Cover design : Noraine Salleh Hudin, Nur Izzah Insyirah Abdul Manan

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Layout : Noraine Salleh Hudin

Salleh Hudin, N. (2017). Urban stress ecology: An individual-based, multi-component study of an urban-dwelling passerine. PhD thesis, Ghent University. Zelzate: University Press.

URBAN STRESS ECOLOGY:

AN INDIVIDUAL-BASED, MULTI-COMPONENT STUDY OF AN URBAN-DWELLING PASSERINE

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Thesis submitted in partial fulfilment of the requirements for the degree of

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The research was financially supported by the Ministry of Higher Education of Malaysia, the Interuniversity Attraction Poles Program SPEEDY (initiated by the Belgian Science Policy Office), and the joint-fund by RSPB and Natural England through the Action for Birds in England Partnership.

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General Introduction 1

Introduction

Human activities have induced changes in land cover, global climate, and biodiversity at extraordinary rates (Steffen et al., 2004). One of the most drastic forms of land-use alteration is caused by urbanization where natural environments are altered through substitution of native flora by built structures, displacement of natural resources by the artificial ones and a higher intensity of human disturbances (Grimm et al., 2008; Marzluff, Bowman, & Donnelly, 2001; McKinney, 2002). Urban expansion is occurring at accelerating pace across the globe and, at present, 0.5-3.0% of the terrestrial areas on the earth has already been converted into urban ecosystems (Liu, He, Zhou, & Wu, 2014). This growth is expected to continue in the future and forecast showed that by 2030 urban land cover will increase up to 1.2 million km^2, almost tripling its size in 2000 (Seto, Güneralp, & Hutyra, 2012).

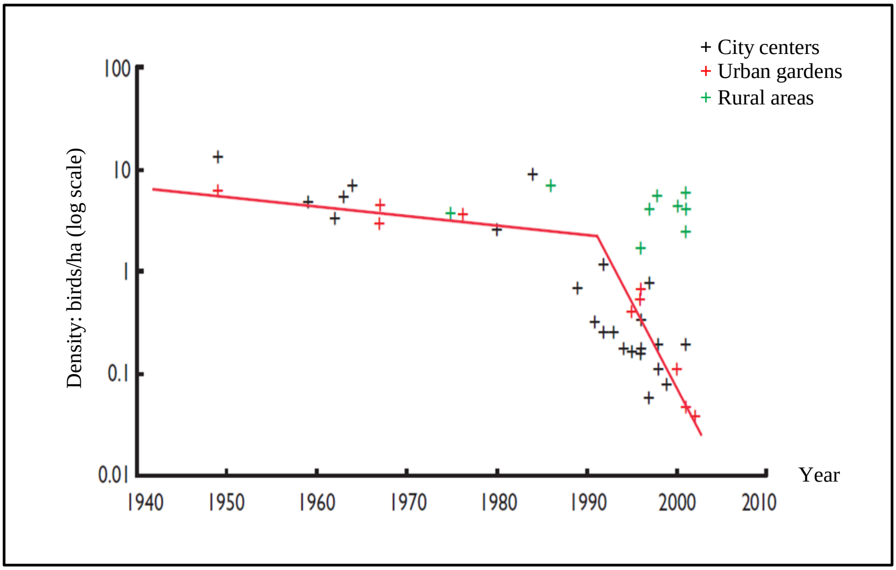
The impacts of urbanization vary with the speed and intensity of environmental changes, and the common consequence is decrease in species richness as observed in mammals, reptiles, birds, amphibians, invertebrates, and plants (McKinney, 2008). At the same time, non-native species may replace the local ones and increase in abundance in local environments (McKinney, 2006). These species are not only less affected by the urbanization processes, instead, they view urban environments as ecological opportunities (Sol, Lapiedra, & González-Lagos, 2013). These urban species have the ability to exploit urban resources, although they can still continue to utilize the natural resources or become fully dependent on anthropogenic resources (Blair, 1996). It is often that these non-native species are the same ones regardless of geographical areas (McKinney, 2006). Therefore, urbanization causes biotic homogenization whereby different cities become increasingly similar in their biotic compositions (McKinney, 2006).

