mediating metabolic processes (Saplosky et al. 2000). When an animal is faced with a stressor, glucocorticoid levels typically rise (Schwabl et al. 1985; Rogers et al. 1993; Kitaysky et al. 1999; Lynn et al. 2003; Bonier et al. 2009; Wingfield 2013; Love et al. 2014). This response may be adaptive, mobilizing stored energy and redirecting energy from long-term investments, such as reproduction, toward immediate survival activities, as well as promoting coping behaviors such as irruptive migration (Wingfield and Ramenofsky 1997; Wingfield and Kitaysky 2002; Breuner et al. 2008). However, chronic activation of the glucocorticoid stress response can be maladaptive, dysregulating the hypothalamic-pituitary-adrenal axis that mediates the glucocorticoid stress response and interfering with reproduction (Sapolsky et al. 2000; Myers et al. 2014). The frequent disturbances and novel stressors present in urban habitats could trigger chronic activation of the stress response. Thus, the ability to appropriately mediate the glucocorticoid stress response to novel and frequent stressors may be key to the successful colonization of urban habitats.

Differences Between Urban and Rural Populations

Birds are one of the most commonly studied urban taxa (Faeth et al. 2011), likely because they are relatively easy to observe and interact with. Among species that inhabit both urban and rural areas, urban individuals exhibit a variety of behavioral and physiological differences from their rural counterparts. Birds sing at higher pitches in urban habitats, which generally have high levels of noise pollution (Slabbekoorn and Peet 2003; Wood and Yezerinac 2006; Hu and Cardoso 2010; Luther and Derryberry 2012). Urban birds appear more tolerant of humans, allowing closer approaches than rural birds (Møller 2010; Scales et al. 2011; Atwell et al. 2012). Breeding often begins earlier in urban populations (Yeh and Price 2004; Partecke et al. 2004) and the breeding season may extend longer (Yeh and Price 2004). Urban populations of migratory species may become more sedentary (Partecke and Gwinner 2007). Territorial aggression levels may increase (Evans et al. 2010; Scales et al. 2011) or decrease (Newman et al. 2006), depending on the species. Circulating glucocorticoid levels also differ in species- and occasionally sex-specific ways (Partecke et al. 2006; Fokidis et al. 2009; Bonier et al. 2007a;

Bonier 2012). However, in most cases we are still unaware of how these differences arise and what effects they have on the individuals exhibiting them. In this work, I chose to focus on several potentially interrelated behavioral and physiological traits that have been previously found to differ between urban and rural groups: glucocorticoids, territorial aggression, and reproductive success and associated parental behaviors.

Previous urban/rural comparisons of multiple avian species have found that both baseline and/or stress-induced glucocorticoid levels sometimes differ between urban and rural populations (Partecke et al. 2006; Bonier et al. 2007a; Fokidis et al. 2009; Zhang et al. 2011; Atwell et al. 2012; but see Schoech et al. 2007). These differences may represent responses to the challenges of urban living or may be a product of selective colonization of urban habitats by individuals with particular stress-response profiles. However, no consistent pattern has emerged across the species studied so far (Bonier 2012). As few studies collect corticosterone samples across multiple years from the same populations or analyze the data with attention to year as a factor when they do so (Newman et al. 2006; Bonier et al. 2007; Fokidis et al. 2009; Hu and Cardoso 2010; Scales et al. 2011; Atwell et al. 2012; but see Schoech et al. 2007 and Partecke and Gwinner 2007), it is also unclear whether differences, when present, are consistent across time.

Territorial aggression has also been observed to differ between urban and rural populations in several species (Newman et al. 2006; Evans et al. 2010; Scales et al. 2011) and has the potential to influence both success in urban habitats and individual fitness. Aggressive behavior may enable individuals to out-compete other species for territories in space-limited urban environments (Duckworth and Badyaev 2007; Groen et al. 2012; Hudina et al. 2012; but see Cunningham and Rissler 2013). Thus, heightened territorial aggression among urban animals may be an example of pre-adaptation to urban habitats. Territorial aggression has also been positively linked to resource availability (Ewald and Carpenter 1978; Fox et al. 1981; Lore et al. 1986; Camfield 2006; Snekser et al. 2009; Maruyama et al. 2010; Di Paola et al. 2012; but see Toobaie and Grant 2013) and to competition for resources within and between species (Perrin et al. 2001; Lacava et al. 2011; Di Paola et al. 2012; Yoon et al. 2012). Such factors could lead to selection on or facultative adjustment of territorial aggression levels after colonization of urban areas. However, aggressive behavior can be costly (Marler and Moore 1988; Duckworth 2006), and thus should generally not be maintained unless its benefits outweigh its costs. The consistency of urban/rural differences in territorial aggression across time is not well understood and the specific features of urbanization driving differences in this behavior have not been clearly identified.

Urban and rural populations also differ in reproductive behavior and physiology. Partecke et al. (2004) found that European blackbirds (*Sturnus vulgaris*) developed their gonads three to four weeks earlier in urban areas than in rural ones, while Yeh and Price (2004) found that dark-eyed juncos (*Junco hyemalis*) breeding in San Diego, CA, extended their breeding season to nearly double that of their nearest rural counterparts by starting earlier and ending later. These differences in reproductive timing could be driven by differences in secondary cues such as higher temperatures (Bornstein 1968) and food availability (Schoech and Bowman 2001) in urban habitats and could lead to reduced gene flow between urban and rural populations. The effects of urbanization on immediate reproductive success are less clear. Although Yeh and Price (2004) found that juncos in their urban population could fledge twice as many broods in a year and that urban nestlings weighed more than rural ones, not all species respond to urbanization this way. Aldredge et al (2012) found that urban-breeding scrub-jays (*Aphelocoma coerulescens*) had reduced hatching success. Richner (1989) found that the nestlings of urban carrion crows

(*Corvus corone corone*) grew more slowly and weighed less at fledging than those of rural birds, and that urban crows fledged fewer nestlings. Thus, while it is clear that urbanization can impact birds' reproductive activities, the costs and benefits of urban breeding need further study.

Phenotypic Plasticity, Selection, and Pre-Adaptation

The differences observed in urban animals could result from one or more factors. Differences in traits between urban and rural animals could result from phenotypic flexibility, in which case these differences would be reversible under the appropriate conditions (Wada and Sewall 2014). Alternatively, these traits may be fixed at differing set points, either genetically or during development. Trait differences between these groups could result from selection on urban populations after colonization of urban habitat or be evidence of individual pre-selection. In the later case, some individuals may possess traits that make them better urban colonizers, and these individuals would thus be disproportionately represented in urban populations. These possibilities are not mutually exclusive and likely interact with each other. For example, urban habitats may select for greater phenotypic plasticity. Similarly, phenotypic plasticity could be considered a pre-adaptation to urban areas (Bonier et al. 2007b). While some studies clearly demonstrate the flexible (Estevez et al. 2002; Rodriguez-Prieto et al. 2008; Bermudez-Cuamatzin et al. 2009; Bermúdez-Cuamatzin et al. 2011; Stevenson and Rillich 2013) or fixed (Parteke et al. 2006; Atwell et al. 2012) nature of certain traits, the basis of urban/rural differences in many traits remains unclear.

Sustainability of Urban Populations

Responses to urbanization are not necessarily adaptive. Behavioral or physiological responses evolved for other habitat types may not be effective in an urban context. Similarly, the novel stimuli and frequent disturbances characteristic of urban areas might elicit unnecessary or disproportionate responses, such as over-activation of the glucocorticoid stress response in the face of repeated unfamiliar disturbances. The presence of a species in urban areas is not by itself indicative of the adaptive value of its responses to urbanization or of a healthy, sustainable urban population. Rather, urban habitats may be sinks within larger metapopulations, sustained by immigration from rural areas. At present, the fitness effects of many of the differences in behavior and physiology observed between urban and rural populations remain understudied. Understanding the influence of urban habitats on reproductive behavior and success is therefore vital to predicting the impact of increasing urbanization on affected populations.

Current Study

Over four consecutive years, I compared urban and rural populations of song sparrows (*Melospiza melodia)*, a native songbird commonly observed in both urban and rural habitats. I focused on four related questions:

1) How do urban and rural individuals differ?
2) What features of urbanization drive these differences?

3) Do differences between urban and rural populations represent adaptive responses to urbanization?

Sparrows were studied at four urban and three rural field sites situated within the New River Valley of southwestern Virginia (37°13’ N, 80°22’ W, ~600-700m elevation). These sites were chosen such that they differed in human population density and percent area of green space. Urbanization can be viewed as a continuum from wilderness areas to heavily urban sites such as megacities; my field sites represent points in the middle of this continuum, rather than its extremes. Thus, my urban sites are not as urbanized as some areas, while my rural sites should not be considered undisturbed. Not all sites were used in every study. Rather, in each study I included as many sites as practically possible, balanced between urban and rural habitats. To determine how populations differed and the habitat features related to these differences, I collected data on baseline and stress-induced corticosterone levels, body condition, and territorial aggression of adult, territory-holding males. I also observed the availability of suitable nesting vegetation, nest placement, and territory distribution, and conducted an experiment in which I provided some territories with supplemental food. To examine the potential sustainability of urban populations and the adaptive value of observed behavioral differences, I observed nest success, parental provisioning, and nestling mass and brood size. These studies and my conclusions are described in detail in the following chapters.

References

Aldredge RA, LeClair SC, Bowman R. 2012. Declining egg viability explains higher hatching failure in a suburban population of the threatened Florida scrub-jay Aphelocoma coerulescens. *J Avian Biol* **43**:369-375.

Atwell JW, Cardoso 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.

Bermudez-Cuamatzin E, Rios-Chelen AA, Gil D, Garcia CM. 2009. Strategies of song adaptation to urban noise in the house finch: syllable pitch plasticity or differential syllable use? *Behavior* **146**:1269-1286.

Bermúdez-Cuamatzin E, Ríos-Chelén AA, Gil D, Garcia CM. 2011. Experimental evidence for real-time song frequency shift in response to urban noise in a passerine bird. *Biol Lett* **7**:36-38

Bonier F, Martin PR, Sheldon KS, Jensen JP, Foltz SL, Wingfield JC. 2007a. Sex-specific consequences of life in the city. *Behav Ecol* **18**:121-129.

Bonier F, Martin PR, Wingfield JC. 2007b. Urban birds have broader environmental tolerance. *Biol Lett* **3**:670-673.

Bonier F, Moore IT, Martin PR, Robertson RJ. 2009. The relationship between fitness and baseline glucocorticoids in a passerine bird. *Gen Comp Endocrinol* **163**:208-213.

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

Bornstein RD. 1968. Observations of the urban heat island effect in New York City. *J Appl Meteor* **7**:575-582.

Breuner CW, Patterson SH, Hahn TP. 2008. In search of relationships between the acute adrenocortical response and fitness. *Gen Comp Endocrinol* **157**:288-295.

Camfield AF. 2006. Resource value affects territorial defense by broad-tailed and rufous hummingbirds. *J Field Ornithol* **77**:12-125.

Chace JF, Walsh JJ. 2006. Urban effects on native avifauna: a review. *Landsc Urban Plan* **74***:* 46–69.

Cunningham HR, Rissler LJ. 2013. Investigating behavioral shifts in aggression between a naturalized and native salamander species of the genus Plethodon. *Herpetol Conserv Biol* **8**:276-287.

Di Paola V, Vullioud P, Demarta L, Alwany MA, Ros AFH. 2012. Factors affecting interspecific aggression in a year-round territorial species, the jewel damselfish. *Ethology* **118**:721-732.

Duckworth RA. 2006. Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. *Behav Ecol* **17**:1011-1019.

Duckworth RA, Badyaev AV. 2007. Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proc Nat Acad* **104**:15017-15022.

Estevez I, Newberry RC, Keeling LJ. 2002. Dynamics of aggression in the domestic fowl. *Appl Anim Behav Sci* **76**:307-325.

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

Ewald PW, Carpenter FL. 1978. Territorial responses to energy manipulations in the Anna hummingbird. *Oecologia* **31**:277-292.

Faeth SH, Bang C, Saari S, Ostfeld RS, Schlesinger WH. 2011. Urban biodiversity: patterns and mechanisms. *Ann NY Acad Sci* **1223**:69-81.

Fahrig L and Rytwinski T. 2009. Effects of roads on animal abundance: an empirical review and synthesis. *Ecol Soc* **14**:21.

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.

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.

Fox SF, Myers R, Rose E. 1981. Dominance and the acquisition of superior home ranges in the lizard uta-stansburiana. *Ecology* **62**:888-893.

Gelb Y and Delacretaz N. 2009. Windows and vegetation: primary factors in Manhattan bird collisions. *Northeast Nat* **16**:455-470.

Groen M, Sopinka NM, Marentette JR, Reddon AR, Brownscombe JW, Fox MG, March-Rollo SE, Balshine S. 2012. Is there a role for aggression in round goby invasion fronts? *Behaviour* **149**:685-703.

Heiss RS, Clark AB, McGowan KJ. 2009. Growth and nutritional state of American crow nestlings vary between urban and rural habitats. *Ecol Appl* **19**:829-839.

Hu Y, Cardoso GC. 2010. Which birds adjust the frequency of vocalizations in urban noise? *Anim Behav* **79**:863-867.

Hudina S, Hock K. 2012. Behavioral determinants of agonistic success in invasive crayfish. *Behav Process* **91**:77-81.

Kitaysky AS, Wingfield JC, Piatt JF. 1999. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. *Funct Ecol* **13**:577-584.

Lacava RV, Brasileiro L, Maia R, Oliveira RF, Macedo RH. 2011. Social environment affects testosterone level in captive male blue-black grassquits. *Horm Behav* **59**:51-55.

Lore R, Gottdiener C, Delahunty MJ. 1986. Lean and mean rats some effects of acute changes in the food supply upon territorial aggression. *Aggressive Behav* **12**:409-416.

Love OP, Madliger CL, Bourgeon S, Semeniuk CAD, Williams TD. 2014. Evidence for baseline glucocorticoids as mediators of reproductive investment in a wild bird. *Gen Comp Endocrinol* **199**:65-69.

Luther DA, Derryberry EP. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. *Anim Behav* **83**:1059-1066.

Lynn SE, Breuner CW, Wingfield JC. 2003. Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. *Horm Behav* **43**:150-157.

Marler CA, Moore MC. 1988. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. *Behav Ecol Sociobiol* **23**:21-26.

Maruyama A, Rusuwa B, Yuma M. 2010. Asymmetric interspecific territorial competition over food resources amongst Lake Malawi cichlid fishes. *African Zool* **45:**24-31.

Marzluff JM, Neatherlin E. 2006. Corvid response to human settlements and campgrounds: causes, consequences, and challenges for conservation. *Biol Conserv* **130**:301-314.

McKinney ML. 2008. Effects of urbanization on species richness; a review of plants and animals. *Urban Ecosyst* **11**:161-176.

Møller AP. 2010. Interspecific variation in fear responses predicts urbanization in birds. *Behav Ecol* **21**:362-371.

Myers B, McKlveen JM, Herman JP. 2014. Glucocorticoid actions on synapses, circuits, and behavior: Implications for the energetics of stress. Frontiers in *Neuroendocrinology* **35**:180-196.

Newman MM, Yeh PJ, Price TD. 2006. Reduced territorial responses in dark-eyed juncos following population establishment in a climatically mild environment. *Anim Behav* **71**:893-899.

Partecke J, Van't Hof TJ, Gwinner E. 2004. Underlying physiological control of reproduction in urban and forest-dwelling European blackbirds Turdus merula. *J Avian Biol* **36**:295-305.

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

Partecke J, Gwinner E. 2007. Increased sedentariness in European blackbirds following urbanization: a consequence of local adaptation? *Ecology* **88**:882-890.

Perrin MR, Ercoli C, Dempster ER. 2001. The role of agonistic behaviour in the population regulation of two syntopic African grassland rodents, the striped mouse Rhabdomys pumilio (Sparrman 1784) and the multimammate mouse Mastomys natalensis (A. Smith 1834) (Mammalia Rodentia). *Trop Zool* **14**:7-29.

United Nations, Department of Economic and Social Affairs, Population Division. 2014. *World Urbanization Prospects: The 2014 Revision, Highlights* (ST/ESA/SER.A/352).

Rheindt FE. 2003. The impact of roads on birds: does song frequency play a role in determining susceptibility to noise pollution? *J Ornithol* **144**:295-306.

Richner H. 1989. Habitat-specific growth and fitness in carrion crows (Corvus corone corone). *J Anim Ecol* **58**:427-440.

Riley SP, Pollinger JP, Sauvajot RM, York EC, Bromley C, Fuller TK, Wayne RK. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. *Mol Ecol* **15**:1733-1741.

Rodriguez-Prieto I, Fernández-Juricic E, Martín J, Regis Y. 2008. Antipredator behavior in blackbirds: habituation complements risk allocation. *Behav Ecol* **20**:371-377.

Rogers CM, Ramenofsky M, Ketterson ED, Nolan V, Wingfield JC. 1993. Plasma corticosterone, adrenal mass, winter weather, and season in nonbreeding populations of dark-eyed juncos (Junco hyemalis hyemalis). *Auk* **110**:297-285.

Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulator, and preparative actions. *Endocr Rev* **21**:55-89.

Scales J, Hyman J, Hughes M. 2011. Behavioral syndromes break down in urban song sparrow populations. *Ethology* **117**:887-895.

Schoech SJ, Bowman R. 2001. Variation in the timing of breeding between suburban and wildlife Florida scrub-jays: do physiologic measures reflect different environments? In: Marzluff, JM, Bowman, R, Donnelly RE (eds). Avian Ecology and Conservation in an Urbanizing World. Kluwer Academic, Norwell, MA pp 291-308.

Schoech SJ, Bowman R, Bridge ES, Boughton RK. 2007. Baseline and acute levels of corticosterone in Florida scrub-jays *(Aphelocoma coerulescens)*: effects of food supplementation, suburban habitat, and year. *Gen Comp Endocrinol* **154**:150-160.

Schwabl H, Wingfield JC, Farner DS. 1985. Influence of winter on endocrine state and behavior in European blackbirds (Turdus merula). *J Comp Ethol* **68**:244-252.

Shochat, E. Lerman SB, Anderies JM, Warren PS, Faeth SH, Nilon CH. 2010. Invasion, competition, and biodiversity loss in urban ecosystems. *BioSci* **60***:* 199–208.

Slabbekoorn H, Peet M. 2003. Ecology: Birds sing at a higher pitch in urban noise – Great tits hit the high notes to ensure that their mating calls are heard above the city's din. *Nature* **424**:267-267.

Snekser JL, Leese J, Ganim A, Itzkowitz M. 2009. Caribbean damselfish with varying territorial quality: correlated behaviors but not a syndrome. *Behav Ecol* **20**:124-130.

Stevenson PA, Rillich J. 2013. Isolation associated aggression – a consequence of recovery from defeat in a territorial animal. *PLoS One* **8**: DOI 10.1371/journal.pone.0074965.

Stracey CM, Robinson SK. 2012. Does Nest Predation Shape Urban Bird Communities? In: Lepczyk CA, Warren PS (eds) Urban Bird Ecology and Conservation. Vol 45. University of California Press, Berkeley, CA, pp 49-70.

Toobaie A, Grant JWA. 2013. Effect of food abundance on aggressiveness and territory size of juvenile rainbow trout, Oncorhynchus mykiss. *Anim Behav* **85**:241-246.

Wada H, Sewall KB. 2014. Itroduction to the symposium – Uniting evolutionary and physiological approaches to understanding phenotypic plasticity. *Integr Comp Biol* **54**:774-782.

Wingfield JC, Ramenofsky M. 1997. Corticosterone and facultative dispersal in response to unpredictable events. *Ardea* **85:**155-166.

Wingfield JC, Kitaysky AS. 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? *Integr Comp Bio* **42**:600-609.

Wingfield JC. 2013. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. *Funct Ecol* **27**:37-44.

Wood WE, Yezerinac SM. 2006. Song sparrow (*Melospiza melodia*) song varies with urban noise. *Auk* **123**:650-659.

Yeh PJ, Price TD. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am Nat* **164**:531-542.

Yoon J, Sillett TS, Morrison SA, Ghalambor CK. 2012. Breeding density, not life history, predicts interpopulation differences in territorial aggression in a passerine bird. *Anim Behav* **84**:515-521.

Zhang S, Lei F, Liu S, Li D, Chen C, Wang P. 2011. Variation in baseline corticosterone levels of tree sparrow *(Passer montanus)* populations along an urban gradient in Beijing, China. *J Ornithol* **152**:801-806.

CHAPTER II: ACROSS TIME AND SPACE: EFFECTS OF URBANIZATION ON CORTICOSTERONE AND BODY CONDITION VARY OVER MULTIPLE YEARS IN SONG SPARROWS (*MELOSPIZA MELODIA*)

Sarah L. Foltz, Jason E. Davis, Kathryn E. Battle, Virginia W. Greene, Brenton T. Laing, Ryan P. Rock, Allen E. Ross, James A. Tallant, Rene C. Vega, and Ignacio T. Moore

This article has been published as: Foltz SL, Davis JE, Battle KE, Greene VW, Laing BT, Rock RP, Ross AE, Tallant JA, Vega RC, Moore IT. 2015. Across time and space: effects of urbanization on corticosterone and body condition vary over multiple years in song sparrows. *J Exp Zool* **323A**:109-120. It is reproduced here with permission from John Wiley and Sons.

ABSTRACT

Animals inhabiting urban areas must simultaneously cope with the unique challenges presented by this novel habitat type while exploiting the distinctive opportunities it offers. The costs and benefits of urban living are often assumed to be consistent across time, but may in fact vary depending on the habitat features influencing them. Here we examine the glucocorticoid levels and body condition of song sparrows (*Melospiza melodia)* resident at urban and rural sites over four consecutive years to determine whether these traits, which may be linked to the relative costs and benefits of these respective habitats, are consistent over time. Glucocorticoid levels and body condition varied by year in both habitat types. While habitat alone did not influence

glucocorticoid levels, there was a significant interaction between year and habitat, indicating that glucocorticoids differ between habitats in some years but not others. There was no discernable effect of habitat alone on body condition. Overall, these data suggest that the costs and benefits of inhabiting urban versus rural habitats differ substantially from year to year.

INTRODUCTION

Urban areas represent a novel habitat type for many species, and these areas are growing in both size and number. Urban habitats are typically characterized by high human population densities, elevated light and noise levels (Longcore and Rich, 2004; Slabbekoorn and Ripmeester, 2008; Hu and Cardoso, 2010), altered predation pressures (Chace and Walsh, 2006; DeCandido and Allen, 2006), and different food sources (Fedriani et al, 2001; Marzluff and Neatherlin, 2006; Heiss et al, 2009). Animal species richness is often lower in heavily urbanized areas (McKinney, 2002; McKinney, 2008), although among groups such as birds and insects, the highest species richness seems to occur in somewhat disturbed zones (Jokimäki and Suhonen '93, Blair '99). Within species, urban individuals can exhibit a number of differences from their rural counterparts, including loss of migratory behavior (Partecke and Gwinner, 2007), extended breeding seasons (Yeh and Price, 2004), increased boldness (Møller, 2010; Scales et al, 2011; Atwell et al, 2012), changes in degree of territorial aggression (Newman et al, 2006; Scales et al, 2011), higher-pitched vocalizations (Slabbekoorn and Peet, 2003; Wood and Yezerinac, 2006; Hu and Cardoso, 2010; Luther and Derryberry, 2012), and differences in plasma stress hormone concentrations (Partecke et al, 2006; Fokidis et al, 2009; Bonier, 2012). Changes in these traits are not necessarily directly correlated with each other, though the same individuals often exhibit differences in more than one trait (Fokidis and Deviche, 2011; Fokidis et al, 2011; Scales et al 2011).

