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ORIGINAL ARTICLE

Variation in baseline corticosterone levels of Tree Sparrow (*Passer montanus*) populations along an urban gradient in Beijing, China

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Abstract Rapid urbanization is a major anthropogenic pressure on bird species that rely on vegetation for food and shelter. Since the baseline corticosterone concentration (BCC) in some bird species has been found to increase slightly in response to environmental challenges, we hypothesized that urbanization could also induce an increase in BCC. To test this hypothesis, we compared the BCC of Tree Sparrow (*Passer montanus*) populations in five urban and two rural habitats and analyzed the relationship between BCC and the degree of urbanization. Here, we show that the BCCs of Tree Sparrow populations were strongly and positively correlated with the degree of urbanization of the habitat. Average BCC of Tree Sparrows from high-rise residential areas and a university campus were significantly higher than those from rural areas, suggesting that Tree Sparrows living in highly urbanized areas have greater environmental challenges than those in rural areas. However, the average BCC of birds from an urban park was not significantly different to that of birds at rural sites and also differed significantly from that of birds at two of the other urban sites. These results suggest that urbanization could pose environmental challenges for Tree Sparrows, a species that appears relatively well-adapted to human-modified environments. The marked variation in BCC between different urban sites indicates that conclusions drawn from data collected at single sites must be interpreted with caution.

Keywords Urbanization · Tree Sparrow · Baseline corticosterone · Variation

Zusammenfassung Eine rasante Urbanisierung kann einen massiven anthropogenen Druck auf Vogelarten ausüben, die auf Vegetation für Nahrung und Schutz angewiesen sind. Da in einigen Arten ein erhöhter Corticosteronspiegel als Reaktion auf selbst geringe Umweltveränderungen gefunden wurde, stellen wir die Hypothese auf, dass Urbanisation zu einer Erhöhung des Hormonspiegels führen kann. Um dies zu testen, haben wir Feldsperlingspopulationen (*Passer montanus*) fünf urbaner und zwei ländlicher Lebensräume verglichen und das Verhältnis zwischen dem basalen Corticosteronespiegel (BCC) und dem Grad der Verstädterung analysiert. Wir zeigen hier, dass der BCC in Feldsperlingen positiv mit dem Grad der Verstädterung des jeweiligen Lebensraumes assoziiert war. Durchschnittliche BCC von Feldsperlingen aus Hochhauswohngebieten und von einem Universitätscampus waren signifikant höher als die von Feldsperlingen aus ländlichen Gebieten. Dies deutet darauf hin, dass Feldsperlinge in stark urbanisierten Gebieten stärkeren Umwelteinflüssen ausgesetzt sind als solche aus ländlicheren Gegenden. Der BCC von Vögeln aus einem städtischen Park war signifikant höher als der BBC von Vögeln vom Lande; er war jedoch signifikant geringer als der von Vögeln von zwei anderen städtischen Umgebungen. Diese Ergebnisse legen nahe, dass Urbanisierung ökologische Herausforderungen darstellt, und das selbst in einer Art, die anscheinend relativ gut an anthropogen veränderte

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Umgebungen angepasst ist. Die auffällige Variation in BCC zwischen verschiedenen städtischen Lebensräumen zeigt, dass Schlussfolgerungen aus Daten einzelner Standorte nur mit Vorsicht interpretiert werden sollten.

Introduction

Urbanization is arguably the most damaging, persistent and rapid form of anthropogenic pressure on animals (Morneau et al. 1999; Park and Lee 2000; Marzluff 2001; Porter et al. 2001; Clergeau et al. 2006; Shochat et al. 2006). The loss of vegetation from urban environments in particular threatens the survival of bird species that rely on vegetation for food and shelter. Although several studies of urban bird species, such as the House Sparrow (*Passer domesticus*) and European Blackbird (*Turdus merula*), have indicated that urbanization affects breeding performance (Partecke et al. 2004; Vincent 2005), body condition (Liker et al. 2008), behavior (Slabbekoorn and Peet 2003; Bautista et al. 2004; Chamberlain et al. 2007) and population dynamics (Baker et al. 2005; Shaw et al. 2008), there is relatively little information on the influence of urbanization on the physiological condition of bird populations (but see Ruiz et al. 2002; Bonier et al. 2007; Partecke et al. 2006; Fokidis et al. 2009).

