Phenotypic signatures of urbanization are scale-dependent: A multi-trait study on a classic urban exploiter

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ABSTRACT

Understanding at which spatial scales anthropogenic selection pressures operate most strongly is a prerequisite for efficient conservation and management of urban biodiversity. Heterogeneity in findings on the strength and direction of urbanization effects may result from a lack of consensus on which spatial scales are most adequate when studying biotic effects of urbanization. Therefore, here, using the house sparrow (*Passer domesticus*) as model, we test the hypothesis that more than one spatial scale will explain variation among phenotypic stress markers. By applying a unique hierarchical sampling design enabling us to differentiate between local and regional effects of urbanization, we here show that the strength and direction of relationships with the percentage of built-up area – a simple structural measure of urbanization - vary among phenotypic stress markers and across the spatial range over which urbanization is measured. While inverse relationships with scaled body mass and bill height of adult house sparrows (*Passer domesticus*) were strongest when the degree of urbanization was quantified at city-level, similar relationships with corticosterone concentrations in feathers were only detected at the scale of individual home ranges. In contrast, tarsus length, wing length, and two measures of feather development were not significantly related to urbanization at any spatial scale. As the suite of phenotypic stress markers applied in this study revealed signatures of urbanization over a broad spatial range, we conclude that measures aimed at mitigating impacts of urbanization on free-ranging populations should best be implemented at multiple spatial scales too.

1. Introduction

Human activities affect the earth's land cover, climate, and biodiversity at ever accelerating rates (Steffen et al., 2015), and urbanization represents one of the most prominent forms of terrestrial land use change (Aronson et al., 2014; Huang, Li, Liu, & Seto, 2019; McKinney, 2006). Biotic impacts of urbanization vary according to the age, size, population density, geographical context and socioeconomic context of cities, along with other factors (Seto, Sánchez-Rodríguez, & Fragkias, 2010). Yet, overall, urban environments are characterized by small and fragmented natural habitat remnants, a high diversity and abundance of non-native species, a low diversity and number of native species, and a loss of phylogenetic diversity within communities (Johnson & Munshi-

South, 2017; La Sorte et al., 2018). In spite of the accumulating empirical evidence for urbanization effects on species diversity and community structure (Alberti, Marzluff, & Hunt, 2017; Batáry, Kurucz, Suarez-Rubio, & Chamberlain, 2018; Marzluff, 2017; Merckx, Souffreau, et al., 2018; Piano et al., 2017), how individuals successfully cope with urban environments remains the topic of considerable debate in urban ecology (Perrier et al., 2018; Sepp, McGraw, Kaasik, & Giraudeau, 2018; Shochat, 2004).

Local variation in environmental conditions is believed to drive trait divergence, and the strong ecological differences between rural and urban habitats can hence be expected to promote intraspecific variation in life-history characteristics across urbanization gradients (Evans, 2010). Many studies indeed report such differences, for example in

body size and mass (Lowe, Wilder, & Hochuli, 2014), vigilance behavior (Sarno, Parsons, & Ferris, 2015) or reproductive phenology (Boggie & Mannan, 2014). Yet, the strength and direction of urbanization impacts may not be universal, as studies conducted on the same species often yield population-specific relationships. For example, while multiple studies report that great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) lay smaller clutches in urban areas (Bailly et al., 2016; Glądalski et al., 2015; Seress et al., 2018), a pan-European assessment revealed that the number of eggs laid was not systematically affected by urbanization (Vaugoyeau et al., 2016). Similarly, while Grégoire (2003) showed marked differences in morphology between rural and urban blackbirds (*Turdus merula*) in eastern France, a large-scale study on eleven paired urban and rural blackbird populations across Europe did not yield consistent patterns in morphological variation (Evans, Sharp, McGowan, & Hatchwell, 2009). Interestingly, a large-scale analysis involving 132 North-American bird species across > 400 landscapes that span the entire range of human influence found that while life-history traits can explain which species likely are affected by urbanization, traits were not closely related to the direction of the relationship between abundance and human influence (Lepczyck et al., 2008). Recently, however, Weeks et al. (2020) found that increasing temperatures associated with climate change are correlated with a consistent reduction in body mass and an increase in wing length among North American migratory birds species.

Heterogeneity in findings may result, at least partly, from a lack of consensus on which spatial scales are most adequate when studying biotic effects of urbanization (Pautasso, 2007) – apart from the fact that biologically relevant scales should always correspond to the spatial ecology of the species of interest (Li & Wu, 2004). Studies can differ in spatial scales over which urbanization is measured as they apply for instance different home-range estimators, or because of differences in the spatial resolution at which geographical data are collected. Yet, even under comparable spatial scales, differences may arise because of complex interactions among processes acting on local and regional scales such as between the intensity of urban heat island effects and city size (Zhou, Rybski, & Kropp, 2017). Likewise, while the availability of roosting or nesting sites may drive urbanization effects at individual home ranges, local presence of competitors, predators or pathogens, or the level of connectivity with other conspecific populations are likely shaped by urbanization effects operating at larger spatial scales. Finally, phenotypic markers of body and feather morphology, haematology, and endocrinology that are commonly applied to measure how birds respond to urbanization likely signal selection pressures operating at multiple spatial scales too, such as is the case for predation pressure, food quality and availability, and micro-climate (Beyer et al., 2010; Kittle, Fryxell, Desy, & Hamr, 2008).