Vertebrates often respond to environmental challenges by elevating plasma levels of glucocorticoid hormones (Sapolsky et al, 2000). The glucocorticoid stress response is commonly studied via several different measures, including baseline plasma concentrations, which are measured immediately upon capture, and stress-induced plasma concentrations, which can be assessed after a standardized handling stressor. Baseline levels of glucocorticoids are involved in maintaining day-to-day energy balance (Saplosky et al, 2000). Stress-induced acute increases are often associated with stressors such as predation attempts, while a more long-term elevation in baseline glucocorticoids could be caused by challenges such as reduced food availability (Schwabl et al, ’85; Lynn et al, 2003), inclement weather (Rogers et al, ’93; Wingfield, 2013) or preparation for the energetic demands of raising young (Kitaysky et al, ’99; Bonier et al, 2009; Love et al, 2014). Increases in glucocorticoid levels are thought to promote survival by mobilizing and redirecting energy away from long-term investments toward more immediately essential processes and by rapidly shifting behavior toward proximate coping activities (Wingfield and Ramenofsky, ’97; Wingfield and Kitaysky, 2002; Breuner et al, 2008). Thus, the glucocorticoid stress response may play an important role in how individuals respond to novel habitats and cope with unfamiliar challenges (Wingfield and Kitaysky, 2002), potentially including those found in urban areas.

Studies on glucocorticoid levels in the context of urbanization remain relatively rare (Bonier, 2012) and findings to date are somewhat contradictory. Some studies have found elevated baseline glucocorticoids in urban birds (Bonier et al, 2007; Fokidis et al, 2009; Zhang et al, 2011), though this pattern is not observed in all species or seasons (Schoech et al, 2007; Fokidis et al, 2009; Atwell et al, 2012). Additionally, urban birds sometimes show lower stress-induced glucocorticoid levels (Partecke et al, 2006; Atwell et al, 2012; but see Fokidis et al, 2009), which may indicate either reduced responsiveness to stressors in the face of repeated stress-response activation (Nelson, 2005) or, conversely, the colonization of urban habitats primarily by individuals with innately lower responsiveness to stressors. These studies all employed a standard procedure of capture and handling by humans followed by restraint in a breathable bag as a stressor, so while not all species exhibited the same reduction in stress response in urban settings, it appears that any differences observed among species are not the result of differing study methodologies. In general, it appears that glucocorticoid levels often differ between urban individuals and their rural conspecifics, and such differences may indicate that these animals experience a meaningful disparity between habitats. However, whether the observed glucocorticoid differences maintain a consistent pattern over time or fluctuate beyond previously observed seasonal shifts, possibly due to labile environmental drivers that differ over years within each habitat, remains unknown.

While glucocorticoid values alone should not necessarily be used to infer an animal's health, body condition can provide a more straightforward metric of stored energy. Limited food availability often results in loss of body mass as animals expend more energy than they take in (Wingfield et al, '83; Kitaysky et al, '99). Thus, low average body condition of individuals in one habitat or year relative to another could indicate poorer habitat quality or environmental conditions. Additionally, glucocorticoids can mediate foraging behavior (Astheimer et al, '92) and are sometimes inversely linked to body condition (Kitaysky et al, '99; Moore et al, 2000; Maute et al, 2013; Hews and Baniki, 2013), so high glucocorticoid levels found in concert with low body condition can be interpreted as potential evidence for poor habitat quality.

To date, it is unclear how consistent phenotypic differences between urban and rural animals are across time. Comparisons of urban and rural animals rarely collect data on the same traits from the same populations across multiple years (Newman et al, 2006; Bonier et al, 2007; Fokidis et al, 2009; Hu and Cardoso, 2010; Scales et al, 2011; Atwell et al, 2012). Those studies that do collect such data do not always analyze the data with an eye toward identifying potential year-to-year variation (Møller, 2010; but see Schoech, 2007 and Partecke and Gwinner, 2007), except in the case of traits for which year is the necessary level of comparison, such as timing of breeding (Yeh and Price, 2004). When we consider that some phenotypic traits, such as glucocorticoid levels, are flexible and responsive to prevailing abiotic and social environmental conditions (Richardson and Fueston, ’75; Ouyang et al, 2011; Davis and Guinan, 2014), the need for such longitudinal comparisons becomes clear. Comparing these labile traits across even a few consecutive years may reveal whether observed differences in them are a product of relatively consistent urban-associated habitat features like ambient light levels or more variable features that may or may not be habitat-specific, such as weather patterns. Longitudinal studies could also help to reveal whether urban and rural habitats mediate environmental challenges differently; one habitat type may buffer its inhabitants from the costs of such stressors to a greater extent that the other. Multi-year comparisons of phenotypic differences between urban and rural individuals are therefore important to our understanding of the long-term impacts of urbanization on urban wildlife.

Our current study was undertaken to determine whether there was a relationship between corticosterone (the main avian glucocorticoid), body condition, and urbanization in song sparrows (*Melospiza melodia)* across multiple consecutive years. We predicted that birds from urban habitats would have consistently higher baseline and lower stress-induced corticosterone levels relative to birds in rural habitats. We also predicted that body condition would be inversely related to baseline corticosterone, such that urban birds would also exhibit lower body condition than their rural counterparts. It is important to note that we did not use undisturbed or pristine wilderness for our rural comparison sites, as few of these exist and the forest cover common to low-disturbance sites in the study region is not generally preferred by song sparrows. Similarly, the urban sites in this study are not megacities or other such extreme cases, but rather towns whose populations average 20,465 people (Community Facts). Instead, our urban and rural sites are defined relative to each other along a continuum of urbanization, with ‘urban’ sites having less green space and higher human population densities than ‘rural’ sites, as described in detail below. Here, we examine relationships between habitat type, body condition, and baseline and stress-induced levels of corticosterone (hereafter referred to as *baseline corticosterone*, and *stress-induced corticosterone,* respectively) in song sparrows at urban and rural sites across four consecutive breeding seasons.

METHODS

Study Populations

Song sparrows are a common songbird native to North America and found in both urban and rural habitats. In our study populations in southwest Virginia, USA (37°13’ N, 80°22’ W, ~700m elevation), song sparrows are non-migratory. Adult males defend a territory during the breeding season, which extends from early April through August (Foltz personal observation). In this region, some males are present on their territories year-round, while others re-establish breeding territories each year, usually beginning in late January or February (Foltz personal observation). From 2010 through 2013, we collected blood samples and measures of body condition from adult, territory-holding male birds at our study sites. All studies were approved by the Virginia Tech Institutional Animal Care and Use Committee.

Our study sites were all located within a 20km radius of Radford, VA, in a patchwork of urban and rural habitat in the New River Valley area of SW Virginia. Urban and rural sites were differentiated based on the portion of their total area comprised of green space and the human population density of the immediately surrounding area, as detailed below. Our 2010 studies were conducted at two urban and two rural sites (Urban 1 and 2; Rural 1 and 2)(See Table 2.1). In 2011, one additional site of each type was added (Urban 3 and Rural 3), and these sites were also used in 2012. In 2013, we monitored these same six sites, but were unable to capture any birds from Urban 1.

Initially, sites were selected based on human population densities of the county (rural) or town (urban) they lay within. We then quantified green space at each site using satellite images from Google Earth (version 6.2) and GE-Path (version 1.4.6). Sites were divided into green space and built space. Green space was comprised of forest, fields, and areas of water $10m^2$ or less (some territories spanned inlets of lakes or rivers). Built space was comprised of buildings, roads, and other man-made structures. We overlaid the area of each site where our study birds were found with a grid of 50x50m numbered plots and selected a number of these plots equal to 5% of total site area, or a minimum of 10 plots for small sites, using a random number generator (Research Randomizer version 4.0). Within each plot, the area of green space was divided by total plot area to obtain a proportion of green space on that plot. These proportions were then averaged to generate a site-wide estimate (Table 2.1). Human population density of our sites was later quantified at a finer scale using census block group population data from the 2010 United States census (2010 Census Interactive Population Search)(Table 2.1). Green space provided an estimate of landscape alterations related to urbanization, while human population density was used as an indirect metric of human activity, and thus disturbances, at these sites. Both human population density and green space were compared between designated urban and rural sites using a MANOVA with post-hoc ANOVAs to differentiate green space from population density.

Corticosterone and Body Condition

All birds were captured during the breeding season. Samples were collected between mid-May and the end of June in 2010, from the end of April to mid-July in 2011, from mid-May to mid-July in 2012, and from early May to late July in 2013. During each field season, we alternated captures among urban and rural sites in an effort to ensure that average capture dates did not differ between habitats.

Birds were captured on their territories using mist nets and playback of pre-recorded male song. To minimize the effects of daily fluctuations in hormone levels, all captures occurred within a four-hour window from 30min before sunrise to 3.5hrs after sunrise. We collected blood within 3min of capture for baseline corticosterone measurement and again at 30min post-capture for stress-induced corticosterone. All blood was collected from the alar wing vein into capillary tubes, placed in a cooler with ice, and transported to the lab. There, samples were centrifuged to separate the plasma, which was then frozen at -20°C until hormone analysis.

Between bleeds, we weighed birds and applied colored and aluminum numbered bands. We also recorded wing and tarsus measurements. Birds were then placed in a cloth bag until it was time for their second blood sample at 30min post-capture, after which they were released back onto their territory. Body condition was assessed via scaled mass analysis, as described by Peig and Green (2009), using weight and tarsus length. Weight and tarsus length were positively correlated across all our samples (linear regression, F-ratio = 8.3371, P=0.0047). We collected analyzable baseline corticosterone samples from 36 males in 2010 (20 rural, 16 urban), 25 males in 2011 (14 rural, 11 urban), 17 males in 2012 (8 rural, 9 urban), and 33 males in 2013 (12 rural, 21 urban). Stress-induced corticosterone samples were collected from 40 males in 2010 (21 rural, 19 urban), 23 males in 2011 (13 rural, 10 urban), 15 males in 2012 (8 rural, 7 urban), and 32 males in 2013 (13 rural, 19 urban). We measured weight and tarsus length for 38 males in 2010 (20 rural, 18 urban), 35 males in 2011 (14 rural, 11 urban), 17 males in 2012 (8 rural, 9 urban), and 33 males in 2013 (12 rural, 21 urban).

Because circulating corticosterone levels can be influenced by breeding condition, it is important to note that birds in both habitats at our sites appear to begin breeding at similar times within each year. We estimated dates of nest establishment (when the first egg was laid) for nests found during the 2011 and 2013 breeding seasons using known periods of incubation for this species and the number of eggs or nestlings in the nest. In 2011, our first urban and rural nests were established on April 26th and April 15th, respectively. In 2013, the first urban and rural nests were established on April 13th and April 19th, respectively. Linear mixed models (one per year) comparing estimated establishment dates for nests found within the first three weeks of the breeding season across habitats and controlling for individual field sites revealed no significant difference in the timing of nest establishment (2011: P=0.9524, 2013: P=0.2888). Breeding is also initiated asynchronously among pairs within sites. Most pairs make multiple nest attempts, some of which fail, contributing to further asynchrony as the breeding season progresses. Thus, birds in both habitats are in a variety of breeding stages at any given time, with observations leading us to assume a random distribution of breeding stage across our samples for both habitats.

Hormone Assays

Total corticosterone levels in plasma samples were measured via direct steroid radioimmunoassays (for a more detailed description, see Malueg et al, 2009). Samples were run in five separate assays, one per year, except in 2011, when samples were split across two assays. Briefly, plasma samples (vol. 10 – 20µl) were incubated overnight with radiolabeled corticosterone. The next day, the samples were extracted with dichloromethane, dried under nitrogen gas, and reconstituted with 600µl of phosphate-buffered saline. 100µl of the reconstituted sample were used to measure extraction efficiency and the remaining sample was split into 200µl duplicates to assay corticosterone levels. Recovery means for these assays were 87.6% (2010), 78.6% (2011A), 73.7% (2011B), 73.6% (2012), and 68.1% (2013). Samples were compared to their assay's standard curve, created by serially diluting known amounts of corticosterone. Inter-assay variation was 23% and intra-assay variations were 15.2% (2010), 16.2% (2011A), 17.2% (2011B), 13.9% (2012) and 25.2% (2013). To control for inter-assay variation when comparing across years, we standardized corticosterone measures using the standards run alongside samples in each assay. Specifically, we calculated the mean standard for each assay and the grand mean of standards for all assays, then divided the grand mean standard by each assay mean standard to create a multiplier for each assay. This multiplier was applied to all samples within a given assay, regardless of where they fell on the assay's standard curve.

Statistical Analyses

All statistical analyses were done in JMP Pro (version 10.0.2). Prior to analysis, corticosterone measures and scaled mass were normalized via square root and natural log transformations, respectively. After building full models including all variables of interest, we employed backward selection, removing fixed factors one at a time beginning with the largest p-value until all remaining factors in the model had p-values of 0.10 or lower. Relationships were considered significant at $p<0.05$.

To determine what factors influenced corticosterone levels, we constructed a repeated-measures multiple linear mixed model (MLMM) using backwards selection. This method accounts for the relationship between baseline and stress-induced corticosterone levels, which we consider non-independent measures because they are samples of the same hormone collected from the same individual within a short time span. In the full model, individuals' corticosterone levels formed the response variable; explanatory variables were habitat type (urban or rural), year (2010, 2011, 2012, 2013), scaled mass, capture date within year, estimated length of playback prior to capture (<5min, 5-15min, >15min) and corticosterone sample time point (baseline or stress-induced). We also incorporated first-level interactions between year and habitat type, year and corticosterone sample time point, and habitat type and corticosterone sample time point. Field site and individual were included as random factors, with individual nested inside site. 2013 was used as the reference year to which all other years were compared, as it was the year with the lowest overall corticosterone levels after correction for inter-assay variation. As we had no basis for considering any of our sample years to be more standard than the others, 2013 was chosen simply because it represented one end of the continuum of our corticosterone data. We then ran post-hoc all-pairs Tukey tests to further explore how all years compared to each other. Habitat type was retained in the final model after backward selection despite having a high p-value because the interaction between site-type and corticosterone sample time point remained significant.

To determine the individual contributions of baseline corticosterone and stress-induced corticosterone to the significance of the explanatory variables identified by backwards selection in our main repeated-measures MLMM described above, we subsequently ran individual MLMMs in which either baseline or stress-induced corticosterone alone was the response variable. To best compare the relationships of baseline and stress-induced corticosterone separately with all of these variables, we did not perform backward selection on these models. Rather, we built each model to contain the same fixed factors, interaction terms, and random factors as the final version of our main repeated-measures MLMM: habitat type (urban or rural), year (2010, 2011, 2012, 2013), capture date within year, and the interaction of year with habitat type as explanatory variables, and individual nested within site as random factors. Corticosterone sample time point and its interactions were omitted because each of these models considers only one of the two possible sample time points. Because these models analyze baseline and stress-induced corticosterone separately, they assume independence of baseline and stressed corticosterone samples, and thus we did not consider this method appropriate to use alone. Rather, we employ it here to shed light on whether correlations found in the full repeated-measures model are primarily associated with either baseline or stress-induced corticosterone alone, or with both measures.

The possible relationships of habitat type and capture year with birds' body condition were also assessed with an MLMM. The response variable was scaled mass. Explanatory variables were habitat type (urban or rural), year (2010, 2011, 2012, 2013), capture date within year, estimated length of playback prior to capture, and the interaction of habitat type with year (fixed factors). Individual was nested within field site and included as a random factor as above. 2011 was the year with the lowest mean scaled mass, and was therefore selected as the reference year for this model. We then compared all years to all other years via post-hoc all-pairs Tukey tests.

RESULTS

Study Sites

Our comparisons of green space and human population density across our urban and rural sites showed a significant overall difference between habitat types (MANOVA, exact F=17.0275, P=0.0230). Post-hoc ANOVAs showed that urban sites had significantly lower proportions of green space than rural sites (exact F=43.6475; P=0.0027). Population densities at the census block level were not significantly different (exact F=4.8142; P=0.0933), though all urban-designated sites had higher population densities than rural-designated ones (Table 2.1). Based on these analyses, we determined that sites adequately fit their original categorical designations, and these designations were used in subsequent analyses.

Corticosterone

Backwards selection of our full repeated measures corticosterone model produced a final model containing year ($P<0.0001$)(Figure 2.1), habitat type ($P=0.8374$)(Figure 2.1), corticosterone sample time point ($P<0.0001$)(Figure 2.1), capture date ($P=0.0016$)(Figure 2.2), and the interactions of year with habitat type ($P=0.0105$) and habitat type with corticosterone sample time point ($P=0.0071$) as explanatory variables (for details, see Table 2.2A). The adjusted R^2 value of this final model was 0.8370, while the adjusted R^2 value of the full model was 0.8437, indicating that the factors removed (scaled mass, time to capture, and the interaction of year and corticosterone sample time point) collectively explained only a very small proportion of the variation in the data, and could safely be removed from the model. Specific parameter estimates comparing our reference year for this model (2013) to other years suggested that 2011 drove the correlation with year ($P=0.0091$) (Figure 2.1). Further examination of the results of our Tukey all-pairs comparisons found that all three preceding years differed significantly from 2013 (2010-2013: $P=0.0013$, 2011-2013: $P<0.0001$, 2012-2013: $P=0.0007$), but not from each other. The significant interaction of year with habitat type indicates that baseline and/or stress-induced corticosterone levels differed between urban and rural habitats in some years but not others; this correlation appears to have been driven by 2010 ($P=0.0024$), based on parameter estimates (Figure 2.1).

The models fitting the same explanatory variables selected from the repeated-measures model against baseline and stress-induced corticosterone separately suggest that the significant effect of year in the original model was driven by both baseline and stress-induced corticosterone (baseline model $P=0.0094$; stress-induced model $P<0.0001$). The stress-induced model also echoes the original finding in that 2011 is the year most different from 2013 ($P=0.0016$); no individual year correlated with baseline corticosterone (2010 P=0.2582; 2011 P=0.3817; 2012 P=2535). As in the repeated measures model, habitat type was not significant in either the baseline or stress-induced models. In both models, the interaction of 2010 with habitat type was significant (baseline model P=0.0027, stress-induced model P=0.0307), indicating that both baseline and stress-induced corticosterone levels differed between urban and rural habitats that year. Capture date had a significant negative correlation with stress-induced corticosterone (P=0.0019), but not baseline corticosterone (P=0.1028). See Tables 2.2B and C for details.

Body Condition

Backwards selection of our model comparing scaled mass to habitat- and time-related variables resulted in a final model that included only year (P=0.0511). The adjusted R^2 value of the full model was 0.7531, while that of the final model was 0.7605. Parameter estimates comparing each year to 2011 suggest that the relationship between year and scaled mass is due mainly to significantly higher scaled mass in 2012 (P=0.0229) relative to 2011 (Figure 2.3) (for details, see Table 2.3). Our comparison of all years via Tukey tests found no significant differences between any one pair of years, but did identify a strong trend toward 2011 birds having lower body condition than 2012 birds (P=0.0525).

DISCUSSION

Our multi-year sampling of corticosterone levels and body condition in urban and rural populations of song sparrows found that patterns are not stable across years. Rather, we found that levels of both baseline and stress-induced corticosterone varied across habitats in a year-dependent manner. Body condition also showed a tendency to differ with year, but was not related to either baseline or stress-induced corticosterone levels.

Both baseline and stress-induced levels of corticosterone often respond to proximate challenges and stressors (Wingfield and Ramenofsky, '97; Kitaysky et al, 2001; Lynn et al, 2003) and therefore may not be expected to remain consistent across years unless the environment is exceptionally stable (Ouyang et al, 2011). Furthermore, it is possible that, despite their close physical proximity in our study, urban and rural habitats do not equivalently mitigate impacts of regional year-to-year environmental fluctuations that may drive the traits we measured. For example, Sheriff et al (2012) found interactions between year and habitat types in arctic ground squirrels. Fecal corticosterone metabolites of squirrels in a variety of elevation-related habitats were compared across two years; metabolite levels were much higher in the first year than the second, but the degree to which levels differed across years was strongly related to habitat type. This pattern is similar to those evident in our urban-rural comparison and suggests that the environmental features influencing corticosterone levels may have differing degrees of impact in urban and rural habitats depending on prevailing regional conditions. That we observe differences between habitats in some years but not others suggests not only that environmental drivers may be fluctuating from year to year, but also that these habitats contain different mitigating factors, such that the final impact of similar conditions on song sparrow populations in each habitat is different. In essence, one habitat may provide birds more opportunities to escape or cope with certain environmental challenges than the other.

While we hesitate to speculate too much in the absence of supporting data, there are several possible environmental and social features that could explain some of the year-to-year differences we see in both corticosterone levels and body condition. One is variation in regional weather conditions such as temperature and rainfall during our study seasons. Temporary harsh or unusual weather events have previously been shown to result in elevation of either fecal corticosterone metabolites (Zav'yalov et al, 2007; Sheriff et al, 2012) or plasma corticosterone (Schwabl et al, '85; Rogers et al, '93; Wingfield and Ramenofsky, '97; Romero and Wikelski, 2001; Ouyang et al, 2012; but see Romero et al, '97 and Brown et al, 2011) and decreased body condition (Zav'yalov et al, 2007; Brown et al, 2011). The winter of 2009-2010, directly preceding the breeding season in which we observed a clear difference between urban and rural birds' baseline and stress-induced corticosterone levels, was an unusually cold and wet year, with Blacksburg, VA recording a record-setting 71 days with one or more inches of snow cover (Chenard, 2010). Variation in predation pressure across years is another possible candidate. Animals exposed to high levels of predation risk exhibit increased corticosterone levels (Silverin '98, Scheuerlein et al, 2001; Cockrem and Silverin, 2002) and reduced body condition (Scheuerlein et al 2001), relative to those that experience lower predation risk. A third possibility, which could be related to differences in stress-induced corticosterone levels, is that demographics within our study populations changed over the course of our study. Studies have found that corticosterone response decreases with age in a number of species (Riegle and Nellor, '67; Heidinger et al, 2006; Wilcoxen et al, 2011). However, we regard this possibility as somewhat unlikely because many of the territories at our study sites were occupied by birds that had been banded in previous years, while other territories were acquired by new, unbanded owners, suggesting a mix of known older birds and younger incoming individuals. Of course, none of these possible contributors are mutually exclusive, and the differences we observed could arise from combinations of these and other variable features of the physical and social environment.

At first glance, the corticosterone values that we report here appear high in comparison to other songbird species and to early values reported for song sparrows (Wingfield, ‘84). However, several other groups working independently from us in various regions of North America, and using similar capture and analysis methods, report average plasma corticosterone values ranging from 17-48ng/mL for baseline and 120-200ng/ml for 30min stress-induced samples (Owen-Ashley and Wingfield, 2006; Newman et al, 2008; Newman and Soma, 2009; Newman and Soma, 2011; Schmidt et al, 2012). Thus we are confident in the data we report.

In summary, the relationships between urbanization and avian physiology are complex and likely influenced by a variety of factors, some of which probably vary across years. Thus, populations that appear different in one year of a study may not always be so. Comparisons of flexible traits between urban and rural populations should observe these groups across multiple years before concluding that the given trait does or does not differ between populations. Similarly, future studies should examine specific features of urbanization that may drive phenotypic differences found in urban populations, most of which are currently unknown, with an eye toward the stability of such features across time. Relatively stable habitat features such as, for example, light levels may be predicted to produce relatively stable trait differences. Conversely, more flexible aspects of habitat quality, such as natural food availability, may fluctuate across years and be mitigated differently in urban and non-urban areas, resulting in trait differences between habitats that appear one year and disappear the next. Thus, we suggest that researchers hoping to identify differences between urban and rural populations or the features that drive such differences conduct their work across several years. This becomes especially important when working with wild, free-living populations, as our findings emphasize that environmental factors beyond the immediate scope of the study may nonetheless have major impacts on study outcomes.