Since there is evidence to suggest that endocrine mechanisms can provide unique insights into how individuals functionally respond to habitat degradation (Marra and Holberton 1998; Ricklefs and Wikelski 2002; Homan et al. 2003; Romero 2004; Brown et al. 2005), understanding the mechanisms through which urbanization affects the endocrine system of birds may provide useful insights for conserving birds in urban environments.

Corticosterone is a hormone that is suspected to facilitate foraging activity, food intake (Astheimer et al. 1992; Landys et al. 2006; Angelier et al. 2007) and the mobilization of stored energy resources (Gray et al. 1990; Sapolsky et al. 2000). Baseline corticosterone concentration (BCC) has been found to increase slightly in some bird species in response to environmental challenges such as decreased food availability (Marra and Holberton 1998; Kitaysky et al. 1999; Schoech et al. 2004; Angelier et al. 2007), fasting (Lynn et al. 2003) and increased brood value (Bokony et al. 2009). Baseline corticosterone levels could, therefore, provide some information on the ability of individuals to cope with environmental challenges (Kitaysky et al. 1999).

Previous research on the European Blackbird (Partecke et al. 2006) found no difference in BCC between captive European Blackbird from urban and rural habitats. Fokidis et al. (2009) also found BCC were generally similar in urban and rural birds according to the research on five free-living passerine species in Phoenix. Bonier et al. (2007), however, demonstrated that free-living male White-crowned Sparrows (*Zonotrichia leucophrys*) in urban habitat had higher baseline corticosterone levels than those in rural habitat from the central coast of California to northern Washington.

Many Chinese cities have undergone very rapid urbanization over the past 20 years. The widespread construction of new, high-rise buildings has greatly reduced the amount of urban vegetation, which may have significantly reduced the amount of habitat available to urban birds such as the Tree Sparrow (*Passer montanus*) (Ruan and Zheng 1991; Zhang et al. 2008). In this paper, we investigate whether there is any evidence that the degree of urbanization has affected average baseline corticosterone levels in the Tree Sparrow.

Materials and methods

Sampling design

Seven sites within the Beijing Municipality were selected as capture sites. These were Yuyuantan park (an urban park), the Minzu university campus (a university campus in a highly urbanized part of Beijing), the Ganjiakou residential area (a low-rise residential area), the Xizhimen residential area (a bungalow-style residential area), the Shijicheng residential area (a high-rise residential area) and two rural sites (Zhangxizhuang and Wangzuo). The minimum distance between sites was over 3 km. The geographic coordinates of the capture sites are shown in Table 1.

We used the "urbanization score" (Liker et al. 2008) to quantify the degree of urbanization of the study sites and their surroundings (see Liker et al. 2008 for details). The mean building density score, the number of cells with high building density, the number of cells with roads, the mean vegetation density score, the number of cells with high vegetation density and the overall urbanization score for each site are listed in Table 1.

A total of 298 adult Tree Sparrows were captured with mist-nets at these seven sites in 2008 and 2009 (Table 2). Birds were captured during the chick-rearing period of the breeding season from 20 to 30 May and 29 June to 11 July, and in winter from 27 December to 2 February. During the breeding season, birds were captured between 6:00 a.m. and 10:00 a.m. but in winter captures took place between 7:00 a.m. and 11:00 a.m. Within 1–3 min of being caught in the net, between 50–100 μl of whole blood was collected from the brachial vein of each bird into heparized microcapillary tubes. Blood samples were stored at 4°C for up to 8 h until centrifuged at 10,000 rpm for 10 min. The blood plasma was then recovered and kept frozen in microcentrifuge tubes until assayed for corticosterone. The blood cells

Table 1 Habitat characteristics of Tree Sparrow (*Passer montanus*) capture sites in Beijing Municipality, China