The importance of selecting appropriate spatial scales when studying ecological processes is well known (McGarigal, Wan, Zeller, Timm, & Cushman, 2016). Studies therefore often characterize environmental gradients across multiple spatial scales (e.g. Fearer, Prisley, Stauffer, & Keyser, 2007; Mahon et al., 2016), yet, when considering urbanization impacts on biodiversity, to date few studies have explicitly considered more than a single scale (e.g. Concepción, Moretti, Altermatt, Nobis, & Obrist, 2015; Merckx, Kaiser, et al., 2018; Merckx, Souffreau, et al., 2018). Indeed, recently, Moll et al. (2019) reviewed the urban ecology literature and found that only about 22% of studies included metrics on multiple spatial scales. To bridge this knowledge gap, we here aim to assess whether, and to what extent, the strength and direction of relationships between urbanization and phenotypic trait variation in a well-known urban exploiter species vary if urbanization is quantified over small (local, home-range level) versus large (regional, city-level level) spatial scales. For multiple reasons, house sparrows (*Passer domesticus*) offer an ideal animal model to address scale dependency of urbanization impacts on species characteristics and life-history traits. House sparrows are common and highly sedentary birds in our study area (northern Belgium) where they occupy habitats

along urbanization gradients ranging from rural farmsteads to highly built-up city centres (De Coster, De Laet, Vangestel, Adriaensen, & Lens, 2015; De Laet & Summers-Smith, 2007). Resulting from their sedentary behaviour, limited dispersal distances and small home ranges, urban and rural house sparrow populations represent separate genetic units (Vangestel et al., 2012) which potentially facilitates local adaptation to urbanization. While it was earlier thought that this archetypical urban exploiter would further benefit from ongoing urbanization (Summers-Smith, 1963), widespread and often severe urban population declines have been reported over the last decades (De Laet & Summers-Smith, 2007; Inger et al., 2015). Following these unexpected declines, a growing number of studies have assessed nutritional, physiological, behavioural and demographic signatures of urbanization on house sparrows and related species (Evans, Ryder, Reitsma, Hurlbert, & Marra, 2015; Jones, Rodewald, & Shustack, 2010; MacGregor-Fors, Quesada, Lee, & Yeh, 2019; Reale & Blair, 2005), results of which are, at least partly, contradictory.

Several correlative and experimental studies reported morphological differences between urban and rural sparrows, whereby the former tend to be smaller, lighter and leaner than the latter (Supplementary Data Table S1). In contrast, physiological markers including but not limited to immune function, haematocrit levels, blood and feather corticosterone, feather growth, fault bars and fluctuating asymmetry often fail to correlate with urbanization metrics in consistent ways (Supplementary Data Table S1). Several hypotheses have been proposed to explain this observed variation in sparrow phenotypes across urbanization gradients. Low-quality urban food is hypothesised to constrain the development of sparrow nestlings (Meillère et al., 2017), hence leading to smaller phenotypes in more urbanized areas. Alternative hypotheses refer to shifts in biotic interactions due to variation in population densities across urban gradients, or to environmental factors such as temperature (urban heat islands), air pollution, human disturbance or electromagnetic radiation (Balmori & Hallberg, 2007; Herrera-Dueñas, Pineda-Pampliega, Antonio-García, & Aguirre, 2017; Shochat, Warren, Faeth, McIntyre, & Hope, 2006). Shochat's credit-or-debit hypothesis, in contrast, posits that the high and constant food availability that comes with urbanization reduces starvation risks and allows adults to maintain leaner body masses (Shochat, 2004) which, in turn, may facilitate their escape from abundant urban predators (Lima, 1986). In all, the pertinent literature lacks consensus on the observed variation in sparrow phenotypes across urbanization gradients.

We here adopt a combined multi-scale multi-marker approach by relating variation in a suite of morphological and physiological markers to a common proxy of urbanization quantified over a range of spatial scales around house sparrow populations. This combined multi-scale and multi-marker approach enabled us to quantify the strength, direction and spatial range over which proxies of nutritional and energetic stress provide signatures of urbanization effects in a typical urban exploiter. Specifically, we test the hypothesis that more than one spatial scale will explain variation among phenotypic stress markers and hence, that disagreements between studies may arise because of heterogeneity in the choice of spatial scale(s) over which urbanization is quantified and of phenotypic traits to measure stress effects. Unravelling the relative role of local, home-range level versus regional, city-wide impacts of urbanization not only offers a better understanding of how species respond to anthropogenic activity (and as such to potential stressors), but is also pivotal to guide targeted and efficient conservation actions for those species struggling to survive in the world's ever expanding urban spaces.

2. Methodology

2.1. Analytic framework

The smallest spatial scale employed here corresponded to minimum

home-range estimates of sparrows ('home-range scale', Heij & Moeliker, 1990), while the largest corresponded to twice the distance whereby the effect of genetic drift exceeds that of gene flow in sparrows ('city-level scale', Vangestel et al., 2012), and hence, where populations can be considered independent. The diversity of the markers we used capture urbanization effects at different stages in the house sparrow's life cycle, including the developmental (i.e. nestling) and moulting periods, which are considered most energetically-demanding, alongside reproduction. Two whole body based markers were used: (i) body size (measured as tarsus length, bill height and wing length) to quantify environmental conditions experienced during development (energy limitations as nestling lead to smaller individuals (Wilder, Raubenheimer, & Simpson, 2016), although body size reductions can be adaptive too (Gardner, Peters, Kearney, Joseph, & Heinsohn, 2011)); and (ii) body condition (i.e. size-corrected body mass) which provides a snapshot of strategic mass regulation at the time of capture and quantifies the capacity of the individual to sustain short-term energetic requirements (Barnett, Suzuki, Sakaluk, & Thompson, 2015). In addition, three feather-based measurements which integrate environmental and nutritional conditions experienced over the period of feather growth were used: (i) Fluctuating Asymmetry (FA), which refers to small random deviations from perfect symmetry and reflects an individual's ability to cope with developmental stress (Lens, Van Dongen, Kark, & Matthysen, 2002); (ii) Growth Bar Width (GBW) refers to daily feather increments and reflects the level of nutritional stress experienced during feather growth (Grubb & Cimprich, 1990; Carl Vangestel & Lens, 2011); and (iii) Feather Corticosterone ($CORT_f$) which provides a retrospective view on the hypothalamic–pituitaryadrenal (HPA) axis (re) activity. This axis is activated when encountering (environmental) stressors but also when the body is at rest, hereby responding to various signals (e.g. circadian, neurosensory, blood-borne, and limbic) (Dickerson & Kemeny, 2004) that lead to the release of corticosterone as the dominant glucocorticoid in the plasma, which is incorporated in the feather during feather synthesis (Romero & Fairhurst, 2016).