Considering the worldwide occurrence of urban sprawl, science is challenged with the needs to predict how urban environments affect species. During the last two decades, numbers of studies have attempted to identify traits that allow survival in urban environments in order to predict future animal species compositions following urbanization processes. Among bird species, Chace & Walsh (2006) showed that urban dwellers are cavity nesters, but Evans et al (2011) found that nesting in cavity does not necessarily constitute the characteristics of species that thrive in cities as long as they do not nest on the ground. These contrasting findings may be because correlative approach relies only on observed distribution, particularly based on binary classification whether the studied traits present in urban dwelling species or not (Evans et al 2011). However, the presence or absence of species in an area can be the results of various factors such as biotic interactions. Such correlative approach also does not consider the differences in species response to urban environments as it assumes that all species in the urban areas are similar in densities (Evans et al 2011).

Recent evidence showed that species survival is mainly driven by appropriate adjustments in exploiting urban resources and dealing with urban-related environmental challenges (Sol, Lapiedra, & González-Lagos, 2013). Specifically, phenotypic adjustments affect individual fitness (Sol et al 2013) and, ultimately, species survival (Maldonado-Chaparro, Read, & Blumstein, 2017). Based on this finding, one would expect that “urban-adaptable” species show different phenotypic response that allows them to deal with urban challenges and survive better in urban environments compared to those from less urbanized areas. If this is true, then the density of these urban species should be higher in urban than in less urbanized areas. Since previous studies also showed that fitness may be influenced by individual intrinsic factors (Newton, 1998) such as sex (Post & Götmark, 2006), age (Baker, 2007), and size (Kotrschal et al., 2015). Therefore, it is possible that avian phenotypic responses vary with individual traits. Studying individual phenotypic response enable identifications of biological processes that limit population survival and thus allow predictions of species persistence in an area. Such information is helpful in designing conservation plan of species in situ.

In birds, one of the most well-known species that are being associated with urban environments is house sparrows (*Passer domesticus)*. Despite their association with human, a long-term study in Europe showed that their urban populations continue to reduce in size whereas the rural populations are apparently recovering during the recent decades (Fig. 1) (De Laet & Summers-Smith, 2007). Knowledge on how the urban house sparrows respond to urban environments may help to understand their urban population decline. Therefore, in this thesis, I studied the differences in phenotypic responses of urban and rural house sparrows where focus was given on variations at individual level.

Fig. 1 Breeding-season density of house sparrows in built-up areas in UK (adapted from Summers-Smith (2003)).



General aims

Two key questions of urban stress ecology are how do urban and rural birds vary in their phenotypes and how do they respond to urban stressors. While literature on the first question is rather established, knowledge on mechanistic urban avian ecology is still in its infancy (Bonier 2012; Shochat et al 2006; Marzluff 2017). Therefore, the aim of this thesis is to gain insights into how birds phenotypically respond to urban environments. As mentioned earlier, the knowledge on birds' responses to urban environments will help to predict species survival in urban environments.

In order to study phenotypic responses to urban challenges (stress), comparative and experimental approaches are necessary. Here, I simulated urban environmental stress by manipulating diet quality and food predictability urban environments differ in food resources (see *Food Resources* section below). Food has important impacts on birds as it affects nearly all aspects of bird ecology such as behavior, reproduction, demography, and distribution (reviewed by Robb et al 2008). In urban habitats, changes in food predictability have been associated with changes in birds' body reserves that may, in turn, affect fitness (Shochat, Lerman, Katti, & Lewis, 2004). Meanwhile, diet quality may influence birds' productivity and survival of nestlings (see Chamberlain et al (2009) and Robb et al (2008) for reviews). Therefore, manipulations of food resources are expected to induce stress to house sparrows. Here, avian phenotypic response will be addressed by investigating whether, and to what extent, nutritional conditions affect the urban and rural birds.