Capture site	Geographic coordinates	Mean building density score	Number of cells with high (>50%) building density	Mean vegetation density score	Number of cells with high (>50%) vegetation density	Number of cells with roads	Urbanization score (PC1)
Shijicheng residential area (S)	39°57′28″N 116°16′33″E	1.81	81	0.67	10	41	1.506
Xizhimen residential area (X)	39°56′17″N 116°21′57″E	1.81	81	1.19	19	20	0.632
Ganjiakou residential area (G)	39°55′06″N 116°20′32″E	1.67	67	1.10	17	21	0.583
Minzu university campus (M)	39°56′54″N 116°19′02″E	1.42	53	1.17	30	18	0.142
Yuyuantan park (Y)	39°54′47″N 116°18′33″E	0.78	29	1.34	47	10	−0.722
Wangzuo (W)	39°48′01″N 116°7′02″E	0.95	38	1.54	59	14	−0.793
Zhangxizhuang (Z)	40°9′06″N 116°33′07″E	0.20	4	1.49	57	9	−1.348

Table 2 Sample sizes of male and female Tree Sparrows caught at each site during breeding and winter seasons in 2008 and 2009

Capture site	2008				2009				Total
	Breeding		Winter		Breeding		Winter		
	Female	Male	Female	Male	Female	Male	Female	Male	
S	6	5	5	5	5	7	5	5	43
X	5	6	5	5	7	5	5	5	43
G	5	5	6	5	5	5	5	6	42
M	5	6	5	5	6	5	5	5	42
Y	5	5	5	5	5	6	5	5	41
W	7	5	5	5	5	5	5	6	43
Z	5	6	7	5	5	5	6	5	44

X Xizhimen, *G* Ganjiakou, *M* Minzu University, *S* Shijicheng, *Y* Yuyuantan, *Z* Zhangxizhuang, *W* Wangzuo

were used to identify the sex of the Tree Sparrows using the molecular methods described by Griffiths et al. (1996).

Corticosterone assay

Tree Sparrow plasma corticosterone levels were measured using an enzyme immunoassay kit from Assay Designs (cat # 901-097). We based our method on that used by Wada et al. (2007), which see for details. We optimized the plasma dilution ratio and the concentration of steroid displacement buffer (SDB) following the optimization protocol used by Wada et al. (2007). Briefly, a plasma dilution of 1:40 with 2% SDB added minimized the interference of plasma compounds in the assay. Each plasma sample was aliquotted into three wells. The inter- and intra-plate coefficients of variation were 10.3 and 7.02%, respectively.

Data analysis

Average BCC of the different Tree Sparrow populations were compared using the univariate Generalized Linear Model (GLM) in SPSS 11.0. The final model was developed by setting BCC as the dependent variable and including the following independent variables as factors: capture site, sex, capture year and season. Season was included as a two-level factor (breeding and winter). Initial

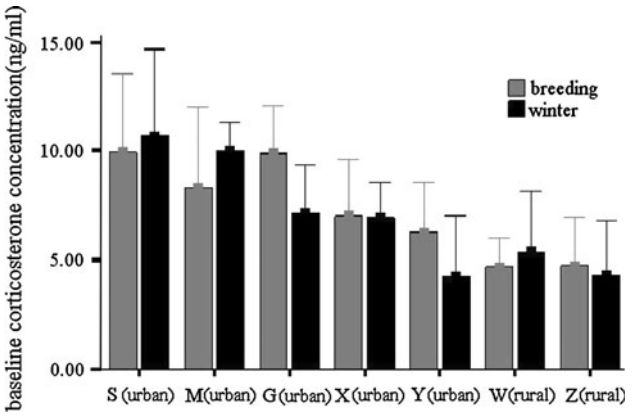

Fig. 1 The plasma baseline corticosterone concentration in Tree Sparrows (*Passer montanus*) at each capture site (mean ± SD)

models included all two-way interactions between capture site and other factors. Non-significant factors and interactions were removed from the models in a backward manner, removing the one with the largest P value in each step. The final model is the model with maximum R^2 value. We used Tukey's Honestly Significant Difference (HSD) post hoc test to conduct pairwise comparisons between capture sites and Pearson Correlation to assess the relationship between the degree of urbanization and average BCC.