3. Study area and sampling procedure

House sparrows were sampled in northern Belgium within a polygon of 4655 km² demarcated by the cities of Gent, Antwerp, Brussels and Leuven. Full details on the nested study design are described in Teyssier et al. (2018). In brief, 18 plots (each measuring 3 by 3 km) within the polygon boundary with a known population of house sparrows was identified, each varying in percentage of built-up area (% BU) as estimated from an object-oriented reference map with precise contours of all buildings, excluding roads and parking infrastructures as a vectorial layer (GRB, https://www.agiv.be/international/en/products/grb-en). All 18 plots were a subset of a larger set of 27 plots designed to quantify urbanization effects on a wider array of taxa (Merckx, Souffreau, et al., 2018). Given that % BU comprised buildings only, areas characterized by 10–15% BU can be considered as highly urbanized. Selected cut-off points were < 5% BU for six rural landscapes (lowest level of urbanization), 5–10% BU for six suburban landscapes (intermediate level of urbanization), and > 10% BU for six urban landscapes (highest level of urbanization). To ensure a seminatural environment in rural landscapes, only rural plots comprising > 20% of ecologically valuable areas were selected (Merckx, Souffreau, et al., 2018). By evenly spreading the 18 plots across the polygon (i.e. similar nearest-neighbor distances), clumping of plots within same urbanization classes was avoided. Within each selected plot, all available house sparrow survey data (i.e. field visits conducted during winter 2012–2013 complemented with data from various citizen science projects (such as e.g. the National House Sparrow Day and the Big Bird Weekend, De Coster et al., 2015)) were tracked to delineate two subplots (each measuring 200 by 200 m) that contained house sparrow populations and were contrasted by the lowest (< 5%) and highest (> 10%) BU values, respectively. Such a hierarchical sampling design, i.e. with two contrasting subplots nested within each of 18 plots based on identical cut-off values of urbanization, resulted in an optimal spread of % BU values at both the local and city-level scale. To test scale-dependency of urbanization effects on phenotypic trait variation (see further), % BU values were recalculated over seven spatial scales (50, 100, 200, 400, 800, 1600, and 3200 m radii) centered at each capture location, based on the same vectorial GRB data layer.

A total of 315 house sparrows were trapped between September 2013 and March 2014. Upon capture, (i) each bird was individually ringed, sexed, and weighed to the nearest 0.01 g using an analytical balance; (ii) its tarsus length, wing length, and bill height were measured to the nearest 0.01 mm using a digital caliper; and (iii) its second outermost right and left tail feathers were plucked and stored in individually-labeled envelopes at room temperature for subsequent analysis of FA, GBW and $CORT_f$. After measurement and sampling, all individuals were released at their original capture site. Both bird ringers in this study (NSH, AT) were holders of a scientific ringing certificate issued annually by the Agency for Nature and Forest. Any trappings on private lands were granted by the respective land owner. All sampling protocols used were approved by the Ethical Committee VIB Ghent site (EC2013-027).

4. Analysis of scaled mass index (SMI)

Body condition was calculated according to the scaled mass index (SMI), which adjusts the mass of all individuals to that which they would have obtained if they had the same body size, using the equation of the linear regression of log-body mass on log-tarsus length estimated by type-2 (standardized major axis; SMA) regression (Peig & Green, 2009). After excluding 3 outliers (i.e., |standardized residual| > 3), the regression slope was 1.51, whereas average tarsus length was 18.74 mm. The scaled mass index was calculated as body mass $\times$ (18.74/tarsus length)$^{1.51}$. A similar scaling was applied to bill height (bill height $\times$ (18.74/tarsus length)$^{0.88}$) and wing length (wing length $\times$ (18.74/tarsus length)$^{0.64}$).

5. Analysis of FA and GBW

A total of 612 retrices (i.e. 306 pairs of left–right homologues) were analyzed for FA by a single person (AT). First, each collected feather was pinned on a separate, white card and the total feather length was measured to the nearest 0.01 mm with a digital caliper. After measuring all feathers, this entire procedure was repeated and feather FA was analyzed through mixed-regression analysis with restricted maximum likelihood (REML) parameter estimation (Van Dongen, Molenberghs, & Matthysen, 1999). In this model, the fixed intercept estimates overall trait size, the fixed slope estimates directional asymmetry (DA), and the random intercepts and slopes estimate the variation in individual trait value and individual FA, respectively. Variance due to measurement error (ME), estimated from the repeated measurements of each left and right homologue feather, was homogeneously distributed between populations (likelihood-ratio tests: all P > 0.05), hence a single error component was estimated. Variance in signed FA ($\delta^2 FA = 0.4066$) was more than tenfold larger than variance in ME ($\delta^2 ME = 0.03085$) and was highly significant (likelihood-ratio test: P < 0.0001). FA measurements were not biased by DA ($F_{1,55} = 0.16$; P = 0.695; denominator degrees of freedom computed using Satterthwaite's formula following Verbeke and Molenberghs (1997). For hypothesis testing, unbiased FA values per individual were calculated as the variance components of the slopes of the individual regression lines in the mixed regression model.