Determining the urbanization level category (eg. urban, suburban, rural) of an area depends on the spatial scale (see *Definition of Urban Environment* section below). Since this study associates phenotypic responses of birds with the urbanization levels of their habitats, hence it is likely that phenotypic response will vary with different scales. Therefore, this thesis will also address how phenotypic response could potentially be affected by spatial scales.

Characteristics of urban environment

Before discussing the mechanisms of how birds respond to urbanization, it is essential to identify how urban environments differ from rural or natural landscapes. In this section, I will discuss six most important features of urban landscapes – the urban physical attributes, climate, water availability, pollution, food resources and predation pressure.

Definition of urban environment

Urban environments are defined as areas where the land is mostly covered by built structures, commonly multi-storey, industrial or commercial buildings (Marzluff et al., 2001). The usage of this descriptive definition in urban studies, however, may not be accurate as it would be based on researcher's perceptions towards the areas. Therefore in recent years, many studies started to classify urbanization level of areas by quantifying the built-up density. Nevertheless, the built-up density changes with changes in spatial scale. Marzluff et al. (2001), for instance, use 100 ha area to define urbanization level in avian studies. However, different species have different space-use sizes (reviewed by Jetz, Carbone, Fulford, and Brown (2004)). For example, the home range of house sparrows is 0.0032-0.49 ha (Vangestel, Braeckman, Matheve, & Lens, 2010) whereas the rock doves (*Columba livia*) have the home range size of 1.34-4.96 ha (Sol & Senar, 1995). It would be ideal that the scales used in quantifying urbanization level correspond to the space-use size of species of interest as it is biologically more relevant to the species. Many studies on passerines used 500 m radius scale to quantify urbanization levels (eg. Angelier, Meillère, Grace, Trouvé, and Brischoux (2016); Bókony, Seress, Nagy, Lendvai, and Liker (2012); Chávez-Zichinelli et al. (2010);

Evans, Ryder, Reitsma, Hurlbert, and Marra (2015); Liker, Papp, Bókony, and Lendvai (2008); Meillère, Brischoux, Parenteau, and Angelier (2015)), while some used different values such as 1 km (Giraudeau & McGraw, 2014) and 10 km radii (Bichet et al., 2013).

Physical attributes

The conversions of natural landscapes into man-made structures, which includes buildings, transportation systems, and soil sealing by artificial pavements, creates the most prominent difference between urban landscapes and natural environments. In Europe alone, 1120 km² of natural landscapes and farmland were replaced by artificial land development per year between 2000 to 2006 (EEA, 2017). By 2030, about 77500 km² of the total areas of the European continent will be transformed into urban landscapes (Seto et al., 2012). Impervious surface may cover 50% of the areas at the urban centers, 30-50% in suburban regions, whereas at rural landscapes the percentage is only less than 20% within 1 km² area (Marzluff et al., 2001; McKinney, 2002). For cavity nesting species, buildings may provide them with nesting sites (Jokimäki, 1999), but, at the same time built structures can also cause increased bird mortality due to collisions (Seress & Liker, 2015). The land conversions may cause habitat loss for native species which intensifies with increasing urbanization level (McKinney, 2002). Through habitat loss, available resources will decrease (Eikaas and McIntosh 2006) and the whole natural landscapes will become fragmented into smaller but many habitat patches (Fahrig, 2003; Scolozzi & Geneletti, 2012).

Climate

The urban habitat structure and land cover caused increased temperature in the urban areas relative to the adjacent landscapes (Collins et al., 2000; Gaston, Davies, & Edmondson, 2010) and this condition is known as “urban heat islands” (Landsberg, 1981). In specific, built structures absorb more heat during the day and release it back into the environments at night (Kuttler, 2008). Moreover, pollution, anthropogenic heat, slower wind speed also contributed to the increased in urban climate (Gartland, 2012). The greeneries which can help to reduce heat up to 5-7°C (Armson, Stringer, & Ennos, 2012), however, are limited in cities. Altogether, these make urban areas 4-10°C warmer than the surrounding rural areas at night although the temperature difference may not be prominent during daytime (Fischer, Oleson, & Lawrence, 2012; Wilby, 2003; Zipperer, Sisinni, Pouyat, & Foresman, 1997). Heat islands increase as cities grow larger, and the hottest areas are the ones with the highest urbanization level and the least green spaces (Gartland, 2012).