ACKNOWLEDGMENTS

We would like to thank the Laboratory for Interdisciplinary Statistical Analysis at Virginia Tech and James S. Adelman for advice on data analysis. This work was supported by the National Science Foundation (IOS 0545735 to I.T.M. and 1311381 to I.T.M. and S.F.) and a graduate fellowship from the Institute for Critical Technology and Applied Science (ICTAS) at Virginia Tech to S.F.

References

2010 Census Interactive Population Search. n.d. In *2010 Census.* Retrieved Sept. 2012 from http://www.census.gov/2010census/popmap/.

Astheimer LB, Buttemer WA, Wingfield JC. 1992. Interactions of corticosterone with feeding, activity, and metabolism in passerine birds. *Ornis Scandinavica* **23**:355-365.

Atwell JW, Cardoso 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.

Blair RB. 1999. Birds and butterflies along an urban gradient: surrogate taxa for assessing biodiversity? *Ecol Appl* **9**:164-170.

Bonier F, Martin PR, Sheldon KS, Jensen JP, Foltz SL, Wingfield JC. 2007. Sex-specific consequences of life in the city. *Behav Ecol* **18**:121-129.

Bonier F, Moore IT, Martin PR, Robertson RJ. 2009. The relationship between fitness and baseline glucocorticoids in a passerine bird. *Gen Comp Endocrinol* **163**:208-213.

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

Breuner CW, Patterson SH, Hahn TP. 2008. In search of relationships between the acute adrenocortical response and fitness. *Gen Comp Endocrinol* **157**:288-295.

Brown, GP, Kelehear C, Shine R. 2011. Effects of seasonal aridity on the ecology and behavior of invasive cane toads in the Australian wet-dry tropics. *Funct Ecol* **25**:1339-1347.

Chace JF, Walsh JJ. 2006. Urban effects on native avifauna: a review. *Landsc Urban Plan* **74**:46-69.

Chenard M. 2010. “A Snowy and Cold Winter: Winter 2009-2010 Summary.” NOAA ‘Bout Weather Spring 2010:3-5.

Cockrem JF, Silverin B. 2002. Sight of a predator can stimulate a corticosterone response in the Great Tit (*Parsus major*). *Gen Comp Endocrinol* **125**:248:255.

Community Facts. n.d. In *American FactFinder*. Retrieved July 17, 2014 from http://factfinder2.census.gov/faces/nav/jsf/pages/community_facts.xhtml.

Davis JE, Guinan JA. 2014. Parental behavior correlates to baseline corticosterone of mates and offspring in nestling eastern bluebirds (*Sialia sialis)*. *Gen Comp Endocrinol* **201**:1-7.

DeCandido R, Allen D. 2006. Nocturnal hunting by peregrine falcons at the Empire State Building, New York City. *Wilson J Ornithol* **118**:53-58.

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.

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.

Fokidis HB, Orchinik M, Deviche P. 2011. Context-specific territorial behavior in urban birds: no evidence for involvement of testosterone or corticosterone. *Horm Behav* **59**:133-143.

Fokidis HB, Deviche P. 2011. Brain arginine vasotocin immunoreactivity differs between urban an desert curve-billed thrashers, Toxostoma curvirostre: relationships with territoriality and stress physiology. *Brain Behav Evol* **79**:84-97.

Heidinger BJ, Nisbet ICT, Ketterson ED. 2006. Older parents are less responsive to a stressor in a long-lived seabird: a mechanism for increased reproductive performance with age? *Proc R Soc B* **273**:2227-2231.

Heiss RS, Clark AB, McGowan KJ. 2009. Growth and nutritional state of American crow nestlings vary between urban and rural habitats. *Ecol Appl* **19**:829-839.

Hews DK, Baniki AJA. 2013. The breeding season duration hypothesis: acute handling stress and total plasma concentrations of corticosterone and androgens in male and female striped plateau lizards (*Sceloporus virgatus)*. *J Comp Physiol [B]* **183**:933-946.

Hu Y, Cardoso GC. 2010. Which birds adjust the frequency of vocalizations in urban noise? *Anim Behav* **79**:863-867.

Jokimäki J, Suhonen J. 1993. Effects of urbanization on the breeding bird species richness in Finland: a biogeographical comparison. *Ornis Fenn* **70**:71-77.

Kitaysky AS, Wingfield JC, Piatt JF. 1999. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. Funct Ecol 13:577-584.

Kitaysky AS, Kitaiskaia EV, Wingfield JC. 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. *J Comp Physiol* **171**:701–709.

Longcore T, Rich C. 2004. Ecological light pollution. *Front Ecol Environ* **2**:191-198.

Love OP, Madliger CL, Bourgeon S, Semeniuk CAD, Williams TD. 2014. Evidence for baseline glucocorticoids as mediators of reproductive investment in a wild bird. *Gen Comp Endocrinol* **199**:65-69.

Luther DA, Derryberry EP. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. *Anim Behav* **83**:1059-1066.

Lynn SE, Breuner CW, Wingfield JC. 2003. Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. *Horm Behav* **43**:150-157.

Malueg AL, Walters JR, Moore IT. 2009. Do stress hormones suppress helper reproduction in the cooperatively breeding red-cockaded woodpecker (*Picoides borealis)*. *Behav Ecol Sociobiol* **63**:687-698.

Marzluff JM, Neatherlin E. 2006. Corvid response to human settlements and campgrounds: causes, consequences, and challenges for conservation. *Biol Conserv* **130**:301-314.

Maute KL, French K, Legge S, Astheimer L. 2013. Seasonal stress physiology and body condition differ among co-occurring tropical finch species. *J Comp Physiol [B]* **183**:1023-1037.

McKinney ML. 2002. Urbanization, biodiversity, and conservation. *Biosci* **52**:883-890.

McKinney ML. 2008. Effects of urbanization on species richness; a review of plants and animals. *Urban Ecosyst* **11**:161-176.

Møller AP. 2010. Interspecific variation in fear responses predicts urbanization in birds. *Behav Ecol* **21**:362-371.

Moore IT, Lerner JP, Lerner DT, Mason RT. 2000. Relationships between annual cycles of testosterone, corticosterone, and body condition in male red-spotted garter snakes, *Thamnophis sirtalis concinnus*. *Physiol Biochem Zool* **73**:307-312.

Nelson RJ. 2005. An Introduction to Behavioral Endocrinology. Sunderland, Massachusetts: Sinauer Associates, Inc.

Newman MM, Yeh PJ, Price TD. 2006. Reduced territorial responses in dark-eyed juncos following population establishment in a climatically mild environment. *Anim Behav* **71**:893-899.

Newman AEM, Pradhan DS, Soma KK. 2008. Dehydroepiandrosterone and corticosterone are regulated by season and acute stress in a wild songbird: jugular versus brachial plasma. *Endocrinology* **149**:2537-2545.

Newman AEM, Soma KK. 2009. Corticosterone and dehydroepiandrosterone in songbird plasma and brain: effects of season and acute stress. *Eur J Neurosci* **29**:1905-1914.

Newman AEM, Soma KK. 2011. Aggressive interactions differentially modulate local and systemic levels of corticosterone and DHEA in a wild songbird. *Horm Behav* **60**:389-396.

Ouyang JQ, Hau M, Bonier F. 2011. Within seasons and among years: when are corticosterone levels repeatable? *Horm Behav* **60**:559-64.

Ouyang JQ, Quetting M, Hau M. 2012. Corticosterone and brood abandonment in a passerine bird. *Anim Behav* **84**:261-268.

Owen-Ashley NT, Wingfield JC. 2006. Seasonal modulation of sickness behavior in free-living northwestern song sparrows (Melospiza melodia morphna). *J Exp Biol* **209**:3062-3070.

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

Partecke J, Gwinner E. 2007. Increased sedentariness in European blackbirds following urbanization: a consequence of local adaptation? *Ecology* **88**:882-890.

Peig J, Green AJ. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* **118**:1883-1891.

Richardson JH, Fueston VM. 1975. Response of two strains of mice to increased social contact and decreased personal space as measured by fluorometric analysis of corticosterone. *Psychol Rep* **37**:124-126.

Riegle GD, Nellor JE. 1967. Changes in adrenocortical function during aging in cattle. *J Gerontol* **22**:83-87.

Rogers CM, Ramenofsky M, Ketterson ED, Nolan V, Wingfield JC. 1993. Plasma corticosterone, adrenal mass, winter weather, and season in nonbreeding populations of dark-eyed juncos (Junco hyemalis hyemalis). *Auk* **110**:297-285.

Romero LM, Ramenofsky M, Wingfield JC. 1997. Season and migration alters the corticosterone response to capture and handling in an Arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *Comp Biochem Physiol [C]* **116**:171-177.

Romero LM, Wikelski M. 2001. Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Niño events. *PNAS* **98**:7366-7370.

Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulator, and preparative actions. *Endocr Rev* **21**:55-89.

Scales J, Hyman J, Hughes M. 2011. Behavioral syndromes break down in urban song sparrow populations. *Ethology* **117**:887-895.

Scheuerlein A, Van't Hof TJ, Gwinner E. 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (Saxicola torquata axillaris). *Proc R Soc B* **268**:1575-1582.

Schmidt KL, Furlonger AA, Lapierre JM, MacDougall-Shackleton EA, MacDougall-Shackleton SA. 2012. Regulation of the HPA axis is related to song complexity and measures of phenotypic quality in song sparrows. *Horm Behav* **61**:652-659.

Schoech SJ, Bowman R, Bridge ES, Boughton RK. 2007. Baseline and acute levels of corticosterone in Florida scrub-jays *(Aphelocoma coerulescens)*: effects of food supplementation, suburban habitat, and year. *Gen Comp Endocrinol* **154**:150-160.

Schwabl H, Wingfield JC, Farner DS. 1985. Influence of winter on endocrine state and behavior in European blackbirds (Turdus merula). *J Comp Ethol* **68**:244-252.

Sheriff MJ, Wheeler H, Donker SA, Krebs CJ, Palme R, Hik DS, Boonstra R. 2012. Mountain-top and valley-bottom experiences: the stress axis as an integrator of environmental variability in arctic ground squirrel populations. *J Zool* **287**:65-75.

Silverin B. 1998. Behavioural and hormonal responses of the Pied Flycatcher to environmental stressors. *Anim Behav* **55**:1411-1420

Slabbekoorn H, Peet M. 2003. Ecology: Birds sing at a higher pitch in urban noise – Great tits hit the high notes to ensure that their mating calls are heard above the city's din. *Nature* **424**:267-267.

Slabbekorn H, Ripmeester EAP. 2008. Birdsong and anthropogenic noise: implications and applications for conservation. *Mol Ecol* **17**:72-83.

Wilcoxen TE, Boughton RK, Bridge ES, Rensel MA, Schoech SJ. 2011. Age-related differences in baseline and stress-induced corticosterone in Florida scrub-jays. *Gen Comp Endocrinol* **173**:461-466.

Wingfield JC. 1984. Environmental and endocrine control of reproduction in the song sparrow, *Melospiza melodia* I. Temporal organization of the breeding cycle. *Gen Comp Endocrinol* **56**:406-416.

Wingfield JC. 2013. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. *Funct Ecol* **27**:37-44.

Wingfield JC, Kitaysky AS. 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? *Integr Comp Bio* **42**:600-609.

Wingfield JC, Ramenofsky M. 1997. Corticosterone and facultative dispersal in response to unpredictable events. *Ardea* **85**:155-166.

Wingfield JC, Moore MC, Farner DS. 1983. Endocrine responses to inclement weather in naturally breeding populations of white-crowned sparrows (Zonotrichia leucophrys pugetensis). *Auk* **100**:56-62.

Wood WE, Yezerinac SM. 2006. Song sparrow (*Melospiza melodia*) song varies with urban noise. *Auk* **123**:650-659.

Yeh PJ, Price TD. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am Nat* **164**:531-542.

Zav'yalov EL, Gerlinskaya LA, Ovchinnikova LE, Evsikov VI. 2007. Stress and territorial structure of a local water vole (Arvicola terrestris) population. *Zool Zhurnal* **86**:242-251.

Zhang S, Lei F, Liu S, Li D, Chen C, Wang P. 2011. Variation in baseline corticosterone levels of tree sparrow *(Passer montanus)* populations along an urban gradient in Beijing, China. *J Ornithol* **152**:801-806.

Tables

Table 2.1: Habitat Features Used to Determine Urbanization of Sites

Field Site	Human Population Density (people/k^2)	Green Space (% of site area)	Years Monitored
Urban 1 (Radford Campus West)	246	59.6	2010 – 2013*
Urban 2 (Virginia Tech Campus)	2563	48.4	2010 - 2013
Urban 3 (Radford Main Campus)	2897	51.6	2011 - 2013
Rural 1 (Claytor Lake State Park)	49	82.5	2010 - 2013
Rural 2 (Kentland Farm)	23	96.0	2010 - 2013
Rural 3 (Heritage Park)	142	98.0	2011 - 2013

*No physiological data presented for 2013, as no birds were captured.

Table 2.2A: Impacts of Urbanization and Year on Collective Corticosterone Levels in Song Sparrows, Repeated-Measures Model

Parameter	Estimate	SE	Z	P-Value	Fixed Effect P-Value
Habitat Type	0.0265	0.1290	0.21	0.8374	0.8374
Year (2010)	0.0857	0.1863	0.46	0.6461	<0.0001
Year (2011)	0.5390	0.2044	2.64	0.0091	<0.0001
Year (2012)	0.4220	0.2439	1.73	0.0858	<0.0001
Corticosterone Sample Time Point	-2.2472	0.0885	-25.40	<0.0001	<0.0001
Capture Date	0.0204	0.0063	-3.21	0.0016	0.0016
Habitat Type x Year (2010)	-0.5754	0.1837	-3.10	0.0024	0.0105
Habitat Type x Year (2011)	0.3667	0.2046	1.79	0.0747	0.0105
Habitat Type x Year (2012)	-0.0268	0.2444	-0.11	0.9127	0.0105
Habitat Type x Corticosterone Sample Time Point	-0.2424	0.0885	-2.74	0.0071	0.0071

SE = standard error. Reference factors for categorical variables are rural (habitat type), 2013 (year), baseline (corticosterone sample time point), and 5-15min (time to capture). Fixed effect p-values show results of test that all parameters associated with the effect are equal to 0, producing a combined p-value for categorical factors. Significance: $P < 0.05$

Table 2.2B: Impacts of Urbanization and Year on Baseline Corticosterone Levels in Song Sparrows

Parameter	Estimate	SE	Z	P-Value	Fixed Effect P-Value
Habitat Type	-0.1891	0.1611	-1.17	0.2434	0.2434
Year (2010)	0.2883	0.2536	1.14	0.2582	0.0094
Year (2011)	0.2464	0.2805	0.88	0.3817	0.0094
Year (2012)	0.3664	0.3191	1.15	0.2535	0.0094
Capture Date	-0.0140	0.0085	-1.65	0.1028	0.1028
Habitat Type x Year (2010)	-0.7742	0.2519	-3.07	0.0027	0.0282
Habitat Type x Year (2011)	0.2634	0.2803	0.94	0.3495	0.0282
Habitat Type x Year (2012)	0.3547	0.3191	1.11	0.2690	0.0282

SE = standard error. Reference factors for categorical variables are rural (habitat type) and 2013 (year). Fixed effect p-values show results of test that all parameters associated with the effect are equal to 0, producing a combined p-value for categorical factors. Significance: $P < 0.05$

Table 2.2C: Impacts of Urbanization and Year on Stress-Induced Corticosterone Levels in Song Sparrows

Parameter	Estimate	SE	Z	P-Value	Fixed Effect P-Value
Habitat Type	0.2023	0.1503	1.35	0.1813	0.1813
Year (2010)	-0.1170	0.2286	-0.51	0.6100	<0.0001
Year (2011)	0.8590	0.2644	3.25	0.0016	<0.0001
Year (2012)	0.4944	0.3078	1.61	0.1114	<0.0001
Capture Date	-0.0260	0.0082	-3.18	0.0019	0.0019
Habitat Type x Year (2010)	-0.4962	0.2264	-2.19	0.0307	0.0437
Habitat Type x Year (2011)	0.3871	0.2647	1.46	0.1467	0.0437
Habitat Type x Year (2012)	-0.2604	0.3081	-0.85	0.4001	0.0437

SE = standard error. Reference factors for categorical variables are rural (habitat type) and 2013 (year). Fixed effect p-values show results of test that all parameters associated with the effect are equal to 0, producing a combined p-value for categorical factors. Significance: $P < 0.05$

Table 2.3: Impacts of Urbanization and Year on Body Condition in Song Sparrows

Parameter	Estimate	SE	Z	P-Value	Fixed Effect P-Value
Year (2010)	-0.2216	0.1797	-1.23	0.2206	0.0511
Year (2011)	-0.4141	0.1986	-2.09	0.0413	0.0511
Year (2012)	0.5471	0.2365	2.31	0.0229	0.0511

SE = standard error. Reference factor for year is 2013. Fixed effect p-values show results of test that all parameters associated with the effect are equal to 0, producing a combined p-value for categorical factors. Significance: $P < 0.05$

Figure Legends

Figure 2.1: Effects of Urbanization and Year on Measures of Corticosterone

Overall, corticosterone levels are higher in 2011 than 2013. The relationship between corticosterone and habitat varies across years, with baseline and/or stress-induced corticosterone being higher in urban (filled points) habitats some years, and in rural (open points) habitats in others. Vertical bars show standard error of means; corticosterone values shown are those standardized across assays. See Materials and Methods: Corticosterone and Body Condition for sample sizes.

Figure 2.2: Relationship Between Corticosterone and Within-Year Capture Date

Stress-induced corticosterone (filled points, solid line) is significantly lower later in the breeding season, and baseline corticosterone (open points, dashed line) shows a similar but non-significant trend. All points in each series correspond to individual birds; corticosterone values shown are those standardized across assays. See Materials and Methods: Corticosterone and Body Condition for sample sizes.

Figure 2.3: Effects of Urbanization and Year on Body Condition

Body condition (scaled mass) across both habitats was lower in 2011 than in 2012. Capped lines show standard error of means. See Materials and Methods: Corticosterone and Body Condition for sample sizes.