The same set of retrices were used for GBW analysis. After measuring total feather length (see earlier), each feather was marked at a distance of 7/10 from its proximal end, and the proximate and distal ends of five consecutive growth bars were marked with an ultrafine mounting pin on a white board. Next, each marked board was scanned

(Océ OP1130) and growth bar widths (GBW) were automatically measured with image analysis software (KS400 Zeiss). The accuracy and repeatability of this method was earlier shown to be very high (Salleh Hudin et al., 2016).

6. Analysis of cort$_f$

A total of 306 s outermost left tail feathers were used for CORT$_f$ analysis using a validated ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) quantification method (Hudin et al., 2018). In brief, a single feather, weighing on average 10 mg, was sampled and dirt (e.g. faeces, mud, etc.) was manually removed by using tweezers. Meticulous cleaning of the feathers eliminated the possibility of dried blood from blood quills being analysed (possibly biasing the analysis), and no blood was visible on any feather sample. Next, each feather was flattened on a polystyrene board along a metal ruler, where needed by pinning it to keep its position, in order to measure its total length (cm). Subsequently, the weight of the feather ($\pm$ 0.00001 g) was determined on an analytical balance XPE205 (Mettler-Toledo, Zaventem, Belgium). Using scissors, the feather was cut perpendicular to the rachis and just above the superior umbilicus to remove the calamus. Again, length and weight of the feather were determined. To obtain a homogenized sample, the feather was cut into fine pieces (< 2 mm) using scissors. Between samples, scissors were rinsed with methanol, followed by ultrapure distilled water and dried with a paper tissue to avoid cross-contamination between samples. The amount of homogenized sample used for analysis was standardized at 10 mg. First, the sample was extracted with 8000 µL of methanol (HiPerSolv Chromanorm, VWR International BVBA, Leuven, Belgium) and 10 µL of a corticosterone-d$_8$ solution of 0.5 µg L^{-1} was added as internal standard. Only products with a certificate of analysis were used. Corticosterone and 11-deoxycorticosterone were obtained from Sigma-Aldrich (Diegem, Belgium) and corticosterone-d$_8$ from CDN Isotopes (Pointe-Claire, Canada). When other amounts of sample were used, the results were corrected accordingly. Next, the sample was vortex-mixed for 30 sec to homogenize and put on an overhead shaker for 60 min at 90 rpm. Subsequently, the sample was centrifuged for 10 min at 3452 g (=4000 rpm on a swing-out) at 7 °C and the supernatant was transferred to a new 12 mL tube. The sample was evaporated to dryness under nitrogen at 60 °C using a Turbovap nitrogen evaporator (Biotage, Sweden) and resuspended in 5000 µL H$_2$O/MeOH (80:20, v/v). Ultra-purification was performed using Grace Pure SPE C$_{18}$-Max (500 mg, 6 mL) columns for solid-phase extraction (SPE) (Grace Davison Discovery Sciences, Lokeren, Belgium). After conditioning a C$_{18}$ SPE column with 3 mL of MeOH followed by 3 mL of H$_2$O, the sample was loaded. The column was washed with 4.5 mL H$_2$O/MeOH (65:35; v/v) and targetted compounds were eluted with 2.5 mL H$_2$O/MeOH (20:80; v/v) into a 12 mL test tube and evaporated to dryness under a stream of nitrogen at 60 °C. The sample was reconstituted in 50 µL H$_2$O/MeOH (80:20; v/v) in a vial with insert and analyzed on an Acquity UPLC-MS/MS Xevo TQS using an Acquity Ultra Performance LC BEH C$_{18}$ (1.7 µm; 2.1 mm $\times$ 100 mm) column (Waters, Milford, USA). Methanol absolute LC-MS as well as formic acid ULC-MS grade from Biosolve BV (Valkenswaard, The Netherlands) and ultrapure water of a Milli-Q from Millipore (Billerica, USA) were used as mobile phase solvents. Since in future research matrix-matched calibration curves are not feasible, calibration curves were made in H$_2$O/MeOH (80:20, v/v). Subsequently, the stock factor was 10,000 and results were corrected for this. Results were reported as the value (ng/mg or µg/kg) $\pm$ the expanded measurement uncertainty (U) (ng/mg or µg/kg) with a coverage factor (k) of 2 (95% confidence interval).

6.1. Statistical analysis

To test at which spatial scale(s) of urbanization phenotypic signatures were strongest for each biomarkers, a set of spatial Generalized Least Squares (GLS) linear models was constructed using the R package nlme (Pinheiro, Bates, DebRoy, Sarkar, & Core, 2018) for each of the markers employed. Each GLS set contained models with % BU calculated over a particular spatial scale as explanatory variable, together with sparrow sex and different combinations of marker-specific covariates (see further). In order to account for possible spatial autocorrelation due to different proximities of capture locations, GLS models were run with five different spatial correlation structures following Zuur et al. (2009, pp 182–188), applying an Akaike Information Criterion (AIC) approach to select the best-fitting full model (i.e. the correlation structure corresponding to the lowest recorded AIC value, see Supplementary Data Table S3). Models with body mass and SMI included time and day of capture as covariates, to account for the fact that mass and condition may change throughout day and year. Day of capture was also included when analyzing raw and scaled wing lengths, as feathers may wear through time. Finally, following Hudin et al. (2018), feather length was included in models assessing CORT$_f$. CORT$_f$ values were log-transformed to attain a normal distribution of model residuals. GLS models per phenotypic marker and per spatial scale considered were ranked according to their AIC values, alongside p-values illustrating which tested relationships were significant at the p < 0.05 level. R-square values of GLS models were obtained using the rsquared function of R package piecewiseSEM (Lefcheck, 2016). The percentage of variation explained by % BU calculated over each spatial scale was conservatively estimated as the difference between the r-square values of the full model and the value of the same model without the factor % BU. Linear and quadratic terms of % BU were included to test for possible non-linear relationships with urbanization. All models assumed a normal error distribution, and normality of residuals was confirmed by Shapiro-Wilk tests (all W > 0.90).