The warmer climate of urban environments is favored especially during winter during which climatic conditions are harsh and food supplies are low (Tryjanowski et al., 2015).

The higher temperature of urban environments allows advanced plant phenology, longer growing season, higher primary productivity, and earlier peak and more rapid development of invertebrates which consequently provide rich food supplies for urban birds (Evans, Chamberlain, Hatchwell, Gregory, & Gaston, 2011; Horak & Lebreton, 1998; Lu, Yu, Liu, & Lee, 2006; Neil & Wu, 2006). Furthermore, as the ambient temperature is higher, birds lose fewer body reserves during the night and thus living in urban environments requires less energetic demands (Ockendon, Davis, Miyar, & Toms, 2009). Despite these advantages, urban heat islands can also negatively affect wild birds as it may increase asynchrony between peak abundance of invertebrate prey and timing of breeding (Seress & Liker, 2015).

Water availability

Alterations of physical conditions from natural landscapes into roads, sidewalks, and parking lots create large areas of impervious surface in cities (Grimm et al., 2008). When natural areas are fully converted into impervious surface, the rate of evapotranspiration reduces from 40% to only 30%, while surface runoff increases from 10% to 55% (Paul & Meyer, 2001). The heat island effects caused increased in precipitation and this large quantity of water is channeled through storm sewers which eventually leaving only small areas of standing water (Gaston, 2010). The presence of water bodies was found to be important for urban dwellers in which it helps to increase urban avian species richness (reviewed by Ferenc, SedláČek, and Fuchs (2014)). For example, available water bodies become a necessity for the presence of some bird species such as spotted towhees (*Pipilo maculatus*) and song sparrows (*Melospiza melodia*; Melles, Glenn, and Martin (2003)). Besides that, the occurrence of winter wrens (*Troglodytes troglodytes*) and common blackbirds (*Turdus merula*) was also positively influenced by the presence of water bodies (Ferenc et al., 2014).

Pollution

Urban environments are also different than any other landscapes due to the higher rates of pollution (Seress & Liker, 2015). Compared to rural birds, individuals from urban populations demonstrate higher oxidative stress and inflammation (Isaksson, Hanson, & Burdge, 2015) which is likely being caused by elevated levels of pollution in urban areas (Stroh, Harrie, & Gustafsson, 2007). Chemical pollutants such as industrial wastes, vehicle emissions, and agricultural discharges may enter biogeochemical and nutrient cycles as well as primary productions (Grimm et al., 2008). Consequently, these pollutant compounds may end up in the tissue of consumers (Seress & Liker, 2015) as evident by the higher amount of heavy metals in the liver of house sparrows from urban areas (Kekkonen, Hanski, Väisänen, & Brommer, 2012). The elevated concentration of heavy metal in birds have been linked to impaired immune response and increased pathogen prevalence in urban birds (Bichet et al., 2013; Gasparini et al., 2014).

Besides that, the night time in urban environments is commonly disturbed by artificial lightings (Boyce, 2014; Schreuder, 2008). The artificial lights, such as streetlights, lighted buildings and towers, security lights, and lights on vehicles, disrupts the natural cycle of light and darkness (reviewed by Longcore and Rich (2004)). Light pollution caused disorientation in migrating birds whereby brightly lit areas attract birds and as birds enter the areas they will hesitate to leave (reviewed by Longcore and Rich (2004)). In these areas birds may collide with buildings, circling lighted structures until exhaustion, and once on the ground, they are exposed to predations (reviewed by Longcore and Rich (2004)). Light pollution is higher in urban areas as shown by a study on blackbirds whereby birds from urban habitats were found being exposed to higher light intensity at night than rural birds (Dominoni, Carmona‐Wagner, Hofmann, Kranstauber, & Partecke, 2014).