Figures

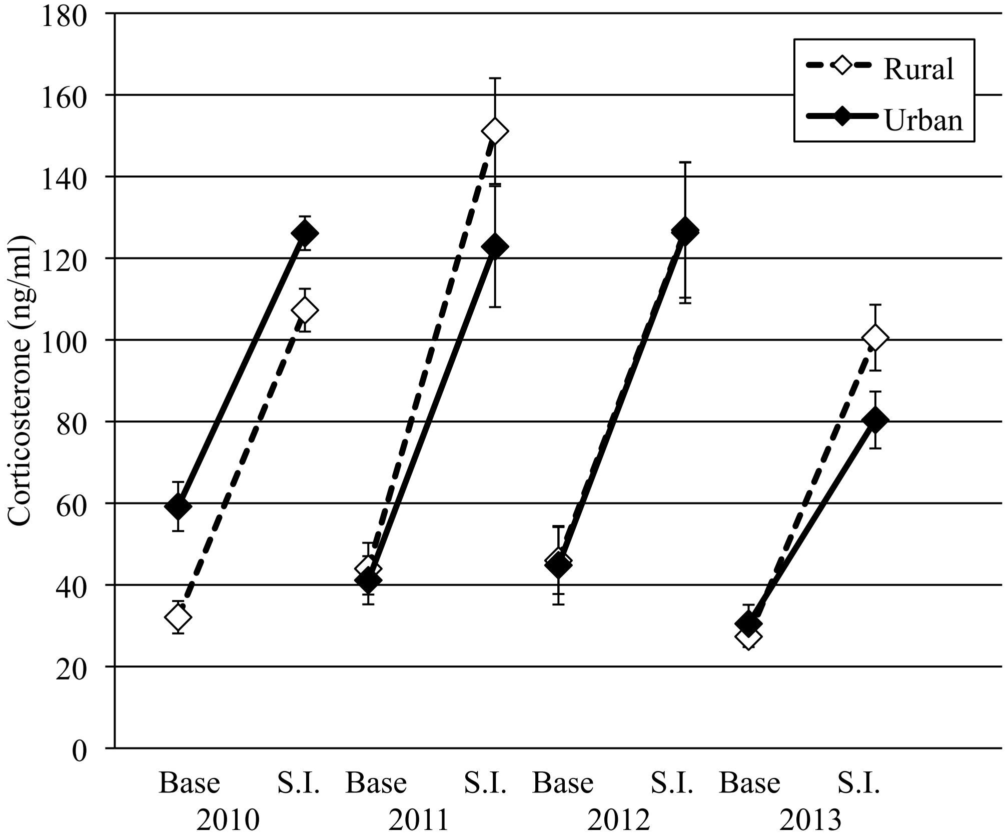

Figure 2.1: Effects of Urbanization and Year on Measures of Corticosterone

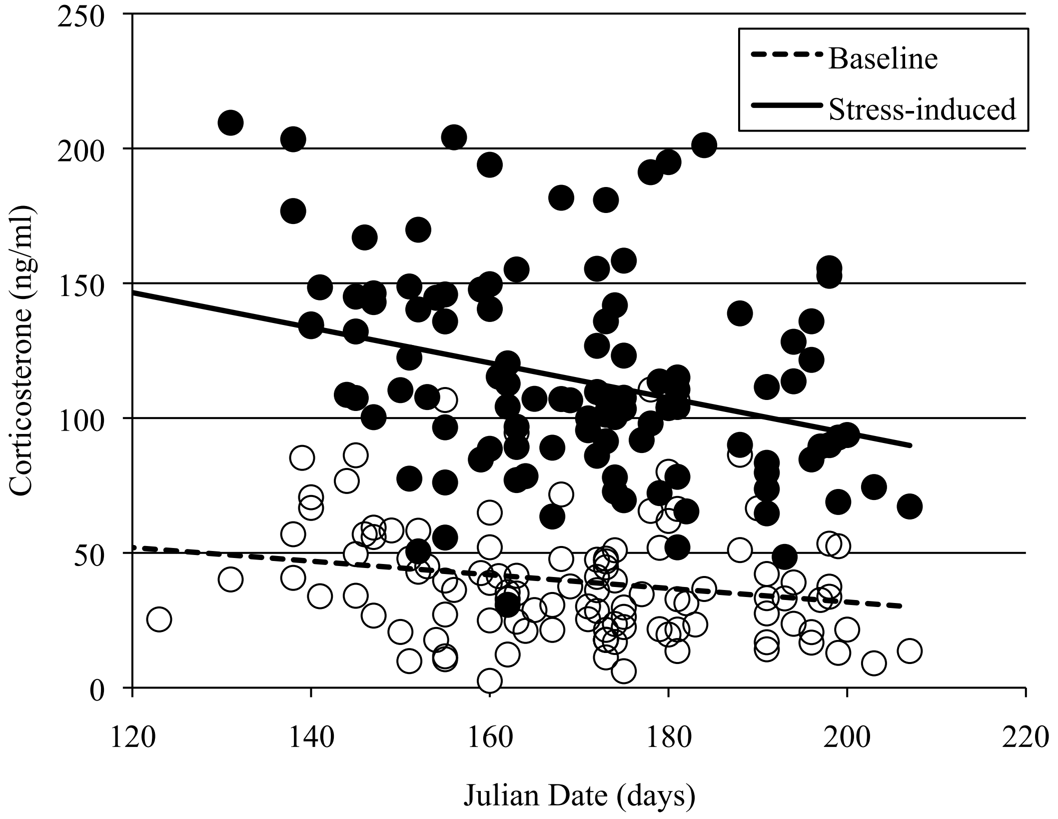

Figure 2.2: Relationship Between Corticosterone and Within-Year Capture Date

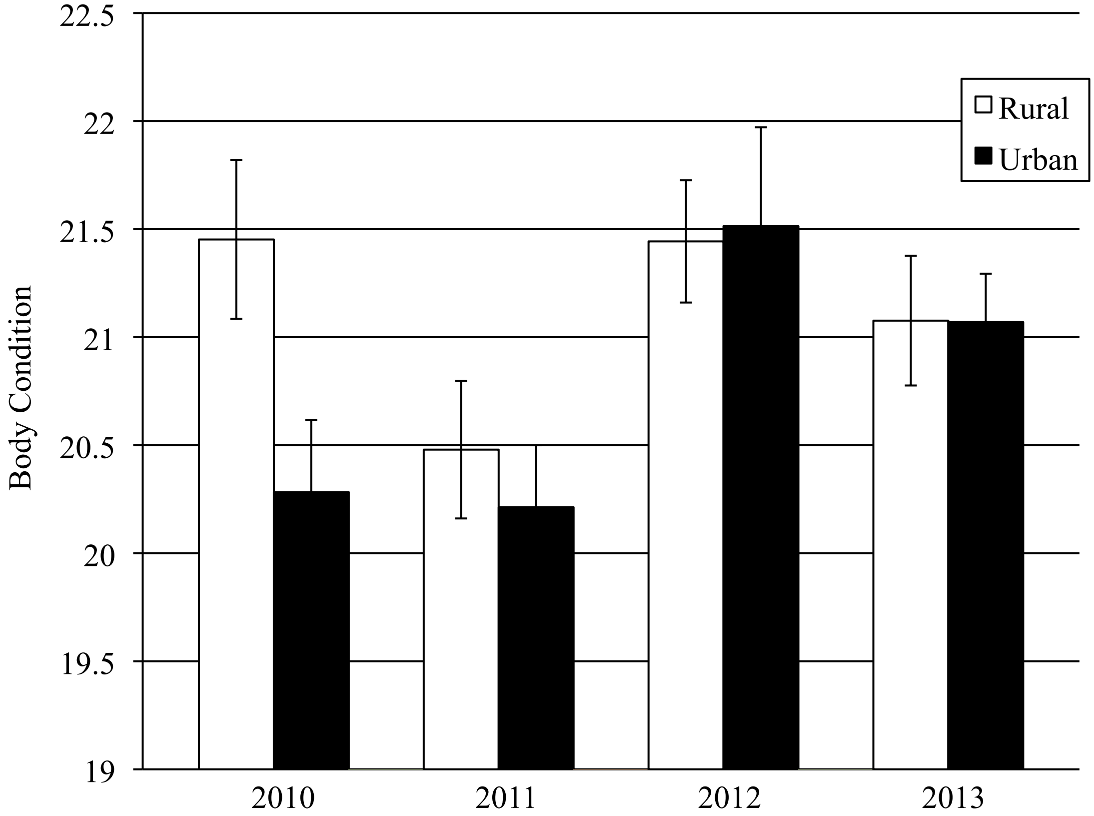

Figure 2.3: Effects of Urbanization and Year on Body Condition

CHAPTER III: GET OFF MY LAWN: INCREASED AGGRESSION IN URBAN SONG SPARROWS IS RELATED TO RESOURCE AVAILABILITY

Sarah L. Foltz, Allen E. Ross, Brenton T. Laing, Ryan P. Rock, Kathryn E. Battle, and Ignacio T. Moore

ABSTRACT

Urban animals often show differences in aggression relative to their non-urban counterparts, but the ultimate and proximate origins of these differences are poorly understood. Here we compared urban and rural song sparrows, a species for which higher levels of aggression in urban populations have previously been reported. First, we confirmed elevated territorial aggression levels in urban birds relative to rural birds over multiple years. To begin to identify the environmental variables contributing to these differences, we related aggression to features of the social and physical environment, specifically population density and the availability of suitable nesting vegetation. Population density was not correlated with territorial aggression levels, but there was a significant relationship between territorial aggression and the interaction of availability of nesting vegetation with habitat type (i.e., rural versus urban). Lastly, we conducted a food supplementation experiment to determine whether potential differences in the relative availability of food between the two habitats might drive differences in aggression. Food supplementation increased territorial aggression significantly, particularly in rural birds. Thus, it appears that the availability of both food and suitable nesting vegetation play a role in determining territorial aggression in song sparrows. The specific combination of these features

found in urban areas may cause the increased levels of territorial aggression seen in these populations.

INTRODUCTION

Animals living in urban habitats display a number of behavioral differences from their rural counterparts, including a reduced tendency to migrate (Partecke and Gwinner 2007), higher tolerance of humans (Møller 2010; Scales et al. 2011; Atwell et al. 2012), increased song pitch (Slabbekoorn and Peet 2003; Wood and Yezerinac 2006; Hu and Cardoso 2010; Luther and Derryberry 2012), and species-specific differences in aggression (Coss et al. 2002; Newman et al. 2006; Scales et al. 2011; Galbreath et al. 2014). Urban-associated features such as light and noise pollution (Longcore and Rich 2004; Slabbekoorn and Ripmeester 2008; Hu and Cardoso 2010), changes in predation pressure (DeCandido and Allen 2006), man-made obstacles (Li et al. 2010; Peralta et al. 2011), differences in vegetation (Miller et al. 2003), and/or changes in available foods (Fedriani et al. 2001; Marzluff and Neatherlin 2006; Heiss et al. 2009) may drive these differences. Many traits observed to differ between urban and rural populations can be plastic and responsive to proximate environmental pressures (Estevez et al. 2002; Rodriguez-Prieto et al. 2008; Bermudez-Cuamatzin et al. 2009; Stevenson and Rillich 2013). Selection may also act on individuals after they colonize urban habitats, causing differences in these traits to arise. A third possibility is pre-adaptation; just as some species possess traits that make them better suited to colonize a given habitat, variation among individuals within a species may lead to some individuals being better suited to urban habitats than others. These possibilities are not mutually exclusive. The behavioral and physiological plasticity that enables species and individuals to respond to environmental changes are often considered pre-adaptations to urbanization (Bonier et al. 2007).

Aggressive behavior may benefit individuals colonizing urban areas. Within a species, more aggressive individuals may outcompete their less aggressive conspecifics for desired resources (Duckworth 2008). However, aggressive behavior can be costly, sometimes resulting in reduced survivorship and/or fitness (Marler and Moore 1988; Duckworth 2006). While both genes and early developmental environment can influence an individual's aggressive responses (Coss et al. 2002; Marks et al. 2005; Eccard and Roedel 2011; Kukekova et al. 2011; McGhee et al. 2013), producing what can be thought of as an aggression "set point", the relative immediate costs and benefits of aggressive behavior may also influence whether an animal expresses such behavior in a given context (Estevez et al. 2002; Stevenson and Rillich 2013). Thus, the tendency to express aggression may be expected to differ between populations facing different environmental pressures, which is often the case when comparing urban and non-urban groups. Habitat quality has been positively associated with territorial aggression in a number of species (Fox et al. 1981; Santangelo et al. 2002; Scales et al. 2013), and heightened conspecific territorial aggression in urban song sparrows has previously been described (Evans et al. 2010; Scales et al. 2011). Taken together, these findings raise the possibility that urban habitats are actually perceived as higher quality than rural areas by song sparrows and that this leads to increased conspecific territorial aggression. However, the specific habitat features that may contribute to this perception have not been clearly identified.

Environmental features that impact aggression can be divided into two categories, social and physical, which overlap and interact with each other. Across taxa, territorial aggression is generally positively related to the availability of resources such as food, space, and suitable vegetation (Ewald and Carpenter 1978; Fox et al. 1981; Lore et al. 1986; Camfield 2006; Snekser et al. 2009; Maruyama et al. 2010; Di Paola et al. 2012; but see Toobaie and Grant 2013) and with intra- and inter-specific competition for these resources (Perrin et al. 2001; Lacava et al. 2011; Di Paola et al. 2012; Yoon et al. 2012 Pusey and Schroepfer 2013). Competition for space and resources can occur even at low population densities if resources are limited. However, aggression tends to increase with population density in many species (Yoon et al. 2012; Pusey and Schroepfer 2013; Yuan et al. 2013; but see Perrin et al. 2001; Bohlin et al. 2002). Thus, while aggressive interactions are often driven by the availability of physical resources, a social dimension is introduced through competition with neighboring conspecifics, which will necessarily be mediated in part by the proximity and density of such neighbors. As urban habitats differ from rural habitats in numerous ways, it is possible that differences in one or more physical or social features may cause differences in territorial aggression to arise.

To investigate whether conspecific territorial aggression differs consistently between urban and rural populations, and if so, what social and physical features of the environment may drive this difference, we conducted a series of observational studies and an experimental manipulation in urban and rural populations of song sparrows (*Melospiza melodia*). Our field sites were spread across a patchwork of farms, parkland, and towns in southwestern Virginia, USA. These sites fall within a larger urban-rural continuum, but do not represent its extremes. Rural sites were comprised of agricultural areas and parks, while urban sites were located within towns whose populations averaged 20,609 people (Community Facts). First, we compared male territorial aggression between habitats across multiple years, predicting that urban males would be more aggressive than their rural counterparts in accordance with previous studies of other song sparrow populations. After confirming that urban birds were consistently more aggressive, we then looked for correlations between our aggression measures and two environmental features: male territory distribution and availability of vegetation suitable for song sparrow nests. We predicted that in both habitat types, males with more and nearer neighbors would be more aggressive than males with few or distant neighbors. We also predicted that high levels of suitable nesting vegetation would correlate with heightened territorial aggression, particularly in urban areas where green space is more limited. Finally, to determine whether food availability (a major component of territory quality) could act as a driver affecting aggression levels, we conducted a food supplementation experiment, predicting that supplemental food would increase territorial aggression in both urban and rural habitats.

METHODS

Study Populations

The song sparrow (*Melospiza melodia*) is a songbird common throughout North America. In our study region of southwestern Virginia, USA (37°13'N, 80°22' W, ~600-700m elevation) they readily inhabit both urban and rural areas and are generally resident year-round, though not all adult males defend territories during the winter. Males re-establish territories or increase territorial behavior beginning in late January and early February, and breeding typically occurs from early April through August, with some fledglings present on their natal territories into

September (SL Foltz, personal observation). From 2011 to 2013, we investigated conspecific territorial aggression in adult, territory holding males during the pre-breeding and breeding seasons (March-July). The Virginia Tech Institutional Animal Care and Use Committee approved all study procedures (protocols 10-052-BIOL, 12-051-BIOL, and 13-032-BIOL).

Birds were studied at 7 field sites (Urban sites: 1-4; Rural sites: 1-3; see Table 3.1) scattered throughout the New River Valley area, all located within 20km of Radford, VA. All 7 sites were used during 2011; in 2012 and 2013, only 6 of the original sites (Urban: 1-3; Rural: 1-3) were used. Urban and rural sites were differentiated based on relative amounts of green space and human population densities (See Foltz et al. 2015 for detailed description). Briefly, we estimated the proportion of site area covered by fields, forest, bare dirt, and other natural features (green space) as opposed to built structures such as buildings, roads, and parking areas using satellite images from Google Earth (version 6.2) and area calculations performed in GE-Path (version 1.4.6). Human population densities were calculated using population data drawn from the 2010 United States Census (2010 Census Interactive Population Search) and areas of the relevant census block groups calculated in GE-Path.

Territorial Aggression Tests

We conducted simulated territorial intrusions (STIs) on focal males' territories between 6am and 10am and used males' behavioral responses to these intrusions to quantify individual territorial aggression. Each STI consisted of the presentation of a 10min loop of two individual songs pre-recorded from a single unfamiliar male. We made multiple looped tracks, each from a different male, such that not all focal birds were presented with the same recording. We randomly selected from among 6 tracks in 2011, 10 tracks in 2012, and 11 tracks in 2013. Recordings were made from males singing at Urban 2, Urban 3 (2011, 2012, and 2013) and Rural 1 (2013) and exclusively from males not used in our studies. Tracks used were different across years. The speaker was positioned face up on the ground on the focal male's territory, 1-2m horizontally from a potential perch (bush or tree) whenever possible. In the few cases where such woody vegetation was not available, we placed the speaker near tall grass or herbaceous plants. During the STI, the observer recorded four behavioral measures: the number of flights made toward or across the speaker, the number of songs sung by the focal male, his closest approach to the speaker, and the time he spent within 4m of the speaker. STIs were performed during the breeding season in 2011 (April 28th – July 17th), 2012 (May 10th – July 12th), and 2013 (April 20th – July 23rd). We completed STIs on 60 males in 2011 (35 rural, 25 urban), 39 males in 2012 (23 rural, 16 urban), and 69 males in 2013 (34 rural, 35 urban). The 37 individuals (19 rural, 18 urban) that were fed in 2012 as part of our supplemental feeding experiment were not included in this data set.

To determine the relationship between male territorial aggression and habitat, we first combined our four observed behavioral measures from STIs into a single aggression score using a principle components analysis (PCA). This PCA included STIs conducted across all three years of sampling. We used the first principle component of this PCA as individuals' aggression scores. We then created a linear mixed model (LMM) in which aggression score was the response variable and explanatory variables were habitat type (urban or rural), year (2011, 2012, 2013), STI date, and the interaction between habitat type and year. Site and individual were included as random factors, with individual nested within site. 2013 was selected as the reference year for this model, as it had the highest mean aggression values and thus represented one end of the continuum of aggression levels. We subsequently performed a Tukey all-pairs comparison to examine relationships between years in further detail. Backward selection was employed to remove non-significant fixed explanatory variables, beginning with the variable with the highest P-value, until the final model contained only variables with P-values less than 0.1, and this method was employed for other regression analyses described below as well. All statistical analyses for this and the following studies were performed in JMP Pro (version 11.0.0) and correlations were considered significant at $P<0.05$.

Neighbor Distribution

In February 2012, during the pre-breeding season when males re-establish their territories, we measured song sparrow distribution to determine whether social factors such as the presence and proximity of neighboring territorial males were related to aggression. Six study sites (3 urban, 3 rural) were surveyed for active song sparrow territories. Observers walked transect lines through the survey area while playing pre-recorded songs to attract the attention of male song sparrows. Each successive transect was parallel to, and 50m from, the previous one. The perches used by responding birds were marked on a satellite image printed from Google Earth. Because our study sites were different sizes, we selected two 300m by 300m plots at each site within areas where birds had previously been observed for territory searches; this allowed us to standardize search effort across sites of different sizes.

Once territories were located, they were numbered and four numbers were randomly selected from each site (N=24 total; 12 urban, 12 rural). Males defending these selected territories were then subjected to STIs as described above. These STIs were in addition to those conducted to test year and habitat effects and were performed during the pre- and early breeding season from mid-March to late-April. Recordings for these STIs were randomly selected from among 7 tracks recorded from males not tested in this study. Immediately after the STI, two observers searched the surrounding area for neighboring birds by walking a circle of radius 75m around the STI point while playing recorded song. The perches used by any males other than the focal male who responded were marked on a satellite image map printed from Google Earth. A second search for neighboring birds was conducted from the same central point two days later. Neighbor locations were put into Google Earth, which we used to measure the distance between the nearest neighboring male and the STI point. Results of the two searches were then averaged to create one estimate of neighbor density and one estimate of nearest neighbor proximity per focal male for analyses.

An LMM was used to compare male territorial aggression to the proximity and density of focal males' neighbors. Aggression scores were generated by taking the first principle component of a PCA combining the STIs done immediately prior to neighbor searches and these formed the response variable. Explanatory variables were the average number of neighbors found within a 75m radius (hereafter *neighbor density*), the average distance to the nearest neighbor (hereafter *neighbor proximity*), habitat type (urban or rural), and recorded song track. Site was included as a random factor. We also compared both the neighbor proximity and neighbor density across habitats at the site level using a MANOVA, a method that accounted for correlation between these measures. Site averages of each measure were used rather than individual focal bird measures in an effort to retain meaningful outliers in the data set while creating a normal distribution. Neighbor proximity was then further normalized via a natural log transformation. Neighbor proximity and neighbor density formed the response variables of the model; habitat type was the explanatory variable.

Availability of Suitable Nesting Vegetation

From early April to mid August of 2013, we measured the availability of vegetation suitable for song sparrows to build nests in to determine whether this physical habitat feature impacts territorial aggression. We searched for and monitored active song sparrow nests at 4 field sites (Urban 2 and 3, Rural 1 and 2). We measured nest height from the ground and the density of the plants in which the nest was placed. Density was measured at 10cm from the nest in each of the four cardinal directions, from ground level to 1m above the ground, by inserting a meter stick into the vegetation vertically and counting the number of points at which branches or leaves made contact with the stick, a method adapted from Zanette et al. (2011). These four density measures, from the cardinal directions, were then averaged. We used these nest vegetation measurements from 35 total nests to determine a range of vegetation heights and densities suitable for song sparrow nests by taking the mean +/- one standard deviation of both measures.

To quantify the availability of suitable nest vegetation present on an individuals' territory, we conducted two perpendicular 50m transects that crossed each other at their midpoints, forming an X, on each territory. The direction of the first transect was randomly determined by throwing out a linear marker (generally a pen) and aligning the transect with the marker's landing position, with the marker at the midpoint of the transect. At the intersection of the transects and at 5m intervals along each arm, we estimated the percent of suitable vegetation present within a circle of 2.5m radius surrounding the sample point, for a total of 21 sample points on each territory. Density of vegetation that met the minimum height requirements was estimated by counting contact points on a meter stick held vertical to the ground, as described above. Vegetation taller than the maximum height range was considered suitable as long as the part within the suitable height range met our density requirements. The percentages of suitable nest vegetation from each of the 21 sample points were then averaged to produce one estimate per territory.

We measured suitable nest vegetation availability on 26 territories (15 urban, 11 rural) belonging to birds whose territorial aggression had previously been tested during the 2013 breeding season. For comparison, we also conducted 16 transects (8 urban, 8 rural) in areas of these sites that were not inhabited by song sparrows. Transect locations in these areas were selected by dividing a satellite map of the unoccupied area taken from Google Earth into 100m by 100m plots and randomly selecting plots for sampling.

We created an MLMM comparing male territorial aggression to suitable nest vegetation, using an aggression score created from the first principle component of a PCA that combined the four behavioral measures of STIs from birds whose territories were mapped for suitable nest vegetation in 2013. This formed the response variable. Explanatory variables were average suitable nest vegetation of the territory, habitat type (urban or rural), STI date, recorded song track, and the interaction of average suitable nest vegetation and habitat type. Site was again included as a random factor.

We also compared the prevalence of suitable nest vegetation across habitats and occupied vs. unoccupied area using two binomial regressions. The response variable of the first regression was habitat type (urban or rural) and that of the second regression was occupancy by song sparrows (yes or no). The explanatory variable of both models was average suitable nest vegetation.

Food Supplementation Experiment

During the breeding season of 2012, we conducted a supplemental feeding experiment to determine how increased food availability on birds' territories would influence male territorial aggression. We divided each of six populations (three urban and three rural) in half, providing supplemental food on half of the territories and leaving the other half unfed as controls. One urban site (Urban 1) was too small to subdivide, so all territories there were used as controls; additional fed territories were added to Urban 3, the nearest of the other two urban sites, roughly 2km away. At all sites, treatments were grouped (fed near fed, unfed near unfed) to minimize unfed birds' opportunities to steal food from neighboring territories.

Beginning in mid-April, fed territories received ~355g of seed mix (1/2 white millet, ¼ thistle, 1/8 sunflower hearts, 1/8 safflower seeds, mixed with ground cayenne pepper to deter foraging mammals) every three days. Our intent was to provide essentially unlimited food, and our initial feeds suggested that this amount of seed was sufficient to last until our next visit. Control territories were also visited on the same schedule. We placed seed on the ground 1-2m from cover in a consistent location on the focal males' territories. Feeding on individual territories ended after all data had been collected from the resident focal male.

Between May 1st (two weeks after feeding began) and June 12th, 2012, we conducted 1hr observations of 24 randomly selected territories (two fed and two unfed per site) to ensure that the focal song sparrows used the seed provided and determine whether the seed attracted other birds (conspecifics or other species), which might then act as competitors. We observed the seed pile on fed territories and a similarly sized area of ground on unfed territories. Control comparison areas were chosen for their similarity to our fed areas; they were within the area that the focal bird had previously been seen using, and located near cover such as a bush or shrub. To conduct the observations, an observer sat 20-25m from the area being observed and recorded the number of visits made by each observed avian species to the seed pile or equivalent area (mammals were only rarely observed, and only one was ever seen feeding). To test whether supplemental feeding affected aggression levels, we performed STIs on 76 focal males (23 rural control, 19 rural fed, 16 urban control, and 18 urban fed) between May 13th and July 15th, 2012 (mean STI date: June 14th). STIs were conducted as described above.

The effect of supplemental feeding on males' territorial aggression was analyzed via a LMM. We first conducted a PCA on the STI data of fed and unfed birds aggression-tested in 2012 to create a single aggression score for each individual; this aggression score was used as the response variable in our model. Explanatory variables were treatment (fed or unfed), habitat type (urban or rural), STI date, recorded song track, and the interaction of treatment and habitat type. Site was included as a random factor. As above, we used backward selection to remove non-significant fixed explanatory variables to create a final model containing only variables with P-values less than 0.1. Two more LMMs compared: 1) the total number of visits by all avian species to fed versus unfed territories in each habitat and 2) the total number of visits by song sparrows to fed vs. unfed territories in each habitat. The response variables were the number of visits of relevant species to the observation site; explanatory variables for both models were treatment (fed or unfed), habitat type (urban or rural), and the interaction of habitat type and treatment. Site was a random factor in both analyses. Backward selection was employed here as well.

RESULTS

Study Populations

All of our urban sites had higher human population densities than our rural sites (Urban mean = 1522.7±1402.12; Rural mean = 71.1±62.4; Table 3.1). Additionally, urban sites had much less green space than their rural counterparts (Urban mean = 54.7%±5.58; Rural mean = 92.2%±8.43; Table 3.1). Categorical designations of urban or rural were used for all further analyses.

Aggression by Habitat and Year

The first principle component of our PCA combining breeding season STI data across all three years explained 57.6% of the variation in our aggression data (see Table 3.2 for eigenvectors). Backward selection of our full model comparing males' territorial aggression scores across habitats and years created a final model that omitted the interaction of habitat type with year. We found significant relationships between aggression score and habitat type ($P<0.0001$), year ($P=0.005$), and STI date ($P=0.001$) (see Table 3.3 for complete statistics). Thus, urban birds were more aggressive than rural birds and this pattern was consistent across years (Figure 3.1). However, overall aggression levels varied by year. Specifically, our Tukey pair-wise comparisons revealed that 2012 aggression levels were significantly different from both 2011 (difference=0.833, SE=0.27, t-ratio=3.11, $P=0.006$) and 2013 (difference=-0.798, SE=0.28, t-ratio=-2.87, $P=0.013$). However, 2011 did not differ significantly from 2013 (difference=0.036, SE=0.24, t-ratio=0.15, $P=0.988$).