Results

According to the final GLM model (adjusted $R^2 = 0.386$, $df = 284$), BCC differed significantly between capture sites ($df = 6$, $F = 9.276$, $P < 0.001$) and there was a significant interaction between capture site and season ($df = 6$, $F = 2.394$, $P = 0.032$). BCC was not significantly affected by sex or year of capture and there was no significant interaction between capture site and these factors.

The average breeding BCC of birds in urban sites was generally higher than those of birds in the two rural populations (Fig. 1). Pairwise comparisons between capture sites showed that the average BCC of birds at three of the urban sites, Ganjiakou, Shijicheng and Minzu University, were significantly higher than those measured at the two rural sites. However, the average BCC of birds at the other two urban sites, Yuyuantan and Xizhimen, was not significantly different from those of birds at the rural sites. There were no significant differences in BCC among the five urban sites or between the two rural sites (Table 3).

The average winter BCC at all urban sites except Yuyuantan was higher than at the rural sites. Pairwise comparisons between capture sites showed that the average winter BCC of birds at Minzu University and Shijicheng differed significantly from those of birds at the rural sites.

Table 3 Comparison of baseline corticosterone concentration in Tree Sparrows from different capture sites

	Urban sites					Rural sites	
	X	G	M	S	Y	Z	W
X	↓winter	0.250	0.865	0.161	0.998	0.482	0.391
G	1.000		0.803	1.000	0.162	0.003**	0.001**
M	0.076	0.151		0.699	0.660	0.024*	0.011*
S	0.021	0.049	0.998		0.108	0.001**	0.000**
Y	0.232	0.198	0.000**	0.000**		0.915	0.998
Z	0.164	0.140	0.000**	0.000**	1.000		1.000
W	0.770	0.700	0.002**	0.001**	0.971	0.966	↑summer

X Xizhimen, *G* Ganjiakou, *M* Minzu University, *S* Shijicheng, *Y* Yuyuantan, *Z* Zhangxizhuang, *W* Wangzuo

* $P < 0.05$, ** $P < 0.01$

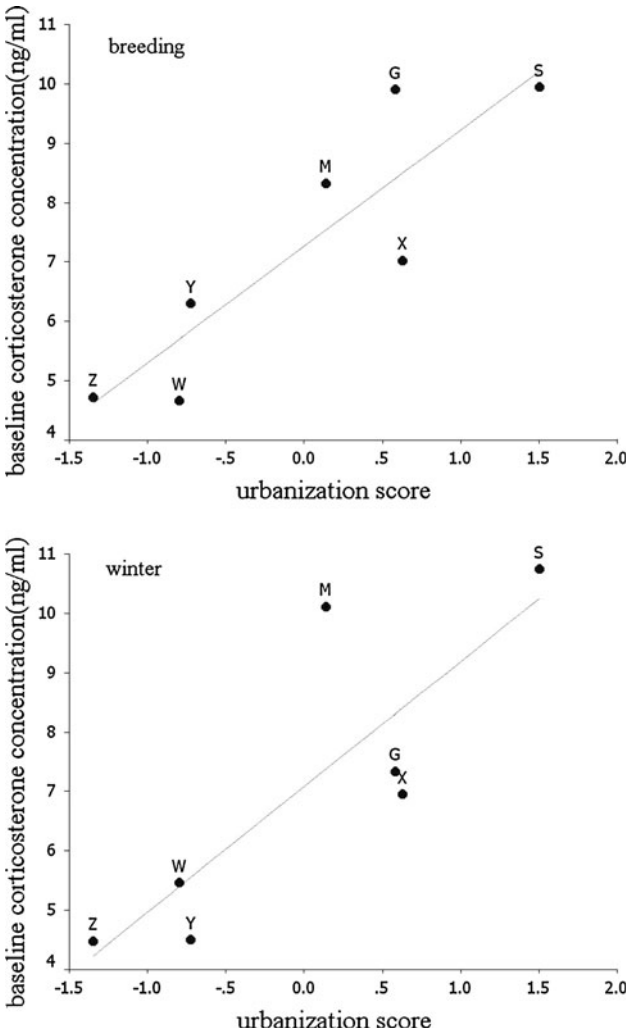

Fig. 2 Relationship between mean plasma baseline corticosterone concentration of Tree Sparrow populations from different capture sites and capture site urbanization scores