Another form of pollution in urban areas is noise which is commonly produced by industrial practices, construction work, transportation, and recreational activities (Slabbekoorn, 2013). These noises are assumed to increase in intensity with increasing human density (Ortega, 2012). A review by Warren, Katti, Ermann, and Brazel (2006) showed that urban areas constitute noisier environments than less urbanized landscapes. With the presence of intense noise in the background, communications in territorial defense and mate attractions, as well as important signals such as begging, alarm and distress calls are being masked (Warren et al., 2006).

Food resources

Because of pollution (Raupp, Shrewsbury, & Herms, 2010), loss of vegetation cover (Shaw, Chamberlain, & Evans, 2008), and changed in plant compositions in urban environments (Southwood, 1961), natural food resources, such as plants and invertebrates, are low in urban areas (Tryjanowski et al., 2015). Luckily, wild bird feeding has becoming very popular among urban residents in which up to 64% of households in the UK feed birds regularly (Davies, Rodriguez, Sweazea, & Deviche, 2012) which results in higher abundance and predictability of food supplies (Shochat, Warren, Faeth, McIntyre, & Hope, 2006). In urban environments, feeders are widely available in the vicinity, foods are spread on the ground by human and can be accessed from garbage bins (Oro, Genovart, Tavecchia, Fowler, & Martínez‐Abraín, 2013; Tryjanowski et al., 2015). Altogether, these contribute to the increase of food availability and predictability for birds in urban environments (Faeth, Warren, Shochat, & Marussich, 2005; Tryjanowski et al., 2015) and thus reduce the risk of starvation (Heiss, Clark, & McGowan, 2009). A review by Robb, McDonald, Chamberlain, and Bearhop (2008) on the effects of supplementary feeding on the reproduction of passerines found that supplementary feeding advances laying date, increase chick growth rate, and improve fledging success.

Despite the plentiful food supplies in urban environments, urban diet may contain different nutritional compositions than in natural food. Protein‐rich invertebrates prey such as beetles, caterpillars, flies, and spiders are low in abundance and diversity and these lead to the lack of protein content in the diet of urban birds (Raupp et al., 2010; Shochat, 2004). Such is observed in American crows (*Corvus brachyrhynchos*) where nestlings from urban areas showed lower protein content in their blood (Heiss et al., 2009). Rather, anthropogenic food and bird feeders mainly comprise of carbohydrates and fat (Meyrier et al., 2017; Pierotti & Annett, 2001). Nevertheless, studies comparing fat contents in food from different urbanization levels found contradicting results whereby urban house sparrows were found having higher fat content in their diet than their rural conspecifics (Gavett & Wakeley, 1986a), whereas Fokidis, Hurley, Rogowski, Sweazea, and Deviche (2011) and Davies et al. (2012) found no significant differences between urban and non‐urban birds.

Predation pressure

While bird feeding can increase the availability of food resources in cities, it may also lead to several negative impacts including increased predation pressure (Robb et al., 2008). Feeders, which form clumped food resources, induce bird aggregations around them and thus may attract predators (Robb et al., 2008). A number of studies have found that predator abundance such as cats and raptors are higher in urban areas than in natural habitats (reviewed by Seress and Liker (2015)). The higher latency to start feeding again in the urban compared to rural house sparrows after being exposed to artificial attacks by their common predators (sparrowhawk (*Accipiter nisus*) and domestic cat (*Felis catus*)) also suggests that urban birds may experience higher predation risk in cities (Seress, Bókony, Heszberger, & Liker, 2011).

Despite the higher predation risk in urban habitats, some studies found that predation rate is lower in urban areas. For instance, an experimental study by Gering and Blair (1999) on nest predation along urbanization gradient found that there were less depredated nests in more urbanized areas which probably due to the high abundance of prey birds such as European starlings (*Sturnis vulgaris*), rock doves and house sparrows. Similarly, a more recent study by Stracey and Robinson (2012) found that although the abundance of predators is higher in urban habitats, nest predation was lower in urban areas. Possible explanations for the mismatch between predation risk and predation rate in urban areas could be that urban predator species have effective ways to deter predators such as through group mobbing behavior, nesting in close-cup nest, and high capability to hide nests (Stracey & Robinson, 2012), and that predators probably rely more on anthropogenic resources rather than prey species (Rodewald, Kearns, & Shustack, 2011).