Neighbor Distribution

The first principle component of our PCA combining STI data from pre- and early-breeding season STIs performed in 2012 explained 49.4% of the variation in our data (Table 3.2). The final model included only habitat type as an explanatory variable (estimate=-0.92, SE=0.13, t-Ratio=-7.29, $P=0.002$), indicating that urban birds had higher territorial aggression than rural birds. Male territorial aggression was not correlated with neighbor proximity, neighbor density, or recorded song track (Figure 3.2). Our MANOVA comparing neighbor proximity and neighbor density across habitat types was not significant (exact F=2.85, degrees of freedom=2, P=0.202).

Availability of Suitable Nest Vegetation

The first principle component of the PCA combining STIs performed in the breeding season of 2013 on males at the four sites where suitable nest vegetation was measured explained 46.1% of the variation in the data (Table 3.2). The final model contained suitable nest vegetation, habitat type, and the interaction of suitable nest vegetation and habitat type as explanatory variables. Only the interaction term was significant (estimate=-0.12, SE=0.05, t-Ratio=-2.42, P=0.025); habitat type and suitable nest vegetation were maintained in the model because they were components of the interaction. In urban birds, territorial aggression increased with available nest vegetation, while in rural birds, aggression decreased (Figure 3.3). Male territorial aggression was not correlated with suitable nest vegetation (estimate=-0.01, SE=0.05, t-Ratio=-0.12, P=0.906, upper 95% CI=0.10, lower 95% CI=-0.11) or habitat type (estimate=-0.46, SE=0.32, t-Ratio=-1.43, P=0.268, upper 95% CI=0.72, lower 95% CI=-1.64) alone, or to STI date or recorded song track. The lack of a direct relationship between aggression and habitat in this analysis is unusual and may be due to the relatively small size of this data set, as there is a fair amount of inter-individual variation in territorial aggression levels even within a given habitat.

Occupied territories had significantly more suitable nest vegetation than unoccupied ones (estimate=-0.26, SE=0.10, χ^2=6.29, P=0.012). However, the proportion of available suitable nest vegetation did not differ significantly between urban and rural territories (estimate=0.03, SE=0.06, χ^2=0.27, P=0.600, upper 95% CI=0.14, lower 95% CI=-0.08).

Food Supplementation Experiment

The first principle component of the PCA combining breeding season STIs performed on fed and unfed territories in 2012 explained 55.3% of the variation in this data set (Table 3.2). The final model examining the effect of supplemental food and habitat type on aggression contained treatment (P=0.002), habitat type (P=0.011), and the interaction of feeding treatment with habitat type (P=0.033) (Table 3.4; Figure 3.4). Recorded song track and STI date had P-values>0.1 and were omitted from the final model. Rural birds responded to supplemental feeding by increasing their levels of aggression significantly, while urban birds' aggression levels do not differ substantially between fed and unfed groups, thereby accounting for the significant interaction term.

Song sparrows visited seed piles during observations at four out of our six observed fed rural territories and all of our six observed fed urban territories. Backward selection of the model examining the effects of supplemental food on visits by song sparrows found that no explanatory factors were significant; that is, neither supplemental feeding nor habitat type affected song sparrow visitation of territories during the period we observed them. The final model examining the effect of supplemental food on visits by all bird species contained only feeding treatment; fed territories received more visits than unfed territories (estimate=1.42, SE=0.50, t-Ratio=2.86, P=0.010). Habitat type and the interaction between habitat type and treatment were not significant and were omitted from the final model. Fed territories were visited by an average of 3.2 species in urban areas and 2.2 species in rural areas, including song sparrows. Unfed territories were visited by an average of 2.2 species in urban areas and 1.7 species in rural areas, including song sparrows.

DISCUSSION

We found that urban male song sparrows were consistently more aggressive in defense of their territories than were rural males. There were no consistent correlations between territorial aggression and social environmental measures (neighbor density and distance), though urban and rural birds' aggression measures showed significant but opposing relationships with our physical environmental measure, the availability of suitable nest vegetation. Our supplemental feeding experiment increased territorial aggression in rural birds, suggesting that food availability, a significant component of habitat quality, is a major contributor to territorial aggression levels.

The high levels of territorial aggression seen in our urban birds, relative to the rural birds, could be a plastic, facultative response to environmental factors or a fixed trait. That is, higher aggression levels may be a response to greater resource availability, or the urban habitat may select for highly aggressive individuals. These possibilities are not mutually exclusive. The results of our feeding study indicate that birds can and do facultatively adjust aggression in response to the availability of food in their environment, and thus that territorial aggression is at least somewhat plastic.

Given that our fed birds showed higher levels of aggression than their unfed counterparts, higher overall levels of aggression in urban song sparrows may be a response to greater amounts of food in this habitat type. Urban birds may have more time and energy to invest in territorial defense and/or urban territories may be perceived as more valuable because they contain more abundant resources. Similarly, the fact that fed urban birds did not increase their aggression as much as fed rural birds did relative to their unfed counterparts may reflect less food-limitation in urban habitats to begin with, and thus a reduced valuation of supplemental food. However, we did not directly measure food availability due to the general and flexible nature of the adult song sparrow diet, and so can only speculate about the exact causes of our findings.

Increased aggression on fed territories may also be a response to increased competition, in the form of other species drawn in by our supplemental food. Fed territories were visited by a significantly higher number of birds of all species than unfed territories. We did not observe song sparrows defending the seed piles from other species, and the number of song sparrows visiting territories did not differ across feeding treatments or habitats. However, we performed our observations at least two weeks after feeding began. Thus, incursions by neighboring song sparrows could have increased immediately after feeding began, triggering increased territorial defense, which would in turn have reduced these incursions such that we saw only increased aggression and no actual incursions two weeks later. A more thorough study of how resident and neighboring song sparrows and co-occurring species respond to food supplementation is needed to entirely rule out inter- and/or intra-specific competition for food as a proximate driver of territorial aggression.

Our attempts to identify features of the social and physical environment other than food that relate to territorial aggression levels produced mixed results. We found no relationship of neighbor density or proximity with males' territorial aggression. Thus, it appears that while social factors such as population density do influence aggressive behavior in some systems (Yoon et al. 2012; Pusey and Schroepfer 2013; Yuan et al. 2013), they are not important determinants of individuals' territorial aggression here. The availability of suitable nest vegetation was related to territorial aggression, but not in the straightforward manner predicted. In line with our original prediction and the effect of habitat quality evident with food supplementation, urban birds on territories with high amounts of suitable nesting vegetation displayed higher levels of aggression relative to those occupying territories with less suitable vegetation. Unexpectedly, however, rural birds showed the opposite pattern; the least aggressive of these males occupied territories with the most suitable nest vegetation. Across habitat types, territories occupied by song sparrows contained significantly more suitable nesting vegetation than nearby unoccupied areas, indicating that the type of vegetation we measured is in fact a relevant resource to song sparrows and may be an important element of habitat quality that affects territory selection. Therefore, it appears that while suitable nest vegetation can be a valued resource, large quantities of it may actually reduce perceived territory value in some circumstances.

Overall, our results show that the high, stable levels of territorial aggression exhibited by our urban song sparrow males are related to resource availability, at least in part. Food availability appears to play an especially strong role in determining aggression levels, and is an environmental feature that is likely impacted by urbanization. Thus, urban development may be impacting animals' behavioral phenotypes via changes in resource availability. These behavioral changes in turn may affect the viability of urban wildlife populations; future studies should evaluate the impacts of behavioral shifts on fitness in urban animals.

ACKNOWLEDGMENTS

We would like to thank the Laboratory for Interdisciplinary Statistical Analysis at Virginia Tech for advice on data analysis. This work was supported by the National Science Foundation (IOS 0545735 to I.T.M. and 1311381 to I.T.M. and S.F.) and a graduate fellowship from the Institute for Critical Technology and Applied Science (ICTAS) at Virginia Tech to S.F.

References

2010 Census Interactive Population Search. n.d. In *2010 Census.* Retrieved Sept. 2012 from http://www.census.gov/2010census/popmap/.

Atwell JW, Cardoso 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.

Bermudez-Cuamatzin E, Rios-Chelen AA, Gil D, Garcia CM. 2009. Strategies of song adaptation to urban noise in the house finch: syllable pitch plasticity or differential syllable use? *Behavior* **146**:1269-1286.

Bohlin T, Pettersson JCE, Johnsson JI. 2002. Is selection for territorial aggression in brown trout density-dependent? *J Fish Biol* **60**:1335-1337.

Bonier F, Martin PR, Wingfield JC. 2007. Urban birds have broader environmental tolerance. *Biol Lett* **3**:670-673.

Camfield AF. 2006. Resource value affects territorial defense by broad-tailed and rufous hummingbirds. *J Field Ornithol* **77**:12-125.

Community Facts. n.d. In *American FactFinder*. Retrieved July 17, 2014 from http://factfinder2.census.gov/faces/nav/jsf/pages/community_facts.xhtml.

Coss RG, Marks S, Ramakrishnan, U. 2002. Early environmental shapes the development of gaze aversion by wild bonnet macaques (Macaca radiata). *Primates* **43**:217-222.

DeCandido R, Allen D. 2006. Nocturnal hunting by peregrine falcons at the Empire State Building, New York City. *Wilson J Ornithol* **118**:53-58.

Di Paola V, Vullioud P, Demarta L, Alwany MA, Ros AFH. 2012. Factors affecting interspecific aggression in a year-round territorial species, the jewel damselfish. *Ethology* **118**:721-732.

Duckworth RA. 2006. Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. *Behav Ecol* **17**:1011-1019.

Duckworth RA. 2008. Adaptive dispersal strategies and the dynamics of a range expansion. *Am Nat* **172**:S4-S17.

Eccard JA, Roedel HG. 2011. Optimizing temperament through litter size in short-lived, iteroparous mammals in seasonal environments. *Dev Psychobiol* **53**:585-591.

Estevez I, Newberry RC, Keeling LJ. 2002. Dynamics of aggression in the domestic fowl. Appl *Anim Behav Sci* **76**:307-325.

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

Ewald PW, Carpenter FL. 1978. Territorial responses to energy manipulations in the Anna hummingbird. *Oecologia* **31**:277-292.

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.

Foltz SL, Davis JE, Battle KE, Greene VW, Laing BT, Rock RP, Ross AE, Tallant JA, Vega RC, Moore IT. 2015. Across time and space: effects of urbanization on corticosterone and body condition vary over multiple years in song sparrows. *J Exp Zool* **323A**:109-120.

Fox SF, Myers R, Rose E. 1981. Dominance and the acquisition of superior home ranges in the lizard uta-stansburiana. *Ecology* **62**:888-893.

Galbreath DM, Ichinose T, Furutani T, Yan WL, Higuchi H. 2014. Urbanization and its implication for avian aggression: a case study of urban black kites (Milvus migrans) along Sagami Bay in Japan. *Landscape Ecol* **29**:169-178.

Heiss RS, Clark AB, McGowan KJ. 2009. Growth and nutritional state of American crow nestlings vary between urban and rural habitats. *Ecol Appl* **19**:829-839.

Hu Y, Cardoso GC. 2010. Which birds adjust the frequency of vocalizations in urban noise? *Anim Behav* **79**:863-867.

Kukekova AV, Johnson JL, Teiling C, Li L, Oskina IN, Kharlamova AV, Gulevish RG, Padte R, Dubreuil MM, Vladimirova AV, Shepeleva DV, Shikhevich SG, Sun Q, Ponnala L, Temnykh SV, Trut LN, Acland GM. 2011. Sequence comparison of prefrontal cortical brain transcriptome from a tame and an aggressive silver fox (Vulpes vulpes). *BMC Genomics* **12**: Article No. 482.

Lacava RV, Brasileiro L, Maia R, Oliveira RF, Macedo RH. 2011. Social environment affects testosterone level in captive male blue-black grassquits. *Horm Behav* **59**:51-55.

Li T, Shilling F, Thorne J, Li F, Schott H, Boynton R, Berry AM. 2010. Fragmentation of China's landscape by roads and urban areas. *Ecology* **25**:839-853.

Longcore T, Rich C. 2004. Ecological light pollution. *Front Ecol Environ* **2**:191-198.

Lore R, Gottdiener C, Delahunty MJ. 1986. Lean and mean rats some effects of acute changes in the food supply upon territorial aggression. *Aggressive Behav* **12**:409-416.

Marks C, West TN, Bagatto B, Moore FBG. 2005. Developmental environment alters conditional aggression in zebrafish. *Copeia* **4**:901-908.

Marler CA, Moore MC. 1988. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. *Behav Ecol Sociobiol* **23**:21-26.

Maruyama A, Rusuwa B, Yuma M. 2010. Asymmetric interspecific territorial competition over food resources amongst Lake Malawi cichlid fishes. *African Zool* **45**:24-31.

Marzluff JM, Neatherlin E. 2006. Corvid response to human settlements and campgrounds: causes, consequences, and challenges for conservation. *Biol Conserv* **130**:301-314.

McGhee KE, Travis J. 2013. Heritable variation underlies behavioural types in the mating context in male bluefin killifish. *Anim Behav* **86**:513-518.

Miller JR, Wiens JA, Hobbs NT, Theobald DM. 2003. Effects of human settlement on bird communities in lowland riparian areas of Colorado (USA). *Ecol Appl* **13**:1041-1059.

Møller AP. 2010. Interspecific variation in fear responses predicts urbanization in birds. *Behav Ecol* **21**:365-371.

Newman MM, Yeh PJ, Price TD. 2006. Reduced territorial responses in dark-eyed juncos following population establishment in a climatically mild environment. *Anim Behav* **71**:893-899.

Partecke JP, Gwinner EG. 2007. Increased sedentariness in European blackbirds following urbanization: a consequence of local adaptation? *Ecology* **88**:882-890.

Peralta G, Fenoglio MS, Salvo A. 2011. Physical barriers and corridors in urban habitats affect colonization and parasitism rates of a specialist leaf miner. *Ecol Entomol* **36**:673-679.

Perrin MR, Ercoli C, Dempster ER. 2001. The role of agonistic behaviour in the population regulation of two syntopic African grassland rodents, the striped mouse Rhabdomys pumilio (Sparrman 1784) and the multimammate mouse Mastomys natalensis (A. Smith 1834) (Mammalia Rodentia). *Trop Zool* **14**:7-29.

Pusey AE, Schroepfer-Walker K. 2013. Female competition in chimpanzees. *Philos T Roy Soc B* **368**: Article No. 20130077.

Rodriguez-Prieto I, Fernández-Juricic E, Martín J, Regis Y. 2008. Antipredator behavior in blackbirds: habituation complements risk allocation. *Behav Ecol* **20**:371-377.

Santangelo N, Itzkowitz M, Richter M, Haley MP. 2002. Resource attractiveness of the male beaugregory damselfish and his decision to court or defend. *Behav Ecol* **13**:676-681.

Scales J, Hyman J, Hughes M. 2011. Behavioral syndromes break down in urban song sparrow populations. *Ethology* **117**:887-895.

Scales J, Hyman J, Hughes M. 2013. Fortune favours the aggressive: territory quality and behavioural syndromes in song sparrows, Melospiza melodia. *Anim Behav* **85**:441-451.

Slabbekoorn H, Peet M. 2003. Birds sing at a higher pitch in urban noise. *Nature* **424**:267-267

Slabbekoorn H, Ripmeester EAP. 2008. Birdsong and anthropogenic noise: implications and applications for conservation. *Mol Ecol* **17**:72-83.

Snekser JL, Leese J, Ganim A, Itzkowitz M. 2009. Caribbean damselfish with varying territorial quality: correlated behaviors but not a syndrome. *Behav Ecol* **20**:124-130.

Stevenson PA, Rillich J. 2013. Isolation associated aggression – a consequence of recovery from defeat in a territorial animal. *PLoS One* **8**:UNSP e74965.

Toobaie A, Grant JWA. 2013. Effect of food abundance on aggressiveness and territory size of juvenile rainbow trout, Oncorhynchus mykiss. *Anim Behav* **85**:241-246.

Yoon J, Sillett TS, Morrison SA, Ghalambor CK. 2012. Breeding density, not life history, predicts interpopulation differences in territorial aggression in a passerine bird. *Anim Behav* **84**:515-521.

Yuan B, Tang C, Wang Z, Lu C, Zou Y. 2013. Density, behavior and habitat selection of red-bellied squirrels (Callosciurus erythraeus castaneoventris) in Longjiang riverside of Yizhou, Guangxi, China. *Asia Life Sci* **22**:549-564.

Zanette LY, White AF, Allen MC, Clinchy M. 2011. Perceived predation risk reduces the number of offspring songbirds produce per year. *Science* **334**:1398-1401.

Tables

Table 3.1: Habitat Features Used to Determine Urbanization of Sites

Field Site	Human Population Density (people/k^2)	Green Space (% of site area)	Years Monitored
Urban 1 (Radford Campus West)	246	59.6	2011 – 2013
Urban 2 (Virginia Tech Campus)	2563	48.4	2011 - 2013
Urban 3 (Radford Main Campus)	2897	51.6	2011 - 2013
Urban 4 (Huckleberry Trail, Christiansburg)	385	59.2	2011
Rural 1 (Claytor Lake State Park)	49	82.5	2011 - 2013
Rural 2 (Kentland Farm)	23	96.0	2011 - 2013
Rural 3 (Heritage Park)	142	98.0	2011 - 2013

Table 3.2: Variation Explained by First Principle Component and its Eigenvectors

Data Set PCA was Performed On	% Variation Explained by 1st Principle Component	Songs Eigenvector	Flights Eigenvector	Time Within 4m Eigenvector	Closest Approach Eigenvector
Aggression by Habitat and Year	57.6	0.45	0.51	0.55	-0.48
Neighbor Distribution	49.4	0.18	0.58	0.54	-0.58
Availability of Suitable Nesting Vegetation	46.1	0.51	0.36	0.54	-0.56
Food Supplementation	55.3	0.48	0.52	0.55	-0.44

Positive and negative eigenvector values indicate their relationship with the principle component. The values above show that songs, flights, and time within 4m of the speaker all increase with increasing values of the first principle component (the aggression score), while the closest approach to the speaker decreases, indicating that more aggressive birds leave less distance between themselves and the speaker.

Table 3.3: Relationships of Habitat and Year to Aggression

Explanatory Variable	Estimate	SE	t-Ratio	P-Value	Fixed Effect P-Value
Habitat (rural)	-0.65	0.115	-5.67	<0.0001	<0.0001
Year (2011)	0.29	0.141	2.06	0.041	0.005
Year (2012)	-0.54	0.164	-3.32	0.001	0.005
STI Date	-0.01	0.004	-3.35	0.001	0.001

Reference year is 2013; reference habitat is urban. Fixed effect p-values show results of test that all parameters associated with the effect are equal to 0, producing a combined p-value for categorical factors. Significance = P<0.05.

Table 3.4: Effect of Food Supplementation on Aggression

Explanatory Variable	Estimate	SE	t-Ratio	P-Value	Fixed Effect P-Value
Treatment (control)	-0.49	0.150	-3.28	0.002	0.002
Habitat Type (rural)	-0.47	0.138	-3.44	0.011	0.011
Treatment x Habitat Type	-0.33	0.150	-2.18	0.033	0.033

Reference treatment is fed; reference habitat is urban. Fixed effect p-values show results of test that all parameters associated with the effect are equal to 0, producing a combined p-value for categorical factors. Significance = P<0.05.

Figure Legends

Figure 3.1: Territorial Aggression Across Habitats and Years

Bars show the mean aggression scores generated via PCA for rural (white) and urban (black) birds in each year sampled. N = 60 in 2011 (35 rural, 25 urban), 39 in 2012 (23 rural, 16 urban), and 69 in 2013 (34 rural, 35 urban). Vertical lines represent standard error of the mean.

Figure 3.2: Relationship of Aggression to Neighbor Distribution

Territorial aggression scores are compared against neighbor proximity (A) and density (B). Dots represent individual birds. N = 24 (12 rural, 12 urban).

Figure 3.3: Relationship of Aggression to Nesting Vegetation Availability

Dots represent individual territories. N = 26 (11 rural, 15 urban). Trend lines illustrate the significant interaction between availability of suitable nest vegetation and habitat type.

Figure 3.4: Effect of Supplemental Feeding on Aggression

Bars show the mean of aggression scores generated by PCA for fed and control (unfed) birds in rural (white) and urban (black) habitats. N = 76 (23 rural control, 19 rural fed, 16 urban control, 18 urban fed). Vertical lines represent the standard error of the mean.