However, the average winter BCC of birds from Yuyuantan park,Ganjiakou and Xizhimen was not significantly different from that of birds at the rural sites. The average winter BCC of birds at Yuyuantan differed significantly from that of birds at two of the other urban sites, but there was no significant difference in winter BCC between birds at the two rural sites (Table 3).

The average BCC of birds at Minzu University campus and Ganjiakou differed significantly between breeding and winter, but no significant seasonal variation in BCC was found between other sites. Average BCC at Minzu University was significantly higher in winter than breeding (t test, $t = -1.850$, $P = 0.032$), but at Ganjiakou the reverse was observed (t test, $t = 2.728$, $P = 0.042$).

The linear relationship between the urbanization scores of the capture sites and average BCC corroborated the above results. Urbanization scores were strongly and positively correlated with average BCC (breeding : $r = 0.857$, $n = 7$, $P = 0.014$; winter: $r = 0.821$, $n = 7$, $P = 0.023$) (Fig. 2).

Discussion

The significant positive correlation between average BCC and urbanization score supports the hypothesis that urbanization can induce an increase in BCC in birds. However, that the BCC of Tree Sparrows in some urban habitat types, such as urban park land, were no different to those of birds at the rural sites.

Although some authors (Marra and Holberton 1998; Romero and Wikelski 2001; Brown et al. 2005) regard high baseline corticosterone levels as indicative of low fitness, some recent papers (Bonier et al. 2009; Bokony et al. 2009) have questioned this conclusion. Since we have no independent measure of fitness, such as reproductive success, the biological significance of the positive correlation between average BCC and urbanization score is unclear. More research is required to demonstrate a relationship between high BCC and fitness and to fully understand the effects of urbanization on avian physiology.

A variety of factors, such as reduced foraging habitat, greater exposure to environmental pollutants, and human disturbance, could potentially be responsible for the higher BCC of urban compared to rural Tree Sparrows. Although we cannot be sure which of these factors, if any, is responsible for the relatively high BCC found in urban Tree Sparrows, differences in BCC between birds at different urban sites suggest that this could be due to differences in the amount of vegetation cover between sites. The fact that the highest BCC was measured in a high-rise residential area with the lowest vegetation score, and the lowest in an urban park, suggests that lack of access to vegetation may be a challenge for urban Tree Sparrows. The mechanism whereby reduced vegetation cover might affect Tree Sparrow populations is unclear, but Vincent (2005) found that the biomass of House Sparrow broods increased with the extent of the vegetated area around nest sites. This suggests that raising nestlings may be more difficult in heavily urbanized habitats.

Although one study of baseline corticosterone level in rural and urban populations of the White-crowned Sparrow (Bonier et al. 2007) provided similar results to ours, the other two studies on captive European Blackbirds (Partecke et al. 2006) and five passerine birds (Fokidis et al. 2009) found no significant difference in BCC between urban and rural populations. There are three possible explanations for this discrepancy. The first is that captivity itself induces stress thereby obscuring any differences in BCC that may have existed between Blackbirds from urban and rural sites. The second is that different bird species differ in their sensitivity to urbanization. The third is that the nature of urbanization, and therefore its effect, may differ from site to site.

In summary, our results indicate that BCC can vary markedly between different urban sites and that data collected from single sites must be interpreted with caution. They also add support to the growing evidence (Bonier et al. 2007) that urbanization can pose environmental challenges to bird species that are commonly associated with, and presumably well adapted to, human modified environments.

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