Phenotypic response to urban environmental conditions

As described in the previous section, urbanization imposes changes in the environmental conditions which may affect urban dwellers. The ability of birds to persist and flourish in the changing environments is largely determined by their capacity to modify their phenotypes in response to changed environmental conditions (McDonnell & Hahs, 2015). Phenotypic response is defined by shifts in traits of individuals when they are exposed to environmental changes where such adjustments in phenotypes increase fitness of individuals under the new environmental conditions. Urban environmental conditions may directly lead to the shifts in physiology, behavior, and morphology of individuals without necessarily involve genetic change, which is known as phenotypic plasticity (Partecke, Gil, & Brumm, 2013). However, ideal urban phenotypes may also require genetic adaptation which is less costly compared to changes in phenotypes through plastic response (Partecke et al., 2013). Genetic adaptation will occur when phenotypic change, which is achieved through plastic response, approaches the ideal urban phenotype and natural selection fixes this heritable trait in the population (Partecke et al., 2013). The genetic fixation of a changed phenotype may take several generations to occur. Since this study only investigated birds' responses to urbanization within a lifetime, therefore I only focused on phenotypic response induced by plasticity. In this section, three components of the phenotypic responses of urban dwellers will be discussed, namely the physiological, behavioral, and morphological responses.

Physiological response

Unfavorable urban characteristics, which is a form of an environmental stressor, are defined as extrinsic factors that challenge individuals and force them to modify their physiology to cope with (Killen, Marras, Metcalfe, McKenzie, & Domenici, 2013). Therefore, in order to maintain normal daily body processes that maintain life, a certain amount of metabolic energy is required by the individuals (allostatic load; McEwen and Wingfield (2010)). Allostatic load, therefore, can be used to represent the degree of chronic stress being imposed by stressors on individuals (Bonier, 2012). Stress occurs when energy demands exceed individual capacity to cope with the unpredictable perturbations that occur in the environments (McEwen, 1998). As urbanization induces dramatic changes to the environmental conditions, therefore it is predicted that stress level may be higher or lower in urban birds (Bonier, 2012). For example, pollution may impose stress to birds and this may result in an increase of stress level. On the other hand, birds may also benefit from the urban environmental changes such as stable resource availability and warmer climate, thus the stress level is decreased.

Past studies on bird stress levels in relation to urbanization levels found mixed results whereby stress levels seem to vary with species (reviewed by Bonier (2012)). Urban habitats caused increased stress in blackbirds (Meillère et al., 2016) while in contrast, urban Inca doves (*Columbina inca)* showed lower stress level than their conspecifics in less urbanized areas (Chávez-Zichinelli et al., 2013). Nevertheless, in many bird species stress levels were similar in urban and non-urban populations (reviewed by Bonier (2012)). Stress levels are also influenced by sex (reviewed by Bonier (2012)) as demonstrated by white-crowned sparrows (*Zonotrichia leucophrys*) whereby urban habitats seemed to caused higher stress level in males, whereas in females stress levels did not differ between urban and rural habitats (Bonier et al., 2006). Variations in stress level are also driven by life history (reviewed by Bonier (2012)). In a study on curve-billed thrashers, urban populations had lower stress level than the desert populations during non-breeding season; however, such difference disappeared during breeding season (Fokidis & Deviche, 2012; Fokidis et al., 2011; Fokidis, Orchinik, & Deviche, 2009b).