7. Results

Quantifying urbanization over incremental spatial scales resulted in progressively lower and more skewed values of percentage built-up area (e.g. 50 m: % BU 19.8%, skewness 0.49; 3200 m: % BU 8.87%, skewness 1.83; Fig. 1). Correlations between % BU calculated across the entire spatial range varied between r = −0.95 and r = 0.93 (mean and standard deviation: −0.38 ± 0.53; see inset in Fig. 1). Within individuals, tarsus length, wing length, bill height and SMI were moderately positively correlated whereas CORT$_f$, GBW and FA were not significantly correlated (Supplementary Data Table A2). Raw and scaled body masses, bill height, wing length and GBW were normally distributed (all Shapiro-Wilk W ≥ 0.96, Fig. 2), whereas raw CORT$_f$ and FA strongly deviated from normality (Shapiro-Wilk W = 0.85 and 0.57, respectively, Fig. 2).

The strength, direction and spatial range over which phenotypic proxies of nutritional and energetic stress were related to urbanization, varied within and among traits (Table 1, Supplementary Data Table S3). Body mass and SMI were inversely related to % BU, most strongly at large spatial scales. Individuals sampled in more built-up areas were leaner, also when correcting body mass for size. Bill height was also inversely related to % BU over all spatial scales except at the smallest one, yet this relationship was no longer statistically significant after allometric scaling. Counter-intuitively, CORT$_f$ was also inversely related to % BU, however, only at the smallest spatial scales that corresponded to individual home range sizes. Finally, tarsus length, wing length, FA and GBW were not significantly related to urbanization across the entire spatial range (Table 1, Supplementary Data Table S3).

8. Discussion

By focusing on a well-studied urban exploiter across a simple structural gradient, we here show that statistical inference of the strength and direction of urbanization impacts on natural populations
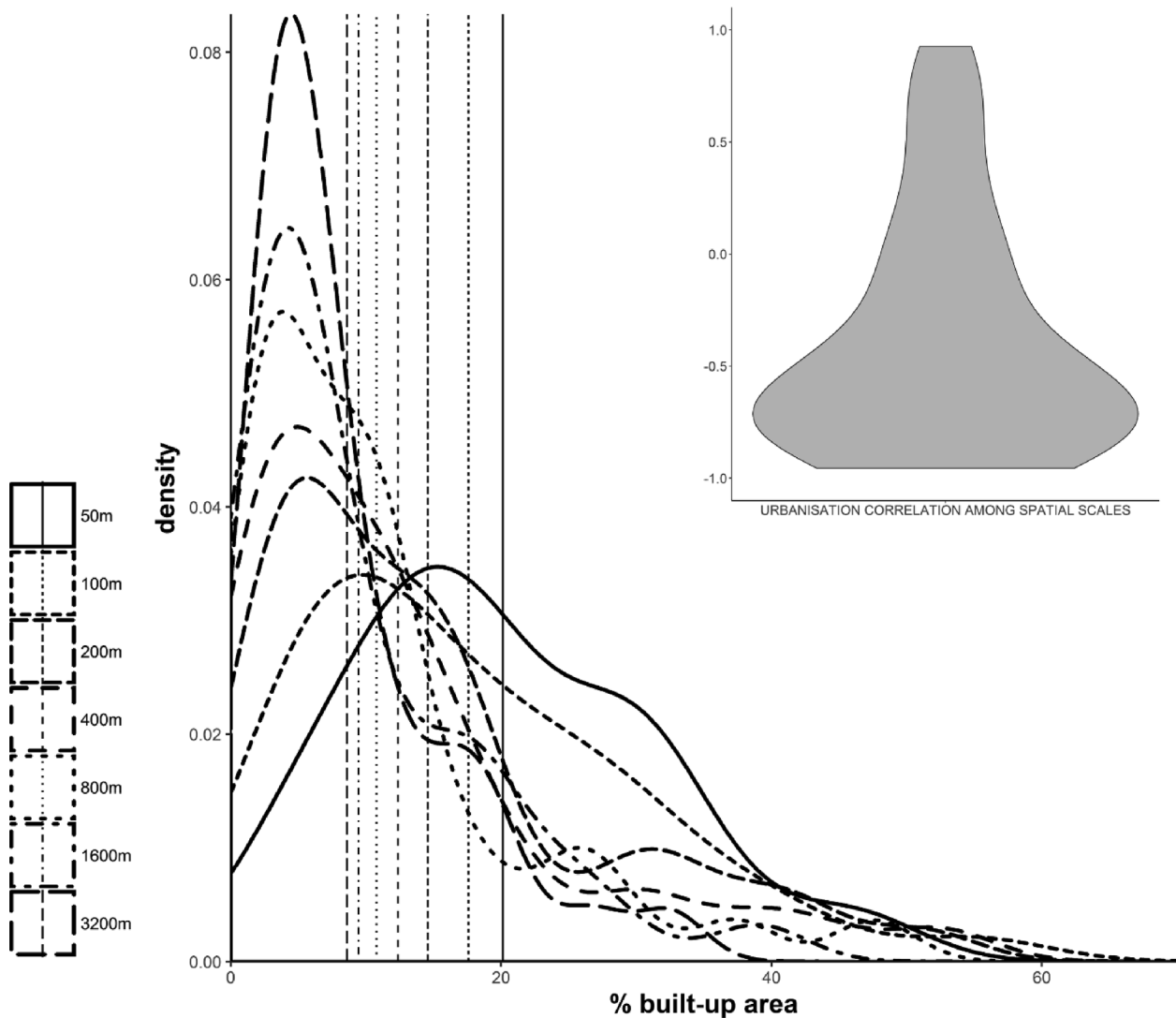
Fig 1. Density plot (i.e. a smoothed, continuous histogram) visualizing the distribution of % BU values across sparrow capture locations, when urbanization is calculated based on progressively larger radii (from 50 to 3200 m). Values along the y-axis represent probability densities (i.e. the probabilities per unit on the x-axis), whereby the total area under the curve integrates to one. Vertical lines indicate the mean % BU at each spatial scale. Inset (y-axis values represent r correlation coefficients) shows that there is substantial variation in the relationship between spatial scales and % BU.

needs to take into account the spatial range over which urbanization is quantified, and the suite of phenotypic traits applied to measure urbanisation impacts. Our results indicate that relationships with house sparrow body mass and condition become most apparent when integrating urbanization measures over much larger, city-level scales. Relationships with CORT$_f$, in contrast, were only present when urbanization was quantified at local scale, and further signalled lower chronic stress levels in more built-up territories. Future generic assessments of species-level responses to urbanization hence preferably incorporate multi-marker and multi-scale designs.