Figures

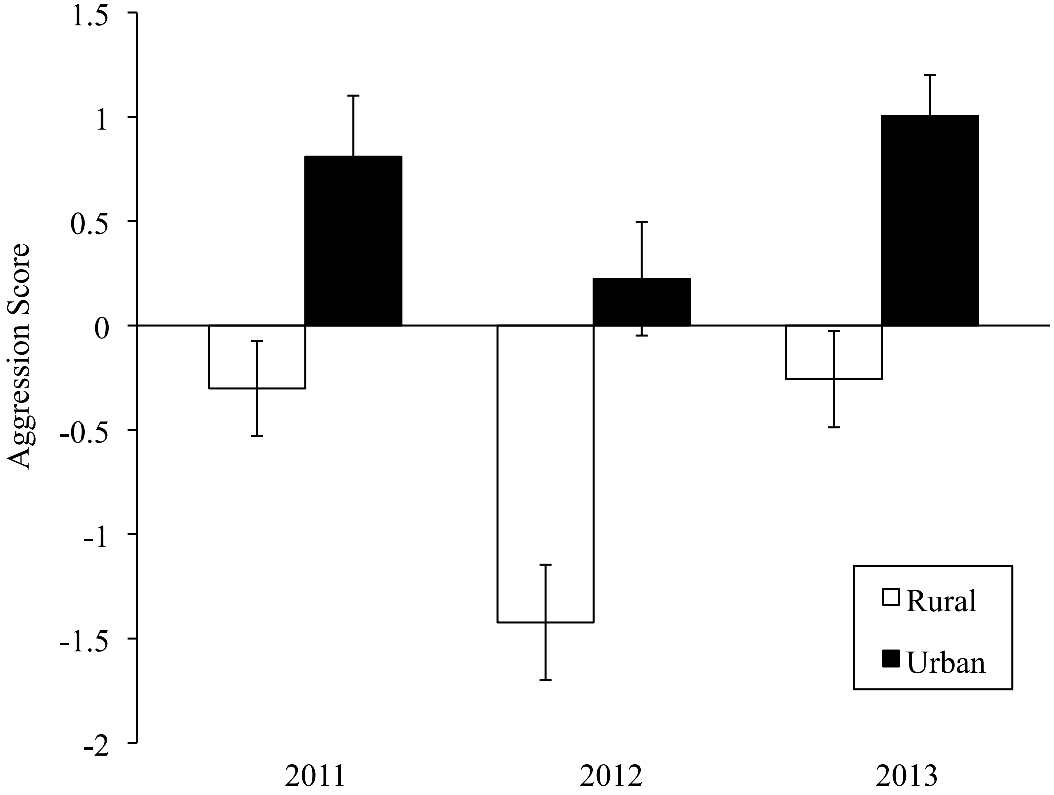

Figure 3.1: Territorial Aggression Across Habitats and Years

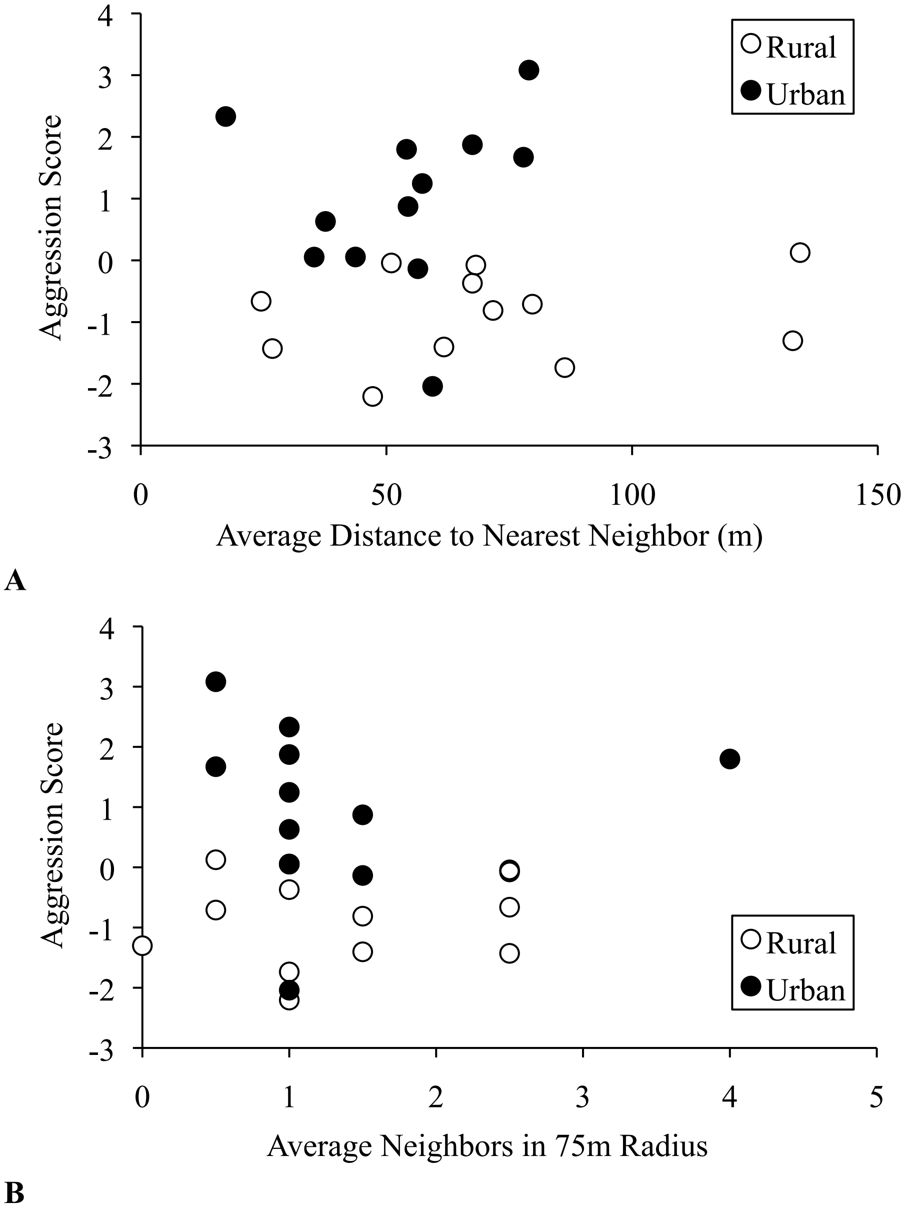

Figure 3.2: Relationship of Aggression to Neighbor Distribution

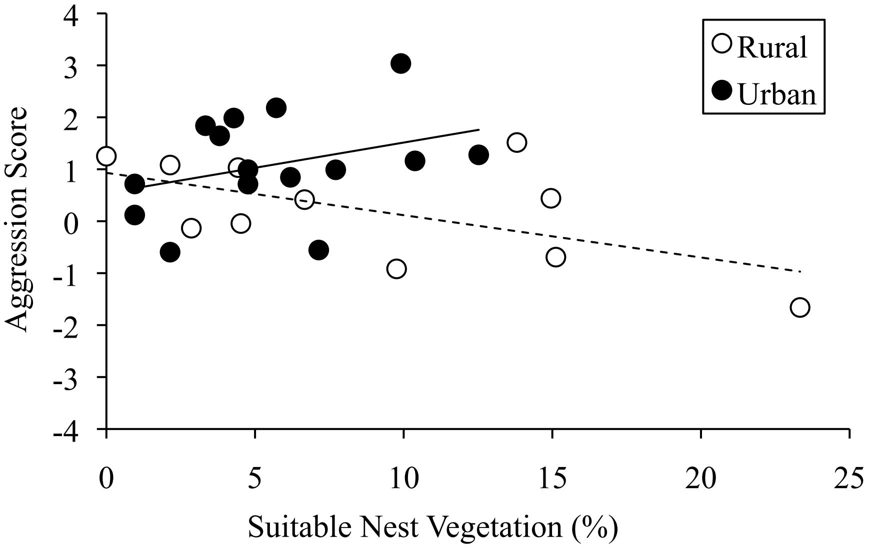

Figure 3.3: Relationship of Aggression to Nesting Vegetation Availability

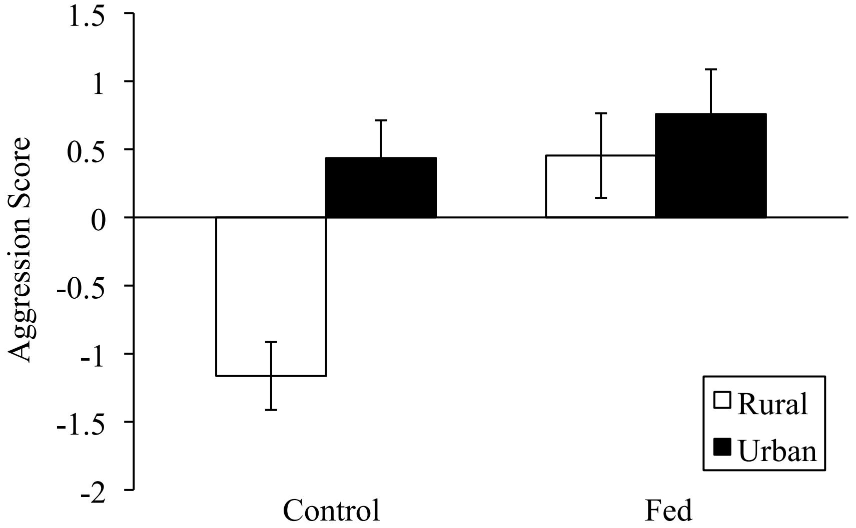

Figure 3.4: Effect of Supplemental Feeding on Aggression

CHAPTER IV: BETTER LATE THAN EARLY: DIFFERENCES BETWEEN URBAN AND RURAL SONG SPARROWS (*MELOSPIZA MELODIA*) IN REPRODUCTIVE SUCCESS

Sarah L. Foltz, Allen E. Ross, Kathryn E. Battle, and Ignacio T. Moore

ABSTRACT

Animals vary widely in their tolerance of urban habitats, with some species avoiding them entirely, while others preferentially inhabit them. However, presence does not necessarily indicate that a species is successful in a habitat. Additionally, populations in urban and rural habitats may demonstrate differences in specific traits, but it is not clear whether or not these differences are adaptive responses to urbanization. Here, we compare reproductive phenology and success of urban and rural song sparrows (*Melospiza melodia)*, a species commonly found in both urban and rural areas. We monitored nests at two urban and two rural field sites in Virginia, USA, across a breeding season. We collected data on placement of nests within the vegetation, nestling mass and number, parental provisioning rates, fledging success, and the territorial aggression of males associated with each nest. Nest placement did not differ between habitats, and neither did nestling mass and number. Male territorial aggression was not a good predictor of variation in nest success. We did find that urban nests had higher overall fledging success and that the success rate at these sites increased across the breeding season, whereas fledging success decreased across the breeding season at the rural sites. We conclude that urban song sparrow populations appear to be relatively successful compared to their rural counterparts and are not simply sinks for excess individuals in the larger population.

INTRODUCTION

For wildlife, urban areas are characterized by a suite of unusual, and in some cases unique, features including high levels of human activity, light and noise pollution (Longcore and Rich 2004; Slabbekoorn and Ripmeester 2008; Hu and Cardoso 2010), altered predator communities and vegetation structure (Miller et al. 2003; Stracey and Robinson 2012), man-made structures (Li et al. 2010; Peralta et al. 2011), and novel foods (Fedriani et al. 2001; Heiss et al. 2009). Many aspects of urbanization pose challenges to native wildlife, while other features present opportunities. Although animal species richness is generally lower in highly urbanized areas (McKinney 2002, 2008), some taxonomic groups such as birds show high species richness in moderately disturbed areas such as the interface between urban and rural habitats (Jokimäki and Suhonen 1993; Blair 1999), and a few species seem to thrive in close proximity to humans (Shochat et al. 2010).

Successful urban species may possess traits that make them better at exploiting urban opportunities and coping with urban-related challenges (Bonier et al. 2007; Møller 2010). However, the presence of a species in urban areas alone does not necessarily indicate that it is well adapted for this habitat type or that its urban populations are self-sustaining. Previous studies have found negative impacts of urbanization on the hatching success of Florida scrub-jays *(Aphelocoma coerulescens)* (Aldredge et al. 2012) and the nestling quality of carrion crows *(Corvus corone corone)* (Richner 1989). Thus, animals living in urban areas may be members of sink populations, sustained predominantly by immigrants from outside source populations.

Therefore, it is necessary to look beyond presence/absence or population densities when determining whether a species is a successful urban colonist, particularly when dealing with highly mobile species whose urban populations may experience significant immigration or emigration.

Within species, individuals living in urban areas can show various differences from individuals in rural habitats, including morphology (Yeh 2004; Lowe et al. 2014), circulating glucocorticoid levels (Partecke et al. 2006; Fokidis et al. 2009; Bonier 2012), song pitch (Slabbekoorn and Peet 2003; Wood and Yezerinac 2006; Hu and Cardoso 2010; Luther and Derryberry 2012), territorial aggression (Coss et al. 2002; Newman et al. 2006; Scales et al. 2011; Foltz et al. submitted), tolerance of human approach (Møller 2010; Scales et al. 2011; Atwell et al. 2012), timing of reproduction (Yeh and Price 2004; Partecke et al. 2004), and migratory tendency (Partecke and Gwinner 2007). These differences between urban and rural populations may arise from differential assortment of individuals into urban and rural habitats based on their individual traits and preferences, from selection on individuals after they arrive in urban areas, or from a plastic, facultative response by individuals to habitat-imposed pressures. However, in many cases it is not yet clear whether these differences represent adaptive adjustments to urban living. In order to clearly understand the long-term impacts of urbanization on wildlife populations, we must connect the differences we have observed in urban animals to their effects on fitness.

To better understand whether urban wildlife populations are adapted or adapting to urban habitats, we conducted a series of inter-related observational studies on urban and rural populations of song sparrows (*Melospiza melodia)*. This species is native to North America and commonly found in moderately urban as well as rural areas in southwestern Virginia. We collected data on nest placement, breeding phenology, nestling condition, nest success, and male territorial aggression during the breeding season of 2013. We have previously described male urban song sparrows exhibiting greater territorial aggression than rural males in these populations (Foltz et al. submitted). We predicted that proximate reproductive cues such as increased food availability (Schoech and Bowman 2001) and warmer temperatures (Bornstein 1968) in urban habitats would lead to earlier breeding in urban populations than rural ones (Boutin 1990; Nager and van Noordwick 1995). We also predicted that nest success and nestling quality would be lower in urban than rural habitats and that nest placement would differ between habitat types due to lack of adequate nest vegetation in urban areas. We further expected that aggressive males would have lower nest success and/or produce lower quality nestlings, investing instead in defending their territories from conspecific males.

METHODS

Study Species and Sites

The song sparrow is a common native North American songbird. In our study region, song sparrows are found in both urban and rural areas and over-winter locally. Some males defend territories all year, while others only exhibit territorial aggression during the breeding season, which lasts from early April through mid-August. Fledglings usually remain on the parental territory for several weeks after leaving the nest, and continue to be fed by one or both parents.

Most pairs in this area make multiple breeding attempts per season and will re-nest if a nest fails. Song sparrow nests are sometimes parasitized by brown-headed cowbirds *(Molothrus ater)* and the presence of a cowbird nestling does not necessarily preclude successful fledging of song sparrow nestlings (SLF, personal observation). Although not all breeding adults in our study areas are banded, we have seen no evidence among our banded birds of individuals or pairs moving to other sites or habitat types within the breeding season.

We conducted our studies at 4 field sites (2 urban and 2 rural) within a 20km radius of the city of Radford, located in southwestern Virginia, USA (37°13'N, 80°22' W, ~600-700m elevation). Urban and rural sites were differentiated by human population densities of the surrounding areas and the amount of total site area comprised of green space, as described in Foltz et al. (2015). Briefly, we estimated the area of each site covered by trees, fields, bare ground, and other natural features (green space), as opposed to built structures such as buildings, roads, and parking areas, using satellite images taken from Google Earth (version 6.2). We performed area calculations in GE-Path (version 1.4.6). Fine-scale population data was drawn from data collected during the 2010 United States Census (2010 Census Interactive Population Search), using the census block groups(s) in each area that overlapped our study sites. Census block group areas ranged from 0.47km^2 to 78.05km^2 (average 15.34km^2).

Nests and Nestlings

To investigate whether timing of breeding and nest success differed between urban and rural habitats, we collected data on song sparrow nests and nestlings during the breeding season of 2013. We began searching for nests at the beginning of April 2013. Nests were found in two ways: by observing adult birds and following them to their nest or by manually searching shrubs that appeared to be likely nest sites based on our previous experience. Once found, lay date was estimated using the known incubation period of song sparrows and the number of eggs or nestlings in the nest. Nests were checked on average every other day for the presence of eggs or nestlings. Monitoring continued until the nest was found empty. At that point, the nest was designated successful if fledglings were seen or heard in the nearby area, or if at least one parent was seen in the area chipping and alarm calling and/or carrying food. Nests were also considered successful if they still contained nestlings 9 or more days after hatching, as song sparrows typically fledge between 9 and 12 days of age (Sibley et al 2001). Failed nests were defined as nests found empty before 9 days post-hatching near which signs of fledglings or parental defense and feeding were not apparent. Two nests fledged only a cowbird nestling and no song sparrow nestlings and were thus recorded as failed nests. Cowbird-parasitized nests were identified after hatching, as cowbird eggs are very similar to song sparrows' eggs in size and markings. One of the 8 rural nests and 7 of the 22 urban nests that survived to the nestling stage contained cowbird nestlings (12.5% and 31.8%, respectively).

When nestlings were between five and seven days of age, we weighed them, measured tarsus length, and applied a numbered band for future identification. Average age of measurement was 5.9 days for urban nestlings and 5.8 days for rural ones. Average nestling mass was calculated for each nest for use in condition analyses. The number of nestlings present in the nest when these measurements were taken was used in later analyses of brood size. Because we often found nests after the eggs had hatched, and because one or more eggs were sometimes removed from nests during incubation (possibly by adult cowbirds or predators), we were unable to ascertain original clutch size. We were able to collect nestling measures from 5 rural and 15 urban nests.

We constructed a binomial regression to compare nest success across habitats. The response variable was nest success (yes or no). Explanatory variables were habitat type, date the nest was found, and the interaction of habitat type and date. Random factors included in the model were site and territory, with territory nested within site.

To further examine nest success between habitats, we compared average nestling mass and brood size using two multiple linear mixed models. The response variables were nestling mass and brood size, respectively. Explanatory variables were habitat type, date of nestling measurement, the interaction of habitat type and date, and cowbird nestling presence. Site was included as a random factor.

All binomial regression models were performed in R (version 3.0.2, platform x86_64-apple-darwin10.8.0) in conjunction with R Studio (version 0.97.312). All other analyses were performed in JMP Pro (version 11.0). We performed backward selection on models when possible, removing explanatory variables one at a time beginning with the one with the highest P-value. Variables with P-values less than 0.10 were retained in the model, and relationships were considered significant at $P<0.05$.

Parental Provisioning

To determine whether parental care differed by habitat type or sex, we collected data on nestling provisioning rates at 5 rural and 15 urban nests. We performed two provisioning rate observations per nest on separate days when nestlings were between 3 and 7 days of age (average age at observation: urban = 5.9 days, rural = 5.7 days). Each observation lasted 45min, a time span long enough in our previous experience to observe multiple feeds, generally by both parents. Observations were performed by a single observer with binoculars, positioned roughly 20m from the nest. When nests were positioned near parking areas, a car was sometimes employed as a blind; otherwise, the observer sat quietly in the open at the best angle of observation. The observer typically remained in one place, but moved when necessary to obtain a better view of parents' identification bands, remaining as far from the nest as possible during these movements. When possible, male and female provisioning rates were differentiated by the presence and/or color of bands on the provisioning adults' legs. All visits to the nest were assumed to be provisioning trips. For each nest, total provisioning rates and rates for each parent were averaged across the two observations and scaled to visits per hour for use in further analyses.

Total mean provisioning rates per hour for each nest were normalized via natural log transformation prior to analysis. We constructed a multiple linear mixed model to compare total mean provisioning rates between habitat types, with total mean provisioning rate per hour as the response variable. Explanatory variables were habitat type, average observation date, and cowbird nestling presence. Site was included as a random factor. We also compared male and female provisioning rates using a simple linear regression model in which mean provisioning rate of the male was the response variable and mean female provisioning rate was the explanatory variable (N=16).

Nest Placement

To determine whether urban and rural birds differ in where they place their nests, we collected data on nest placement and visibility from 45 nests (14 rural, 31 urban). After the nests we located were no longer active, we measured characteristics of the nest and its surrounding vegetation. Specifically, we measured nest height (from the ground to the top edge of the cup), the density of the vegetation supporting and surrounding the nest, the distance from the nest to the nearest edge of the surrounding vegetation, and how visible the nest was from outside the vegetation. Vegetation density was measured following the protocol of Zanette et al. (2011). Briefly, we inserted a meter stick vertically into the vegetation at 10, 50, and 100cm from the nest in each of the cardinal directions (north, south, east, and west) and counted the number of times that parts of the vegetation touched the stick. We also performed this measurement horizontally in each direction, placing the stick so that one end touched the nest and thereby measuring density from 0 to 100cm out from the nest in each cardinal direction. Vegetation density measurements were averaged together to create a mean estimate of vegetation density within one meter of the nest for use in further analyses. To measure the area of the nest visible to the human eye from 1m away, we placed a 10cm by 10cm card gridded into 1cm^2 squares on the nest so that the card faced the nearest edge of the surrounding vegetation. We then took a photo 1m away from the nest from the direction of the nearest edge of the surrounding vegetation. The number of complete squares visible in the photo out of 100 was later counted.

To compare nest location across habitats, we constructed a series of linear mixed models with nest height, mean vegetation density, and distance from the nest to the nearest vegetation edge as their respective response variables. Mean vegetation density was normalized via natural log transformation, and distance from the nest to the nearest vegetation edge was normalized by taking each data point to the power of 0.5. In each model, habitat type (urban, rural) was the explanatory variable, and site and territory were included as random factors, with territory nested within site. Visibility of the nest to the human eye was also compared across habitats, but proved difficult to normalize due to skew in the data and so was converted to a bivariate categorical form (high, low) using the median visibility (10%) as the dividing point. We then constructed a binomial regression with the same explanatory and random factors as above. Backward selection was not performed on these models, as only one explanatory variable was included in the original.

We also examined the effect of nest location on nest success across both habitats by constructing a binomial regression model in which nest success (yes, no) was the response variable. Nest height, mean vegetation density, distance of the nest from the nearest vegetation edge, and visibility of the nest to the human eye were the explanatory factors, while territory was included as a random factor.

Male Aggression

To explore whether male territorial aggression imposes a fitness cost, we performed simulated territorial intrusions (STIs) to measure individual males' territorial aggression. We then compared this behavior to nest success and nestling quality across habitats. These aggression data were taken from a previous study and were performed as described in Foltz et al.

(submitted). Here we use those data to investigate relationships between territorial aggression and reproductive success.

For each of the following analyses, we created a single aggression score for each individual male by combining the four behaviors recorded during STIs via a principle components analysis (PCA) (see Foltz et al. submitted for details). The first principle component of these analyses formed the aggression score for individual males and this score was used in all further analyses. To examine the relationship between aggression and nestling quality, we constructed two multiple linear models with brood size (N=20: 5 rural, 15 urban) and average nestling weight (N=20: 5 rural, 15 urban), respectively, as response variables. Aggression score, date of nestling measurement, cowbird nestling presence (yes/no), and playback track (1-11) were the explanatory variables in both models. We also looked at the impact of male aggression on nest success in each habitat by building a binomial regression with nest success (yes/no) as the response variable and aggression, date the nest was found, and the interaction of aggression and date as the explanatory factors. Birds' individual territory was a random factor. We ran separate models for urban (N=38) and rural (N=17) habitats to control for the previously observed relationship between habitat and aggression. For these models, we conducted separate PCAs for urban and rural birds. Lastly, we examined whether more aggressive males provided less parental care via a MLM in which the provisioning rates of 15 males formed the response variable and explanatory variables were male aggression score, average observation date, cowbird nestling presence, and playback track.

RESULTS

Study Sites

Urban sites had much higher human population densities than rural sites as calculated from US 2010 census block group data (urban mean=2730 people/km^2, SE=167; rural mean=36, SE=13). Urban sites also had substantially less green space than rural sites (urban mean=50% green space, SE=2%; rural mean=89% green space, SE=7%). See Table 4.1 for details.

Nests and Nestlings

We estimated that the first urban and rural nests were established on April 13th and 19th, respectively (previously published in Foltz et al. 2015). In all, we found and monitored 55 nests (17 rural, 38 urban), of which 32 failed (13 rural, 19 urban). Nests were found on 35 unique territories (13 rural, 22 urban). The additional 18 nests were re-nestings by some of these same pairs. We found nests on 49% of the urban territories searched and 45% of the rural territories searched. The majority of our nest failures (22 out of 32) appeared to be attributable to nest predation. Failed nests were considered predated when they were found empty and often damaged, sometimes with eggs or pieces of eggshell nearby. Non-predated nest failures were attributed to nest parasitism by cowbirds (2 nests), nestling injury (1 nest), infertility or insufficient incubation (2 nests), inclement weather (1 nest), and unclear causes (4 nests). In the cases of failure by nest parasitism, only the cowbird nestling fledged; other eggs failed to hatch and/or song sparrow nestlings did not survive to fledging. The injured nestling may have been a casualty of a parasitism attempt, but the injury did not appear consistent with predation. Of our predated nests, 2 urban nests showed signs of predator damage while 5 were left intact (71% undamaged). Four rural nests were damaged and 6 were left intact (60% undamaged). We failed to record data on nest damage for 5 nests (3 urban, 2 rural) and these nests were excluded from the above percentages.

Our binomial regression model comparing nest success across habitats required no backward selection, as all original explanatory variables (habitat type, date the nest was found, and their interaction) were retained in the final model. Both habitat type (P=0.01) and the interaction of habitat type and date (P=0.01) were significant (Figure 4.1). Date alone was not (P=0.10), but was kept in the model due to its role in the interaction term. Specifically, urban habitats had a higher proportion of successful nests than rural habitats. Nests became more likely to succeed in urban habitat as the breeding season progressed, while in rural habitat nest success declined later in the breeding season (Figure 4.1). See Table 4.2 for details.

Backward selection of our model comparing brood size and average nestling mass to habitat type, date of nestling measurement, and the interaction of habitat type and date found no significant relationships with any of these explanatory variables. All explanatory variables had p-values greater than 0.1 and were removed.