Behavioral response

Urban habitats commonly offer novel food resources including human leftover, artificial feeders and non-native ornamental plants and trees (Tanner, Salall, & Jackson, 2011). In order to acquire food successfully, it would be advantageous if urban birds possess modified behavior that allows them to quickly accept the new food resources, which otherwise may expose them to the risk of starvation (Sol et al 2013). Because urban food resources are temporally predictable, Griffin, Netto, and Peneaux (2017) showed that urban environments favor increased learning so that birds can take advantage of this predictable variation. However, urban food resources can also be less predictable in terms of spatial distribution due to heterogeneous urban configurations (Griffin et al., 2017). Thus, increased neophilic behavior, which is the degree of attractions to novelty, would facilitate urban birds in dealing with this complexity (Griffin et al., 2017). Besides that, Griffin et al. (2017) also found that the abundant food supplies in urban environments reduce the need for innovativeness in foraging activities among established urban birds. Social foraging behavior of urban birds may also change when birds have access to supplementary feeding such as reduced mixed-species foraging activities as it will only beneficial during food scarcity (Robb et al., 2008) and reduced territorial defense because birds cross territorial boundaries to access feeders (Wilson, 2001). In contrast, supplementary feeding can also increase territorial defense behavior if the success rate to monopolize this clumped food resources is high (Robb et al. (2008) and the references therein).

Due to the stable food availability and warmer climate in urban environments, urban birds can start breeding earlier in the season and thus have the opportunities to breed within a longer period of optimal conditions (reviewed by Lowry, Lill, and Wong (2013)). Field observations on white-winged choughs (*Corcorax melanorhamphos*) found that urban birds initiated breeding earlier than birds from non-urban habitats (Beck & Heinsohn, 2006). Likewise, a common garden experiment on urban and forest blackbirds discovered that urban blackbirds had a more advanced gonadal development which indicates an earlier onset of breeding activities which consequently resulted in a prolonged breeding season (Partecke & Gwinner, 2007). For a multiple brooded birds, these adjustments may allow birds to produce more clutches and young within a breeding season, and thus increase their fitness (Adriaensen & Dhondt, 1990).

Urbanization also caused alterations in bird communication. In response to increased noise in cities, birds may communicate in higher frequency (Sol et al. (2013) and the references therein) to avoid overlapping with the low-frequency range of background noise which will result in the masking of acoustic signals (Nemeth & Brumm, 2010). This shift in vocal frequency may benefit birds as high-frequency songs can reach larger distance (Nemeth & Brumm, 2010). Communication distance can also be improved by singing louder (reviewed by Gil and Brumm (2013) and Slabbekoorn (2013). As an example, the communication distance of great tits (*Parus major*) enlarged from 61 to 67 m when singing in high frequency, but singing louder increased the distance greatly from 61 up to 91 m (Nemeth &

Brumm, 2010). Birds also change their singing routine within a day to avoid overlapping between singing activities with the noise peaks in urban habitats (reviewed by Gil and Brumm (2013). Such changes have been observed numbers of urban dwelling birds including great tits, blue tits (*Cyanistes caeruleus*), and common blackbirds where they sang earlier than their counterparts that live in the adjacent forests (Bergen & Abs, 1997).

Morphological response

The majority of studies reporting birds' morphological response along urbanization gradient have been focusing on body mass and body size (reviewed by Gil and Brumm (2013)). In comparison with rural populations, urban house sparrows in Hungary seemed to have reduced body mass (Bókony et al., 2012). A very similar study by Meillère et al. (2015) on house sparrows in France also yielded identical results. It was suggested that the lower body mass in the urban populations was caused by the loss of need to store high body reserve as food resources are highly predictable in the urban environments (Bókony et al., 2012; Meillère et al., 2015). Body mass may also be influenced by predation risk whereby increased predation pressure may cause reduced body mass to allow better take-off, increase velocity, and improve maneuverability (Lind, Fransson, Jakobsson, & Kullberg, 1999; Witter & Cuthill, 1993; Witter, Cuthill, & Bonser, 1994). Besides that, body condition was not related to urbanization levels in house sparrows which indicates that urban habitats do not exert nutritional stress on adult birds (Bókony et al., 2012; Meillère et al., 2015). On the other hand, gray catbird nestlings from more urbanized areas exhibited lower body condition and it was caused by lead contamination (Roux & Marra, 2007).