The house sparrow is a commonly used animal model to quantify anthropogenic effects on free-ranging bird populations (Supplementary Data Table S1). When it became apparent in the early 1980 s that urban populations of this traditional city dweller were in strong decline across large parts of Europe (De Laet & Summers-Smith, 2007; Summers-Smith, 2003), possible mechanisms underlying their ability (or failure) to cope with contemporary urbanization became the subject of intense empirical research. Results presented here suggest that the heterogeneous use of spatial scales and biomarkers among these studies may, at least partly, explain the lack of overall consensus in their findings. The fact that body mass and condition chiefly respond to urbanization

at larger spatial scales suggests (plastic) responses to underlying environmental drivers whose effects increase with urban sprawl or human population densities, such as those related to urban heat (Brommer, Hanski, Kekkonen, & Väisänen, 2015; Seress & Liker, 2015) or strategic mass regulation balancing starvation and predation risks (Cox & Cresswell, 2014; Shochat, 2004). Earlier studies indeed confirmed that larger cities capture and generate more heat (Zhou et al., 2017) and provide more anthropogenic food, either through direct provisioning at bird feeders or as communal waste (Jones & Reynolds, 2008; Reynolds, Galbraith, Smith, & Jones, 2017). In support of this rationale, Hudin et al. (2016) showed that house sparrows trapped in southern French cities were consistently leaner than individuals trapped in more rural settings, even after scaling their body mass to body size. Yet, when subsequently exposing these individuals to predictable food supplies under controlled aviary conditions, body masses of rural sparrows decreased while those of urban birds remained unchanged, to the extent that initial differences between both groups disappeared, as predicted under the credit-or-debit hypothesis. We hence argue that phenotypic markers such as body mass and condition primarily capture coarse-grained effects of urbanization, rather than processes operating at individual home-range scales.
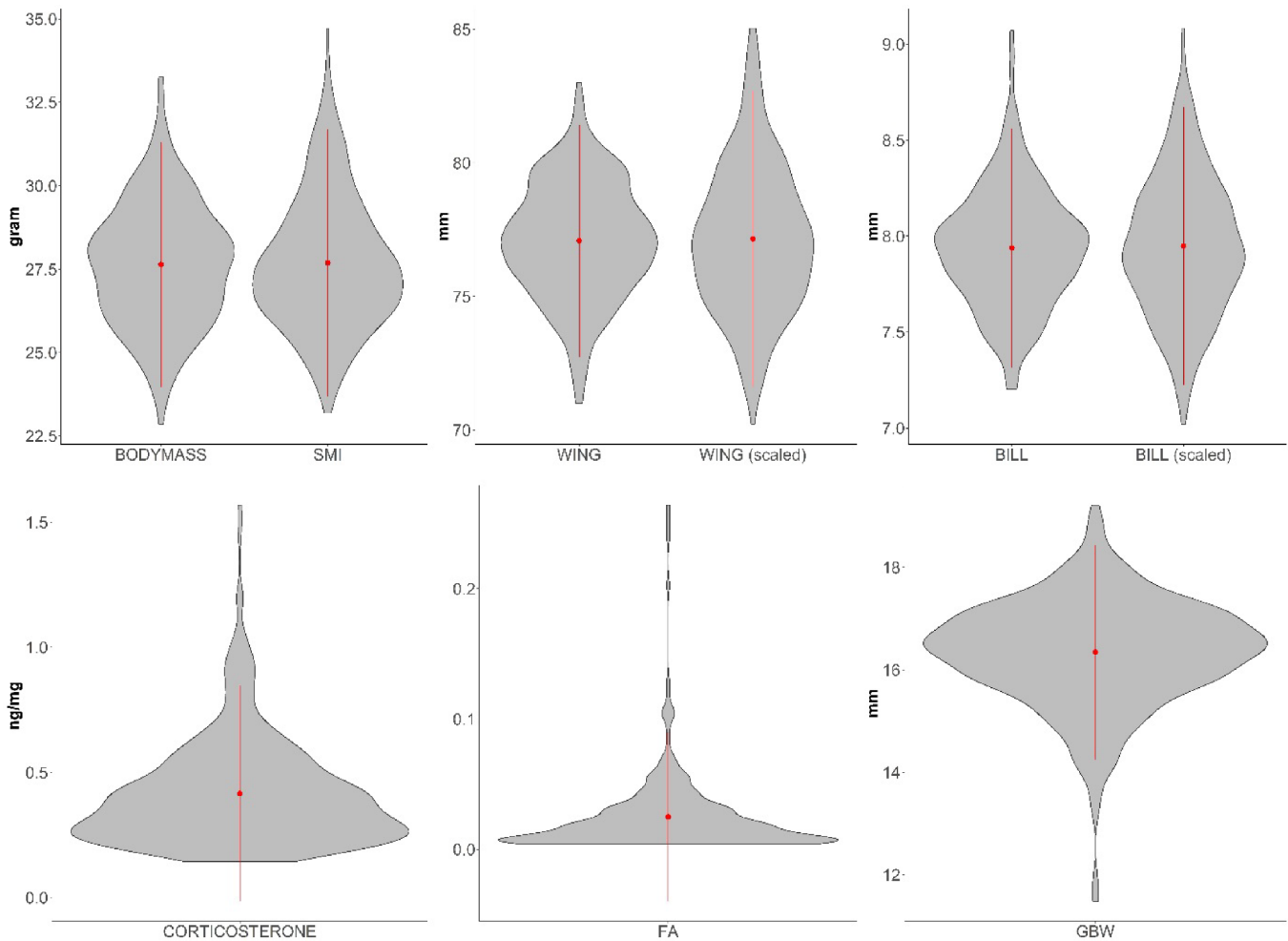
Fig. 2. Violin plots illustrate how phenotypic markers of environmental stress are distributed among all sparrows analysed. Violin plot outlines illustrate kernel probability density, i.e. the width of the area represents the proportion of the data located there.