Parental Provisioning

Backward selection on our model comparing total mean provisioning rates across habitats produced a final model in which only habitat type remained as a significant explanatory variable. Provisioning rates were higher in rural habitats than urban ones (F-ratio=5.04, P=0.04)(Figure 4.2). Our comparison of male to female mean provisioning rates found a positive trend (F=3.81, P=0.07) between male and female feeds.

Nest Placement

None of the measures we took of nest location differed by habitat type (nest height: t-ratio=0.58, P=0.57, mean vegetation density: t-ratio=-0.77, P=0.45, distance from the nest to the nearest vegetation edge: t-ratio=-0.32, P=0.75, visibility of the nest: Z-ratio=0.002, P=0.10). Backward selection of the binomial regression model comparing nest location to nest success also found no relationship between nest success and any of the location variables measured.

Male Aggression

The first principle component of the PCA of our data set comparing male aggression and nestling quality explained 45.5% of the variation in the data (Table 4.3). Backward selection of the model comparing nestling mass to male aggression produced a final model containing no significant explanatory variables; only average nestling mass (t-ratio=-2.00, P=0.06) remained in the model. Thus, aggressive males tended to have lower-weight nestlings (Figure 4.3). The model comparing nestling number to male aggression found no relationship of nestling number with any explanatory variables.

The PCA for the data set comparing nest success to aggression in urban habitats produced a first principle component that explained 49.0% of the variation in the data (Table 4.3). Backward selection on this model found that only the date the nest was found was significantly related to aggression (t-ratio=2.97, P=0.003). The separate PCA for our data comparing nest success to aggression in rural habitats produced a first principle component that explained 60.1% of the data variation (Table 4.3). No explanatory variables remained in this model after backward selection. Thus, male aggression does not appear to be related to nest success in either urban or rural habitats.

The first principle component of our PCA for the data set comparing male provisioning rates and territorial aggression explained 44.7% of the variation in the data (Table 4.3). Our model based on this comparison contained only aggression score and average observation date after backward selection. There was a non-significant negative trend between male provisioning rate and aggression score (F-ratio=3.50, P=0.08), while the relationship between male provisioning rates and observation date was positive and significant (F-ratio=13.64, P=0.003).

DISCUSSION

Initiation of breeding did not appear to differ significantly between habitat types (Foltz et al. 2015). Song sparrows breeding in urban habitats had higher overall nest success than those breeding in rural habitats. Moreover, rural nests were only successful early in the breeding season, while successful urban nests were spaced more evenly across the breeding season.

Our earliest urban and rural nests were established less than a week apart, indicating that song sparrows initiated breeding at the same time in both of these habitats. This is in contrast to a number of previous studies of other songbird species that found an earlier onset of breeding in urban populations (Partecke et al. 2004; Yeh and Price 2004; Aldredge et al. 2012). Although it is likely that urban habitats contain more food suitable for adult song sparrows, such as seed from feeders, nestling song sparrows are typically fed insects. It may be that the availability of foods suitable for nestlings and other proximate breeding cues, such as ambient temperature, do not differ significantly between our study habitats.

As we did not see great variation in nest placement between habitats, the most straightforward potential explanation for greater nest success in urban areas is that urban-breeding song sparrows face a lower risk of nest predation relative to rural song sparrows, particularly later in the breeding season. We did not directly assess nest predator abundance at our sites but our nest failure data provide some clues. Changes in nest failure rates over the course of the breeding season have been shown in a number of previous studies (Arcese et al. 1996; Rogers et al. 1997; Benson et al. 2010; Soderstrom et al. 2001; Shitikov et al. 2013). Causes of such changes, when known, often included competition with nest parasites (Arcese et al. 1996; Rogers et al. 1997) and depredation by predators (Benson et al. 2010). Patterns of nest failure in relation to date varied with cause, species, and habitat (Arcese et al. 1996; Soderstrom et al. 2001; Shitikov et al. 2013) such that there does not appear to be a “normal” pattern of within-season nest failure. Arcese et al. (1996) found that song sparrow nest failure rates at island study sites in British Columbia, Canada, were highest during the middle of the breeding season, which coincided with the breeding season of brown-headed cowbirds in that region. Changing rates of cowbird parasitism at our sites could account for the decrease in urban nest failures we observed at the end of the breeding season, as cowbirds nestlings were found in nests early in the season, but none were observed during the last month or so of monitoring. However, rural nest were also parasitized early in the breeding season, so nest depredation by cowbird adults and competition from cowbird nestlings do not explain their increasing failure rate as the season progressed.

Studies that examine predator communities in relation to urbanization and urban-related factors such as housing density generally conclude that urban areas often contain a higher overall abundance of predators, including nest predators (Jokimäki and Huhta 2000; Sorace 2002; Richmond et al. 2011; Stracey and Robinson 2012). The abundance of mammalian and some avian nest predators has been found to increase with urbanization (Richmond et al. 2011; Stracey and Robinson 2012), as does the incidence of songbird nest predation by snakes, birds, and house cats (Stracey 2011; Reidy and Thompson 2012). However, rates of nest predation also appear to be species-specific (Wilcove 1985; Gering and Blair 1999; Thorington and Bowman 2003; Ryder et al. 2010; Stracey and Robinson 2012; Friesen et al. 2013). Various life history characteristics of the species in question, such as body size, response to predators, nest type, and nest placement, may also play a role in determining nest predation risk in urban habitats (Klug et al. 2010; Stracey and Robinson 2012). For example, Stracey and Robinson (2012) found that large-bodied birds that often mobbed nest predators were more common in urban habitats, while small-bodied birds that built open-cup nests were nearly absent. They suggest that this is because large, mobbing species are better able to protect their nests from urban predators and open-cup nests more vulnerable than enclosed ones. Thus our findings are somewhat surprising, as song sparrows are relatively small, build open-cup nests, and do not (at least based on our observations) generally engage in mobbing behavior.

The predator communities of urban habitats also typically differ in their taxonomic make-up from those of rural areas (Jokimäki and Huhta 2000; Ryder et al. 2010; Stracey 2011; Fischer et al. 2012). Variation in levels of snake predation in particular could explain the opposing relationships with within-season date seen in our study habitats. Snake activity correlates positively with songbird nest predation (Sperry et al. 2008, 2012; Weatherhead et al. 2010) and is often also positively correlated with temperature (Sperry et al. 2008; Weatherhead et al. 2010; Cox et al. 2013). Thus, the amount of nest predation by snakes at our study sites would be expected to increase across the breeding season as average temperatures increase, which could account for the decreasing nest success observed at rural sites. Relatively few snakes in urban habitats, meanwhile, could partially account for the opposing pattern of within-season nest success we see in urban areas. However, our nest damage data do not support the hypothesis that urban areas have fewer snakes. Snakes are unlikely to damage nests during predation attempts, while mammalian and avian predators often do. A larger percentage of our predated urban nests were left undamaged, suggesting if anything more snake predation in urban areas than rural ones.

Prey switching has been proposed as an explanation for the lower rates of nest predation sometimes observed in urban habitats (Stracey 2011). Thus, rather than a direct effect of differences in predator abundance and type, it may be that community structure of our urban habitats provides predators with a wider array of food sources, thereby reducing predation pressure on song sparrow nests. If some of this prey becomes more abundant later in the breeding season, this could also account for the decrease in predation across the season seen in our urban habitats. On the whole, it seems likely that multiple factors are working in conjunction in both habitats to create the opposing relationships of nest predation across the breeding season seen in our study.

We initially predicted that habitat differences in resource availability might impact nest success. However, we found no differences in nestling mass or number, or in nest placement between our habitats, suggesting that resources are sufficiently available in both habitats to enable successful breeding.

Our data suggest a possible relationship between male aggression and nestling mass, potentially mediated by impacts of aggression on paternal feeding rate, though both of these relationships were non-significant trends. Previous studies have found that there can be trade-offs between aggression and paternal care in males, apparently mediated by testosterone (Ketterson et al. 1992; McGlothlin et al. 2007; but see Van Duyse et al. 2002). This relationship seems to run counter to our finding of higher reproductive success in urban populations, where males are also more aggressive on average. However, we measured reproductive success in terms of whether or not nestlings fledged, and it is possible that aggressive males simply fledged lower-weight nestlings. Though we found no significant difference between habitats in average nestling mass, we were unable to collect data from all successful nests and our sample size is small. Further work, ideally with larger samples sizes, is needed to reveal whether aggressive male song sparrows do feed their offspring less often than their less-aggressive counterparts, and whether this reduction in feeding leads to lower nestling mass.

Urban areas are not necessarily detrimental to wildlife and may be able to support sustainable populations of native species. However, the specific habitat features that enable increased nest success in song sparrows are likely to be somewhat species-specific, and so other species may not experience the same benefits we see here. Differences between urban and rural areas in terms of resource availability, predator communities, and other features relevant to nest success are also relative; urban habitats are scattered across a wide array of ecosystems, and so the relative costs and benefits of urban versus rural living may vary among these areas. Nevertheless, our findings suggest that at least some species benefit from urban habitats and these species could become increasingly tied to this habitat type as their urban-dwelling populations out-perform rural ones. Additionally, the opposing patterns of within-season nest success we observed could drive urban and rural populations to shift the timing of their respective breeding seasons in opposite directions, potentially encouraging reproductive isolation of these groups over time. Overall, our study illustrates that urbanization has the potential to impact species' abundance, distribution, and even identities over the long run in ways that we are only beginning to understand.

ACKNOWLEDGMENTS

We would like to thank the Laboratory for Interdisciplinary Statistical Analysis (L.I.S.A.) at Virginia Tech for advice in analyzing our data. This work was supported by the National Science Foundation (IOS 0545735 to I.T.M. and 1311381 to I.T.M. and S.F.) and a graduate fellowship from the Institute for Critical Technology and Applied Science (ICTAS) at Virginia Tech to S.F.

References

2010 Census Interactive Population Search. n.d. In *2010 Census.* Retrieved Sept. 2012 from http://www.census.gov/2010census/popmap/.

Aldredge RA, LeClair SC, Bowman R. 2012. Declining egg viability explains higher hatching failure in a suburban population of the threatened Florida scrub-jay Aphelocoma coerulescens. *J Avian Biol* **43**:369-375.

Arcese P, Smith JNM, Hatch MI. 1996. Nest predation by cowbirds and its consequences for passerine demography. *Proc Natl Acad Sci* **93**:4608-4611.

Atwell JW, Cardoso 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.

Benson TJ, Brown JD, Bednarz JC. 2010. Identifying predators clarifies predictors of nest success in a temperate passerine. *J Anim Ecol* **79**:225-234.

Blair RB. 1999. Birds and butterflies along an urban gradient: surrogate taxa for assessing biodiversity? *Ecol Appl* **9**:164-170.

Bonier F, Martin PR, Wingfield JC. 2007. Urban birds have broader environmental tolerance. *Biol Lett* **3**:670-673.

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

Bornstein RD. 1968. Observations of the urban heat island effect in New York City. *J Appl Meteor* **7**:575-582.

Boutin S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. *Can J Zool* **68**:203-220.

Coss RG, Marks S, Ramakrishnan U. 2002. Early environment shapes the development of gaze aversion by wild bonnet macaques (Macaca radiata). *Primates* **43**:217-222.

Cox W, Thompson F, Reidy J. 2013. The effects of temperature on nest predation by mammals, birds, and snakes. *Auk* **130**:784-790.

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 J, Cleeton S, Timothy P. 2012. Urbanization and the predation paradox: the role of trophic dynamics in structuring vertebrate communities. *BioSci* **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.

Foltz SL, Davis JE, Battle KE, Greene VW, Laing BT, Rock RP, Ross AE, Tallant JA, Vega RC, Moore IT. 2015. Across time and space: effects of urbanization on corticosterone and body condition vary over multiple years in song sparrows. *J Exp Zool* **323A**:109-120.

Foltz SL, Ross AE, Laing BT, Rock RP, Battle KE. Get off my lawn: increased aggression in urban song sparrows is related to resource availability. Submitted to *Behav Ecol.*

Friesen LE, Casbourn G, Martin V, Mackay RJ. 2013. Nest predation in an anthropogenic landscape. *Wilson J Ornithol* **125**:562-569.

Gering JC, Blair RB. 1999. Predation on artificial bird nests along an urban gradient: predatory risk of relaxation in urban environments? *Ecography* **22**:532-541.

Heiss RS, Clark AB, McGowan KJ. 2009. Growth and nutritional state of American crow nestlings vary between urban and rural habitats. *Ecol Appl* **19**:829-839.

Hu Y, Cardoso GC. 2010. Which birds adjust the frequency of vocalizations in urban noise? *Anim Behav* **79**:863-867.

Jokimäki J, Suhonen J. 1993. Effects of urbanization on the breeding bird species richness in Finland: a biogeographical comparison. *Ornis Fenn* **70**:71-77.

Jokimäki J, Huhta E. 2000. Artificial nest predation and abundance of birds along an urban gradient. *Condor* **102**:838-847.

Ketterson ED, Nolan V, Wolf L, Ziegenfus C. 1992. Testosterone and avian life histories – effects of experiemtally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis)*. *Am Nat* **140**:980-999.

Klug PE, Jackrel SL, With KA. 2010. Linking snake habitat use to nest predation risk in grassland birds: the dangers of shrub cover. *Oecologia* **162**:803-813.

Li T, Shilling F, Thorne J, Li F, Schott H, Boynton R, Berry AM. 2010. Fragmentation of China's landscape by roads and urban areas. *Ecology* **25**:839-853.

Longcore T, Rich C. 2004. Ecological light pollution. *Front Ecol Environ* **2**:191-198.

Lowe EC, Wilder SM, Hochuli DF. 2014. Urbanization at multiple scales is associated with larger size and higher fecundity of an orb-weaving spider. *PloS One* **9**:e105480.

Luther DA, Derryberry EP. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. *Anim Behav* **83**:1059-1066.

McGlothlin JW, Jawor JM, Ketterson ED. 2007. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. *Am Nat* **170**:864-875.

McKinney ML. 2002. Urbanization, biodiversity, and conservation. *Biosci* **52**:883-890.

McKinney ML. 2008. Effects of urbanization on species richness; a review of plants and animals. *Urban Ecosyst* **11**:161-176.

Miller JR, Wiens JA, Hobbs NT, Theobald DM. 2003. Effects of human settlement on bird communities in lowland riparian areas of Colorado (USA). *Ecol Appl* **13**:1041-1059.

Møller AP. 2010. Interspecific variation in fear responses predicts urbanization in birds. *Behav Ecol* **21**:365-371.

Nager RG, van Noordwijk A. Proximate and ultimate aspects of phenotypic plasticity in timing of great tit breeding in a heterogeneous environment. *Am Nat* **146:**454-474.

Newman MM, Yeh PJ, Price TD. 2006. Reduced territorial responses in dark-eyed juncos following population establishment in a climatically mild environment. *Anim Behav* **71**:893-899.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol S* **37**:637-669.

Partecke J, Van't Hof TJ, Gwinner E. 2004. Underlying physiological control of reproduction in urban and forest-dwelling European blackbirds *Turdus merula. J Avian Biol* **36**:295-305.

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

Partecke J, Gwinner E. 2007. Increased sedentariness in European blackbirds following urbanization: a consequence of local adaptation? *Ecology* **88**:882-890.

Peralta G, Fenoglio MS, Salvo A. 2011. Physical barriers and corridors in urban habitats affect colonization and parasitism rates of a specialist leaf miner. *Ecol Entomol* **36**:673-679.

Reidy JL, Thompson FR III. 2012. Predator identity can explain nest predation patterns. *Stud Avian Biol - Ser* **43**:407-413.

Richmond S, Nol E, Burke D. 2011. Avian nest success, mammalian nest predator abundance, and invertebrate prey availability in a fragmented landscape. *Can J Zoolog* **89**:517-528.

Richner H. 1989. Habitat-specific growth and fitness in carrion crows (Corvus corone corone). J *Anim Ecol* **58**:427-440.

Rogers CM, Taitt MJ, Smith JNM, Jongejan G. Nest predation and cowbird parasitism create a demographic sink in wetland-breeding song sparrows. *Condor* **99**:622-633.

Ryder TB, Reitsma R, Evans B, Marra PP. 2010. Quantifying avian nest survival along an urbanization gradient using citizen- and scientist-generated data. *Ecol Appl* **20**:419-426.

Scales J, Hyman J, Hughes M. 2011. Behavioral syndromes break down in urban song sparrow populations. *Ethology* **117**:887-895.

Schoech SJ, Bowman R. 2001. Variation in the timing of breeding between suburban and wildlife Florida scrub-jays: do physiologic measures reflect different environments? In: Marzluff, JM, Bowman, R, Donnelly RE (eds). Avian Ecology and Conservation in an Urbanizing World. Kluwer Academic, Norwell, MA pp 291-308.

Shitikov DA, Dubkova EV, Makarova TV. 2013. The demography of Yellow Wagtails *Motacilla flava* on abandoned fields in northern European Russia. *Bird Study* **60**:518-526.

Shochat E, Lerman SB, Anderies JM, Warren PS, Faeth SH, Nilon CH. 2010. Invasion, competition, and biodiversity loss in urban ecosystems. *BioSci* **60**: 199–208.

Sibley DA. 2001. New World Sparrows. In The Sibley Guide to Bird Life and Behavior (C. Elphick, J.B. Dunning, Jr, and D.A. Sibley, Editors). Alfred A. Knopf, New York, USA.

Slabbekoorn H, Peet M. 2003. Birds sing at a higher pitch in urban noise. *Nature* **424**:267-267

Slabbekorn H, Ripmeester EAP. 2008. Birdsong and anthropogenic noise: implications and applications for conservation. *Mol Ecol* **17**:72-83.

Soderstrom B. 2001. Seasonal change in Red-backed Shrike *Lanius collurio* territory quality – the role of nest predation. *Ibis* **143**:561-571.

Sorace A. 2002. High density of bird and pest species in urban habitats and the role of predator abundance. *Ornis Fenn* **79**:60-71.

Sperry JH, Peak RG, Cimprich DA, Weatherhead PJ. 2008. Snake activiy affects seasonal variation in nest predation risk for birds. *J Avian Biol* **39**:379-383.

Sperry JH, Barron DG, Weatherhead PJ. 2012. Snake behavior and seasonal variation in nest survival of northern cardinals Cardinalis cardinalis. *J Avian Biol* **43**:496-502.

Stracey CM. 2011. Resolving the urban nest predator paradox: the role of alternative foods for nest predators. *Biol Conserv* **144**:1545-1552.

Stracey CM, Robinson SK. 2012. Does Nest Predation Shape Urban Bird Communities? In: Lepczyk CA, Warren PS (eds) Urban Bird Ecology and Conservation. Vol 45. University of California Press, Berkeley, pp 49-70.

Thorington KK, Bowman R. 2003. Predation rate on artificial nests increases with human housing density in suburban habitats. *Ecography* **26**:188-196.

Van Duyse E, Pinxten R, Eens M. 2002. Effects of testosterone on song, aggression, and nestling feeding behavior in male great tits, Parsus major. *Horm Behav* **41**:178-186.

Weatherhead PJ, Carfagno GLF, Sperry JH, Brawn JD, Robinson SK. 2010. Linking snake behavior to nest predation in a Midwestern bird community. *Ecol Appl* **20**:234-241.

Wilcove DS. 1985. Nest predation in forest tracts an dthe decline of migratory songbirds. *Ecology* **66**:1211-1214.

Wood WE, Yezerinac SM. 2006. Song sparrow (*Melospiza melodia*) song varies with urban noise. *Auk* **123**:650-659.

Yeh PJ. 2004. Rapid evolution of a sexually selected trait following population establishment in a novel habitat. *Evolution* **58**:166-174.

Yeh PJ, Price TD. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am Nat* **164**:531-542.

Zanette LY, White AF, Allen MC, Clinchy M. 2011. Perceived predation risk reduces the number of offspring songbirds produce per year. *Science* **334**:1398-1401.

Tables

Table 4.1: Habitat Features Used to Determine Urbanization of Sites

Field Site	Human Population Density (people/k^2)	Green Space (% of site area)	Years Monitored
Urban 1 (Virginia Tech Campus)	2563	48.4	2011 - 2013
Urban 2 (Radford Main Campus)	2897	51.6	2011 - 2013
Rural 1 (Claytor Lake State Park)	49	82.5	2011 - 2013
Rural 2 (Kentland Farm)	23	96.0	2011 - 2013

Table 4.2: Nest Success Compared Across Habitat Types

Variable	Estimate	Standard Error	t-Ratio	P-Value
Habitat Type	-21.36	8.18	-2.61	0.01
Date Nest Found	-0.10	0.06	-1.63	0.10
Habitat Type x Date Nest Found	0.16	0.06	2.55	0.01

Table 4.3: Variation Explained by First Principle Components of Aggression PCAs and Their Eigenvectors

Dataset	% Variation Explained	Songs	Flights	Time w/ 4m	Closest Approach
Aggression and Nestling Quality	45.5	0.41	0.27	0.59	-0.64
Aggression and Nest Success, Urban	49.0	0.50	0.37	0.52	-0.58
Aggression and Nest Success, Rural	60.1	0.49	0.50	0.50	-0.51
Aggression and Parental Care	44.7	0.45	0.24	0.60	-0.61

Positive eigenvector values indicate a positive relationship with the overall principle component; eigenvectors with negative values load in the opposite direction and have a negative relationship with the principle component. The eigenvectors above indicate that songs, flights, and time within 4m of the speaker all increase with increasing values of the first principle component (the aggression score), while the closest approach to the speaker decreases with aggression, indicating that aggressive birds approach closer.

Figure Legends

Figure 4.1: Nest Success Differs with Habitat Type and Date

19 of 36 total urban nests succeeded (53% success rate); 4 of 17 total rural nests succeeded (24% success rate). Each point represents a nest.

Figure 4.2: Provisioning Rates are Higher in Rural Habitats

Vertical bars show standard error of the mean.

Figure 4.3: Aggressive Males in Both Habitats Tend to Have Lower-Weight Nestlings

Each point represents an individual male (N=20).