Scale-dependency of relationships between urbanization and $CORT_f$, as revealed in our study, present an additional challenge for interpreting $CORT_f$, to infer stress effects in free-ranging populations. Studies testing how urbanization affects corticosterone in birds so far proved inconclusive, with studies reporting both positive correlations or no relationships at all (see Supplementary Table S1 for summary). Hereby should be noted that the pertinent literature is mainly based on data obtained using antibody based screenings assays, whereby results are potentially biased by differences in antibody, cross-reactivity with compounds with chemical-physical similar properties, etc., and not by quantification methods (Aerts, 2018). For example, Bonier (2012) showed that corticosterone levels in single species can be higher, lower, or similar between urban and rural populations, and may vary with sex, life-history, and year. Because of current housing regulations, urban sparrows may locally suffer from insufficient nesting opportunities in new or recently-renovated housing estates, hence resulting in fine-grained mosaics of population density (Moudrá, Zasadil, Moudrý, & Šálek, 2018). Inverse relationships between sparrow densities and human population densities as we found here were previously reported in our study area too (De Coster et al., 2015), and lower incidences of social conflicts under reduced house sparrow densities may underlie the observed relationship with $CORT_f$ in our study (Hawley, Lindström, & Wikelski, 2006). Moreover, by using flight initiation distance as a measure of house sparrow sensitivity to human disturbance, Vincze et al. (2016) provided evidence for stronger habituation in urban sparrows. As the latter were earlier shown to reduce CORT levels in the blood (Angelier, Meillère, Grace, Trouvé, & Brischoux, 2016; Partecke,

2014) this may provide an additional explanation for the inverse relationship between the extent of urbanization and $CORT_f$ concentrations in our study. Yet, more research is needed to exclude other alternative explanations, such as the possible role of atmospheric pollution (Meillère et al., 2016).

In accordance with a suite of other studies (Chávez-Zichinelli et al., 2010; Meillère et al., 2015, 2016; Salleh Hudin et al., 2018; Seress et al., 2012), feather-based proxies of developmental (FA) or nutritional (GBW) stress failed to correlate with urbanization measured across any spatial scale. This provides additional evidence that house sparrows populating highly-urbanized areas are not (severely) constrained by food quality of quantity, and therefore likely are not of inferior quality either. In support of this, Bókony, Kulcsár, and Liker (2010) showed that wild-caught urban house sparrows were not competitively inferior to rural ones when housed in mixed-flock aviaries, despite the lower body mass of the former. Together with our finding that $CORT_f$ levels tend to be lower in urban sparrows, and that such individuals show evidence of strategic mass regulation, results of this study fit into the emerging view that urban environments are not necessarily of inferior quality to more rural ones, at least not for urban exploiters (Meillère et al., 2017).

Understanding at which spatial scales anthropogenic selection pressure operates most strongly across urban areas is a prerequisite for efficient conservation and management of urban biodiversity (Pickett et al., 2017). For example, at community level, it has been shown that avian biodiversity tends to be negatively related to the degree of urbanization measured at small (local) scale, but positively when

Table 1
Summary of the main results of the GLS analyses relating phenotypic markers of stress to urbanisation at different spatial scales. Arrows indicate direction of relationship (↘: negative, ↗ positive), colours indicate significance levels (red: p-value < 0.05, orange: 0.05 < p-value < 0.10, black: p-value > 0.10). See Supplementary Data Table S3 for detailed statistical results.