Figures

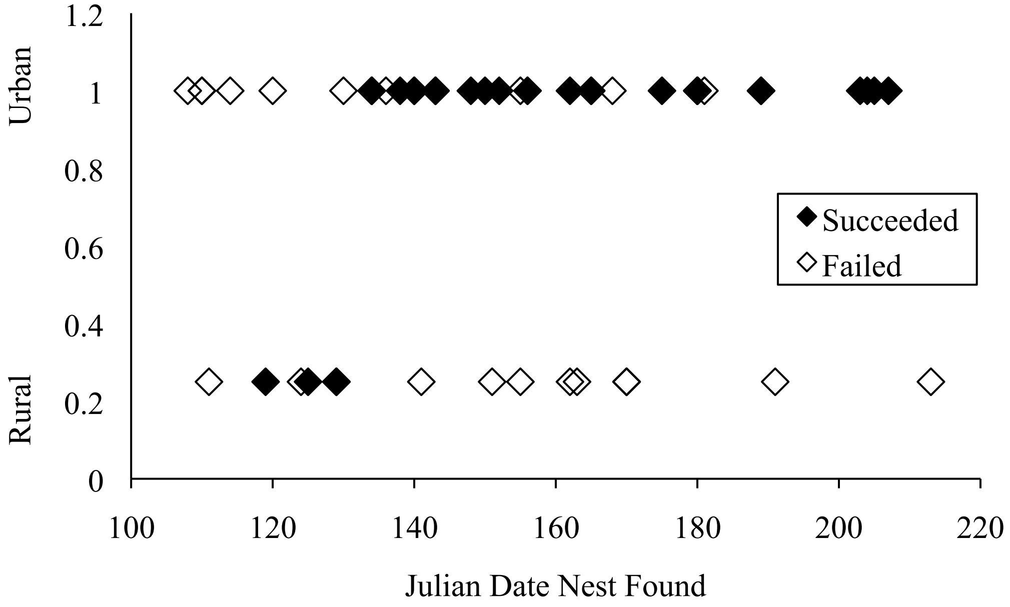

Figure 4.1: Nest Success Differs with Habitat Type and Date

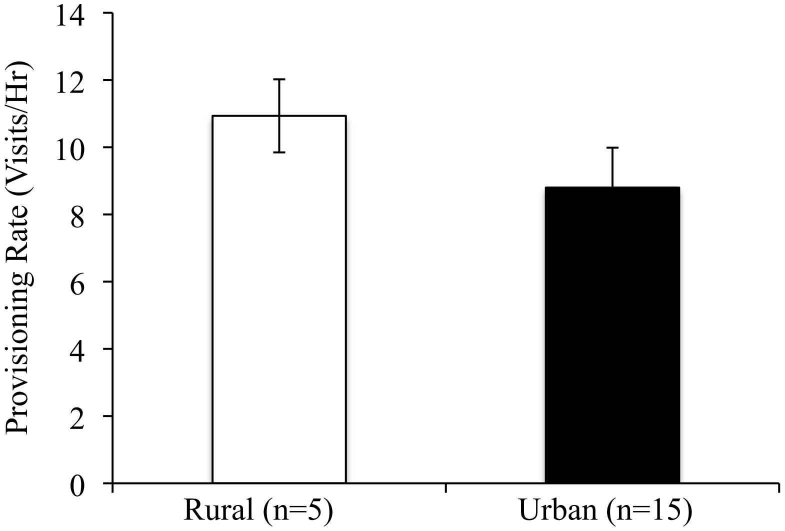

Figure 4.2: Provisioning Rates are Higher in Rural Habitats

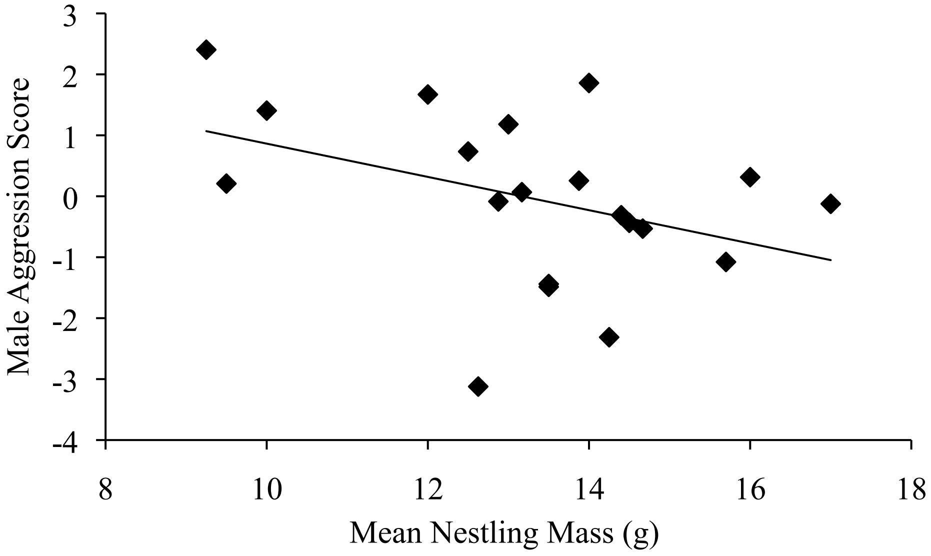

Figure 4.3: Aggressive Males in Both Habitats Tend to Have Lower-Weight Nestlings

CHAPTER V: CONCLUSIONS

Sarah L. Foltz

How do urban and rural song sparrows differ?

Over the course of my research it became clear that urban and rural song sparrows differ from each other in numerous ways, though these differences were not always consistent over time and their significance was not always clear. Urban birds exhibited consistently higher territorial aggression across three consecutive years of study. Corticosterone levels differed between the two groups in some years, though not all, and not in a consistent direction. Urban birds enjoyed greater reproductive success than their rural counterparts, though they provisioned their nestlings less often. Lastly, the reproductive success of song sparrows breeding in urban habitats increased over the course of the breeding season, while in rural habitats it decreased, although breeding appeared to be initiated in both habitats simultaneously. Thus, it is safe to say the urban habitats do in fact shape song sparrow physiology and behavior. A key question is whether these differences in physiology and behavior are shaped through selection, selective colonization, or phenotypic plasticity.

What habitat features influence these differences?

Urban habitats differ in innumerable ways from other habitats. Which specific features of urban habitats drive the differences in behavior and physiology I investigated? The food-supplementation experiment showed that food availability has a significant influence on territorial aggression, especially in rural populations. Rural birds on food-supplemented territories became as aggressive as urban birds. In other words, by increasing the quality of their territories by adding food, I was able to make rural birds defend their territories as aggressively as urban birds usually do. Territorial aggression levels of fed urban birds were not significantly higher than their unfed urban counterparts. It may be that urban birds are already maintaining the highest possible aggression levels and/or that additional food does not substantially increase their valuation of their territories. Urban areas likely contain more food sources for dietary generalists such as song sparrows in the form of bird feeders and even fruiting ornamental plants.

The availability of suitable nest vegetation was also correlated with territorial aggression, though urban and rural birds showed opposite relationships. Urban birds were most aggressive on territories with high amounts of suitable nest vegetation, while rural birds were most aggressive on territories with the least suitable nest vegetation. The greater abundance of overall green space in rural habitats may partially explain these opposing relationships, although I found no significant difference in the availability of suitable nest vegetation specifically between habitat types. Taken together, my findings suggest that quality of habitat is a driving feature behind territorial aggression and that song sparrows may perceive urban territories as higher quality and therefore worthy of stronger defense.

In addition to the drivers and correlates of urban/rural differences for which I have direct evidence, I suspect that urban-associated differences in predation risk contribute to the differences I observed. Given the appearance of failed nests observed during the reproduction study, predation is likely a major determinant of reproductive success in these populations. Further, it seems likely based on my observations and the findings of previous studies that risk of nest predation and predator community structure may vary substantially between habitats (Jokimäki and Huhta 2000; Ryder et al. 2010; Stracey 2011; Fischer et al. 2012). Within each habitat, differences in the relative availabilities of prey species may cause the same predatory species to focus on different prey (Miller et al. 2006; Randa et al. 2009; Stracey 2011). A combination of these differences likely give rise to the differences we observed in reproductive success both between habitats and over the course of the breeding season. Knowledge of the species and relative abundances of predators in these habitats will further our understanding of the reproductive success and potential sustainability of urban wildlife populations.

Are urban habitats necessarily deleterious to native wildlife?

Many animal species are not observed in urban areas, visit them only rarely, or exist there in lower densities than in their native habitats (Faeth et al. 2011; Scheffers and Paszkowski 2012). These differences in density suggest that urban areas are poor habitats for wildlife. However, urban habitats offer a number of potential benefits and opportunities to species or individuals that are able to cope with the unique challenges found in them. For example, the presence of bird feeders and discarded food provides many species, avian and otherwise, with more abundant and stable sources of food (Contesse et al. 2004).

During my first year of study, baseline corticosterone levels were higher in urban individuals than in rural ones. This could be interpreted as evidence for relatively lower individual condition, potentially due to poor habitat quality. However, the relationship between baseline corticosterone levels and fitness is not consistent across life history stages or individuals (Bonier et al. 2009), and thus I hesitate to draw this conclusion, especially in the absence of a strong relationship between cort and body condition, which was measured in these same individuals. The fact that this pattern did not reappear in subsequent years also suggests that corticosterone levels are not intrinsically higher in urban populations, a situation that could be interpreted as indicating that urban habitats attract lower-quality birds. Instead, the year-to-year fluctuations corticosterone levels that I saw suggest that corticosterone is responding to more proximate and variable environmental pressures such as weather, food availability, or predation.

My aggression studies showed that urban song sparrows had relatively high levels of territorial aggression and that this behavior was apparently related to habitat quality, specifically food and suitable nest vegetation availability. Coupled with my finding that the density of song sparrow territories was no lower in urban areas than in rural ones, these results suggest that song sparrows may perceive urban habitat as more desirable than rural habitat. The study of reproductive success supported this apparent perception. Urban birds had higher overall reproductive success, indicating that urban habitats are probably not sinks. This is in contrast to Aldredge et al. (2012), who found that scrub-jays (*Aphelocoma coerulescens)* had lower reproductive success in urban areas. Additionally, unlike both Richner (1989) and Yeh and Price (2004), I found no difference in mass or number of nestlings between urban and rural nests, even though parental feedings were less frequent at my urban sites. However, there was a trend toward lower average mass in nestlings of more aggressive males, and territorial aggression is on average higher among urban males. Further work is necessary to confirm this relationship, as my data set is small. However, such a finding would be in line with other studies showing that males with high levels of testosterone, a hormone associated with territorial aggression in song sparrows (Wingfield 1994), feed their offspring less often (Ketterson et al. 1992; McGlothlin et al. 2007; but see Van Duyse et al. 2002).

In addition to higher overall reproductive success, urban birds' reproductive success was highest later in the breeding season. In contrast, nests at rural sites were more likely to succeed earlier in the season. Currently, my populations appear to initiate breeding at approximately the same time of year. However, these opposing patterns of reproductive success could eventually drive urban birds to breed later in the year and rural birds to breed earlier. This could in turn lead to reproductive isolation of populations in neighboring urban and rural habitats. Such isolation would be deleterious to small populations. In larger ones, it could lead to the evolution of species or subspecies of urban specialists.

Overall, my data suggest that some native animal species can do well in urban habitats, but that adjustments to life in urban habitats are not entirely cost-free. Whether the benefits outweigh the costs of urban living likely varies from species to species, and possibly from year to year.

Phenotypic plasticity, selection, and pre-adaptation

My corticosterone and aggression data show that some of the differences observed between urban and rural song sparrows are at least partially due to phenotypic flexibility, in the sense that changes in these traits are reversible responses to environmental pressure (Wada and Sewall 2014). Corticosterone levels varied from year to year in birds from both habitats, and differences between habitats were evident in some years but not others. This result suggests both that levels are primarily responding to transient environmental pressures and that urban birds' corticosterone levels are not fixed, either genetically or during development, at a set point significantly above or below that of rural birds. Given that both baseline and stressed corticosterone levels were similar across habitats in several study years, it also does not appear that song sparrows in my urban populations exhibit an attenuated stress response. This result is in contrast to studies of urban dark-eyed juncos (*Junco hyemalis;* Atwell et al. 2012) and European blackbirds (*Turdus merula;* Partecke et al. 2006), both of which found attenuated stress responses in urban birds brought into a common-garden captive setting. It appears that while my song sparrows experience a difference in stressors between urban and rural populations in some years, alteration of the stress response is not necessary to colonize urban sites such as those I studied here. The Partecke et al. (2006) and Atwell et al. (2012) studies were both conducted in populations from much larger urban sites (Munich and San Diego, respectively); the higher degree of urbanization at and surrounding these sites may account for the difference in results. Species may also play a role here, as the urban/rural pattern of corticosterone response to urban habitats appears to be somewhat species-specific (Fokidis et al. 2009; Bonier et al. 2012).

I was able to increase levels of territorial aggression in rural song sparrow males to levels typically seen in urban males by providing supplemental food. Thus, territorial aggression is also a flexible phenotypic trait, at least in rural populations. However, urban birds had consistently higher aggression levels than rural birds when unfed and aggression within this group did not increase significantly with feeding. Therefore, it is possible that urban birds possess less phenotypic flexibility in aggression than their rural counterparts. It is also possible that birds from both habitats are similar in their phenotypic flexibility where aggression is concerned, but that urban birds oscillate around a higher set point than rural birds. Aggressive behavior is known to have genetic and developmental components (Coss et al. 2002; Marks et al. 2005; Eccard and Roedel 2011; Kukekova et al. 2011; McGhee et al. 2013) that could create such a set point. Differences in aggression flexibility or set point, if they exist, could have arisen via post-colonization selection on urban populations, from preferential colonization of urban areas by individuals with inherently less flexible or higher aggression, or from developmentally plastic responses to these different habitats. It is clear from my data that the phenotypic flexibility of certain traits play an important role in the differentiation of the behavior and physiology of urban and rural individuals, but other mechanistic influences on these traits cannot be ruled out.

Future Directions

The field of urban ecology remains relatively open, with much work remaining to be done. There are three main lines of inquiry that follow from the work presented here that I find particularly interesting, the first of which I touched on briefly but which is deserving of much more in-depth study:

1) Impacts of urban/rural behavior differences on fitness:

Many studies have compared the expression of various behaviors in urban and rural populations, but the potential costs of urban-induced behavioral alterations are less commonly investigated (Mockford and Marshall 2009; Slabbekoorn 2013; Read et al. 2014). While a shift in a given trait may be a response to urbanization, it is not necessarily an adaptive one.

Additionally, adjustments to a trait that address one problem created by urban living may lead to other issues. For example, upward shifts in the pitch of urban birds song have been well documented, and are thought to enable urban birds to hear each other over low-frequency traffic noise (Slabbekoorn and Peet 2003; Wood and Yezerinac 2006; Bermudez-Cuamatzin et al. 2009; Hu and Cardoso 2010; Luther and Derryberry 2012). However, recent studies suggest that these changes may decrease song recognition or preference between birds from areas with differing noise levels, potentially isolating urban populations (Mockford and Marshall 2009). In this study, I addressed reproductive success with respect to urban/rural differences in aggression and found no significant effect, but I have no data on the relationship between aggression and survival. Studies examining both reproductive and survival effects of traits that differ with urbanization to determine lifetime fitness are rare and necessary. Understanding the relative benefits and costs of shifting traits on individuals, populations, and species is important if we are to grasp the overall impact of urbanization on wildlife.

2) Relative impacts of different types of urbanization:

Urban centers contain multiple types of land use, including residential neighborhoods, business districts, industrial areas, and parks. These different land uses create different micro-habitats that vary in their supply of resources such as vegetation, food, and shelter. However, many studies on the influences of urban animals either do not differentiate between urban micro-habitats or consider only one urban habitat type. Studies intentionally comparing individuals across different urban land uses remain relatively rare (Wang 2009; Zhang et al. 2011). However, those studies that do compare individuals across different urban contexts have found differences in behavior and physiology (Bermudez-Cuamatzin et al. 2009; Wang 2009; Zhang et al. 2011). Depending on a species' needs, it may be relatively successful in some urban sub-habitats, but not in others. In extreme cases, zoning may produce islands of suitable habitat and populations of urban animals that are fairly isolated within them. Knowledge of the relative suitability of various urban sub-habitats could be used to inform city planning in growing urban areas.

3) Epigenetic mechanisms underlying trait urban/rural differentiation:

Urban habitats are not only full of novel challenges for incoming animals; they present a moving target. As technology advances, the challenges posed by urban habitats change, and often change rapidly. Animals that can respond quickly to changes in habitat stand a better chance of colonizing and persisting in urban areas. Epigenetic processes may be the key to responding quickly. The epigenome can be altered within an individual's lifetime (Francis et al. 1999; Szyf et al. 2005; Verhoeven 2010), enabling rapid response to shifting environmental pressures. Some facets of the epigenome are also heritable (Morgan et al. 1999; Johannes et al. 2009), such that selection might act on these rather than on the underlying genome. Because the underlying genome remains unchanged, alterations to traits made via epigenetic processes are potentially reversible, an ideal situation in an unstable environment. Investigating the mechanisms that animals use to rapidly adjust trait expression will inform our knowledge of how animals adapt to all unstable habitats.

In Conclusion

The research detailed here has added to the behavioral and physiological differences observed between urban and rural conspecifics and has revealed some of the ways in which these differences are created and maintained. It has also hinted at the potential costs of these responses to urban living. Lastly, it shows that urban habitats are not necessarily poor habitats for wildlife and that some species may be very successful in this novel habitat type. I hope that this work will serve as a foundation for future studies that will not only be useful in conservation efforts but also enhance our basic understanding of how animals colonize and adapt to novel habitats.

References

Aldredge RA, LeClair SC, Bowman R. 2012. Declining egg viability explains higher hatching failure in a suburban population of the threatened Florida scrub-jay Aphelocoma coerulescens. *J Avian Biol* **43**:369-375.

Atwell JW, Cardoso 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.

Bermudez-Cuamatzin E, Rios-Chelen AA, Gil D, Garcia CM. 2009. Strategies of song adaptation to urban noise in the house finch: syllable pitch plasticity or differential syllable use? *Behavior* **146**:1269-1286.

Bonier F, Martin PR, Moore IT, Wingfield JC. 2009. Do baseline glucocorticoids predict fitness? *Trends Ecol Evol* **24**:634-642.

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

Contesse P, Hegglin D, Gloor S, Bontadina F, Deplazes P. 2004. The diet of urban foxes (Vulpes vulpes*)* and the availability of anthropogenic food in the city of Zurich, Switzerland. *Mamm Biol* **69**:81-95.

Coss RG, Marks S, Ramakrishnan, U. 2002. Early environmental shapes the development of gaze aversion by wild bonnet macaques (Macaca radiata). *Primates* **43**:217-222.

Eccard JA, Roedel HG. 2011. Optimizing temperament through litter size in short-lived, iteroparous mammals in seasonal environments. *Dev Psychobiol* **53**:585-591.

Faeth, S.H., Bang, C., Saari, S., Ostfeld, R.S., Schlesinger, W.H. 2011. Urban biodiversity: patterns and mechanisms. *Ann NY Acad Sci* **1223**:69-81.

Fischer J, Cleeton S, Timothy P. 2012. Urbanization and the predation paradox: the role of trophic dynamics in structuring vertebrate communities. *BioSci* **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.

Francis D.D., J. Diorio, D. Liu, M.J. Meaney. 1999. Nongenomic transmission across generations in maternal behavior and stress responses in the rat. Science 286:1155-1158.Lynn SE, Breuner CW, Wingfield JC. 2003. Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. *Horm Behav* **43**:150-157.

Hu Y, Cardoso GC. 2010. Which birds adjust the frequency of vocalizations in urban noise? *Anim Behav* **79**:863-867.

Johannes F., E. Porcher, F.K. Teixeira, V. Saliba-Colombani, M. Simon, N. Agier, A. Bulski, J. Albuisson, F. Heredia, P. Audigier, D. Bouchez, C. Dillmann, P. Guerche, F. Hospital, V. Colot. 2009. Assessing the Impact of Transgenerational Epigenetic Variation on Complex Traits. *PLoS Genet* **5**:e1000530.

Jokimäki J, Huhta E. 2000. Artificial nest predation and abundance of birds along an urban gradient. *Condor* **102**:838-847.

Ketterson ED, Nolan V, Wolf L, Ziegenfus C. 1992. Testosterone and avian life histories – effects of experiemtally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (Junco hyemalis). *Am Nat* **140**:980-999.

Kukekova AV, Johnson JL, Teiling C, Li L, Oskina IN, Kharlamova AV, Gulevish RG, Padte R, Dubreuil MM, Vladimirova AV, Shepeleva DV, Shikhevich SG, Sun Q, Ponnala L, Temnykh SV, Trut LN, Acland GM. 2011. Sequence comparison of prefrontal cortical brain transcriptome from a tame and an aggressive silver fox (Vulpes vulpes). *BMC Genomics* **12**:482.

Luther DA, Derryberry EP. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. *Anim Behav* **83**:1059-1066.

Marks C, West TN, Bagatto B, Moore FBG. 2005. Developmental environment alters conditional aggression in zebrafish. *Copeia* **4**:901-908.

McGhee KE, Travis J. 2013. Heritable variation underlies behavioural types in the mating context in male bluefin killifish. *Anim Behav* **86**:513-518.

McGlothlin JW, Jawor JM, Ketterson ED. 2007. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. *Am Nat* **170**:864-875.

Miller DA, Grand JB, Fondell TE, Anthony M. 2006. Predator functional response and prey survival: direct and indirect interactions affecting a marked prey population. *J Anim Ecol* **75**:101-110.

Mockford EJ, Marshall RC. 2009. Effects of urban noise on song and response behavior in great tits. *Proc Roy Soc B-Biol Sci* **276**:2979-2985.

Morgan H.D., H.G.E. Sutherland, D.I.K Martin, E. Whitelaw. 1999. Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* **23**:314-318.

Partecke J, Gwinner E, Bensch S. 2006b. Is urbanization of European blackbirds (*Turdus merula*) associated with genetic differentiation? *J Ornithol* **147**:549-552.

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

Randa LA, Cooper DM, Meserve PL, Yunger JA. Prey switching of sympatric canids in response to variable prey abundance. *J Mammal* **90**:294-603.

Read J, Jones G, Radford AN. 2014. Fitness costs as well as benefits are important when considering responses to anthropogenic noise. *Behav Ecol* **25**:4-7.

Richner H. 1989. Habitat-specific growth and fitness in carrion crows (Corvus corone corone). *J Anim Ecol* **58**:427-440.

Ryder TB, Reitsma R, Evans B, Marra PP. 2010. Quantifying avian nest survival along an urbanization gradient using citizen- and scientist-generated data. *Ecol Appl* **20**:419-426.

Scheffers, B.R. and Paszkowski, C.A. 2012. The effects of urbanization of North American amphibian species: identifying new directions for urban conservation. *Urban Ecosyst* **15**:133-147.

Slabbekoorn H, Peet M. 2003. Birds sing at a higher pitch in urban noise. *Nature* **424**:267-267

Slabbekoorn H. 2013. Songs of the city: noise-dependent specral plasticity in the acoustic phenotype of urban birds. *Anim Behav* **85**:1089-1099.

Stracey CM. 2011. Resolving the urban nest predator paradox: the role of alternative foods for nest predators. *Biol Conserv* **144**:1545-1552.

Szyf M., I.C.G. Weaver, F.A. Champagne, J. Diorio, M.J. Meaney. 2005. Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front Neuroendocrin* **26**:139-162.

Van Duyse E, Pinxten R, Eens M. 2002. Effects of testosterone on song, aggression, and nestling feeding behavior in male great tits, Parsus major. *Horm Behav* **41**:178-186.

Verhoeven K.J.F., J.J. Jansen, P.J. van Dijk, A. Biere. 2010. Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol* **185**:1108-1118.

Wada H, Sewall KB. 2014. Itroduction to the symposium – Uniting evolutionary and physiological approaches to understanding phenotypic plasticity. *Integr Comp Biol* **54**:774-782.

Wang, Y., Chen, S., Blair, R.B., Jiang, P., Ding, P. 2009. Nest composition adjustments by Chinese bulbuls Pycnonotus sinensis in an urbanized landscape of Hangzhou (E. China). *Acta Ornithol* **44**:185-192.

Wingfield JC. 1994. Regulation of territorial behavior in the sedentary song sparrow, Melospiza melodia morphna. *Horm Behav* **28**:1-15.

Wood WE, Yezerinac SM. 2006. Song sparrow (Melospiza melodia) song varies with urban noise. *Auk* **123**:650-659.

Yeh PJ, Price TD. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am Nat* **164**:531-542.

Zhang, S., Fumin, L., Liu, S., Li, D., Chen, C., Wang, P. 2011. Variation in baseline corticosterone levels of tree sparrow (Passer montanus) populations along an urban gradient in Beijing, China. *J Ornithol* **152**:801-806.