body mass	sign	AIC	R-square (%)	Δ AIC	SMI	sign	AIC	R-square (%)	Δ AIC
50m	↘	1267.515	1.1	6.797	50m	↘	1331.504	1.1	4.828
100m	↘	1264.650	1.7	3.932	100m	↘	1329.900	1.0	3.224
200m	↘	1263.956	2.2	3.238	200m	↘	1327.722	1.5	1.046
400m	↘	1264.753	2.4	4.035	400m	↘	1332.243	1.3	5.567
800m	↘	1263.596	2.7	2.878	800m	↘	1332.554	1.5	5.878
1600m	↘	1261.242	3.8	0.524	1600m	↘	1331.337	1.7	4.661
3200m	**↘**	**1260.718**	**4.1**	**0.000**	**3200m**	**↘**	**1326.676**	**1.9**	**0.000**
wing length (raw)	sign	AIC	R-square (%)	Δ AIC	wing length (scaled)	sign	AIC	R-square (%)	Δ AIC
50m	↘	1296.307	0.1	1.843	50m	↘	1506.927	0.0	0.354
100m	↘	1296.712	0.5	2.248	100m	↘	1506.573	0.1	0.000
200m	↘	1294.464	0.5	0.000	200m	↘	1506.670	0.1	0.097
400m	↘	1296.603	0.3	2.139	400m	↘	1507.059	0.0	0.486
800m	↘	1297.492	0.1	3.028	800m	↘	1506.985	0.0	0.412
1600m	↘	1297.869	0.0	3.405	1600m	↗	1507.146	0.0	0.573
3200m	↗	1297.459	0.0	2.995	3200m	↗	1506.645	0.1	0.072
bill height (raw)	sign	AIC	R-square (%)	Δ AIC	bill height (scaled)	sign	AIC	R-square (%)	Δ AIC
50m	↘	174.600	1.2	2.207	50m	↘	277.194	0.9	0.007
100m	↘	174.051	1.3	1.657	100m	↘	277.984	0.6	0.797
200m	↘	172.742	1.8	0.349	200m	↘	277.187	0.8	0.000
400m	↘	172.749	1.7	0.356	400m	↘	277.836	0.7	0.649
800m	↘	173.578	1.4	1.185	800m	↘	277.810	0.7	0.623
1600m	↘	173.779	1.9	1.385	1600m	↘	277.623	0.6	0.436
3200m	**↘**	**172.394**	**1.8**	**0.000**	3200m	↘	277.192	0.7	0.005
GBW	sign	AIC	R-square (%)	Δ AIC	FA	sign	AIC	R-square (%)	Δ AIC
50m	↗	938.859	0.3	0.454	50m	↘	-1207.097	0.4	1.525
100m	↗	939.536	0.1	1.132	100m	↘	-1208.622	0.5	0.000
200m	↗	939.536	0.1	1.132	200m	↘	-1207.020	0.5	1.602
400m	↗	939.007	0.3	0.602	400m	↘	-1207.746	0.5	0.876
800m	↗	938.479	0.3	0.075	800m	↘	-1206.701	0.3	1.921
1600m	↗	938.441	0.4	0.037	1600m	↘	-1194.032	0.3	14.590
3200m	↗	938.404	0.4	0.000	3200m	↘	-1207.319	0.4	1.303
CORTf	sign	AIC	R-square (%)	Δ AIC	tarsus	sign	AIC	R-square (%)	Δ AIC
50m	↘	412.503	0.8	1.641	50m	↘	784.354	0.0	0.868
100m	**↘**	**410.861**	**1.3**	**0.000**	100m	↘	783.486	0.1	0.000
200m	↘	412.322	1.0	1.460	200m	↘	783.642	0.1	0.156
400m	↘	413.639	0.4	2.778	400m	↘	783.880	0.1	0.394
800m	↘	414.395	0.2	3.534	800m	↘	783.503	0.1	0.017
1600m	↘	414.133	0.3	3.272	1600m	↘	783.775	0.1	0.289
3200m	↘	413.817	0.3	2.955	3200m	↘	783.716	0.1	0.230

measured over larger, regional spatial scales (Pautasso, 2007). At species level, habitat selection can be expected to be performed at multiple spatial scales too (McGarigal et al., 2016). For instance, introduced ring-necked parakeets (*Psittacula krameri*) reach their highest densities in city parks embedded within densely built-up landscapes. While the focal habitat supplies tree cavities for nesting, the surrounding urban environment provides ample foraging opportunities at backyard bird feeders (Strubbe & Matthysen, 2007). Phenotypic stress markers applied in this study revealed signatures of urbanization over a broad spatial range, from differences in CORT$_f$ levels at home-range scale to shifts in body condition at city-level scale. Along similar lines, de Satgé et al. (2019) recently showed that urbanisation at different scales additively reduces reproductive success of great tits (*Parus major*). Reduced breeding success is likely caused by urbanisation effects on food availability, as localised absences of native vegetation lowers the presence of important arthropod species, while regional pollution levels can affect prey nutritional value (Isaksson & Andersson, 2007). This implies that, in order to be effective, actions aimed at mitigating impacts of urbanization on free-ranging populations need to be implemented at multiple spatial scales too. At local (e.g. home-range) levels, increasing habitat quality by increasing native tree and shrub

cover may help increasing reproduction and survival (Moudrá et al., 2018). Yet, concurrent measures at regional scales – such as limiting atmospheric pollution through traffic reductions (Herrera-Duenas, 2017), or creating dispersal corridors facilitating movement of individuals through the urban matrix (Vangestel, Braeckman, Matheve, & Lens, 2010) - are paramount for long-term regional population persistence. Moreover, applied research into population effects of urbanization may additionally benefit from a more mechanistic quantification of 'urbanization'. Indeed, organisms do not directly consume or interact with percentages of built-up area. Rather, this widely-used index of urbanization represents a non-interactive (i.e. static, scenopoetic) niche variable which is well-suited for grasping broad ecological properties of a species (Soberón, 2007) but less so for testing underlying ecological relationships. A better understanding of mechanistic drivers of animal condition, survival, reproductive success, and ultimately population dynamics, may be achieved by relating phenotypic biomarkers to estimates of food availability, the strength of biotic interactions, or resource-consumer dynamics in more direct ways (Comte, Cucherousset, & Olden, 2017; Larson, Olden, & Usio, 2010). As opposed to single, static proxies of urbanization, such bionomic (Soberón, 2007) dynamic variables are amenable to experimental manipulation, and hence, more

useful for hypothesis development and testing (Sol, González-Lagos, Moreira, Maspons, & Lapiedra, 2014). By applying such approach, we may ultimately start to understand under which conditions urban areas are not by default ecological sacrifice zones (Rosenzweig, 2003) where even generalist, synanthropic species struggle to flourish, but can be designed or modified to sustainably support more diverse communities of organisms (Ahern, 2013; Grimm et al., 2008).

Author contribution

D.S. led the writing of the ms and conducted statistical analysis, N.S.H. helped prepare first manuscript drafts and conducted field work, A.T. performed field work, P.V. was instrumental in selecting suitable sites with house sparrow populations and coordinated the field work, J.A. was responsible for all corticosterone analyses, L.L. devised the study design. All authors commented on and helped improving the ms.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.landurbplan.2020.103767.

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