Wing length is determined by numbers of factors such as nutritional limitations and predation pressure. Seress et al. (2012) found that house sparrow nestlings from suburban areas had reduced wing length than nestlings in rural areas. Through cross-fostering experiment, they found that rearing conditions, rather than hatching conditions, affect nestling morphology (Seress et al., 2012). In the same study, a common garden experiment which explored the effects of parents' food provisioning at different urbanization levels revealed that urban nestlings suffered nutritional inadequacy and this might explain the shorter wings of nestlings from the more urbanized areas (Seress et al., 2012). The importance of nutritional conditions on wing length has also been studied by Ibáñez-Álamo and Soler (2010). However, in their study, they did not find significant differences in wing length of urban, rural, and forest blackbirds (Ibáñez-Álamo & Soler, 2010). Ibáñez-Álamo and Soler (2010) suggested that nutritional constraint and predation risk are the important selection pressures in the urban areas and forests, respectively, while in the rural areas both factors exert intermediate effects simultaneously. Under strong predation pressure, birds may increase their wing length to reduce wing loading (body mass/wing area) so that they can increase their take-off speed and maneuvering ability which help them to escape from predators (Burns & Ydenberg, 2002; Witter et al., 1994). On the other hand, Hedenström and Rosén (2001) found that in small-bodied birds, having shorter wings will allow them to perform tight turn during fast horizontal flight which is effective in escaping from predators.

Tarsus length was lower in urban birds than in rural birds (Bókony et al., 2012; Meillère et al., 2015). The shorter tarsi were also observed in the nestlings of house sparrow and American crow from more urbanized areas (Heiss et al., 2009; Seress et al., 2011). Rather than being an adaptation to urbanization, the smaller size of birds in urbanized areas probably reflects poor nutritional conditions during developmental phase (Bókony et al., 2012; Heiss et al., 2009; Meillère et al., 2015; Seress et al., 2011). On the contrary, Evans, Gaston, Sharp, McGowan, and Hatchwell (2009) found that that urban blackbirds had greater body mass, longer tarsi, and longer wings than in the rural populations. However, Evans et al. (2009) suggest that these results are site-specific as no consistencies were found when comparing different study sites.

Previous studies or bill morphology reported variations between urban and rural populations. For example, Evans et al. (2009) found that urban blackbirds had stubbier bills (greater bill length and width ratio) than rural blackbirds, Badyaev, Young, Oh, and Addison (2008) found that urban house finches (*Carpodacus mexicanus*) are greater at bill height, length, and width than their desert conspecifics although birds from both areas had similar body size, while Hutton and McGraw (2016) showed that *Haemorhous mexicanus* (also known as house finches) from urban areas had longer, but not wider or higher, bills than finches from rural areas. Likewise, commensal house sparrows also had longer and pointier bills than their non-commensal subspecies (*P. d. bactrianus*; Riyahi et al. (2013)). Bill morphology in granivorous species has been linked to bite force which could be translated as the needs of seed handling whereby handlings of larger and harder seeds, such as those provided at feeders in urban habitats relative to grass seeds in non-urban habitats, require stronger bite force and thus larger bills (Badyaev et al., 2008; Herrel, Podos, Huber, & Hendry, 2005).

Study species

The model species in this study is house sparrow, a member of the family Passeridae. Being native in Eurasia, Africa, and the Middle East, house sparrows at present are widely distributed around the world as they were also introduced in America, Australia, and New Zealand in the last 2000 years (Anderson, 2006; Cocker, Tipling, Elphick, & Fanshawe, 2013). In all of these regions, house sparrows occur ubiquitously in close associations with human, ranging from farms and villages, suburban areas with gardens, to cities and industrial areas (Summers-Smith, 1988).

This sexually dimorphic species is known as being invasive in many places. House sparrows have caused damages on standing grains and cultivated plants such as vegetables and fruit trees (reviewed by Anderson (2006)). Several studies reported that the loss caused by house sparrows per field of wheat, barley, sorghum, and millet was as high as 4.6%, 9.3%, 18.6%, and 100%, respectively (Anderson (2006) and references therein). In Europe, crop destructions by house sparrows occurred frequently especially in barley and wheat fields and the estimated total grain loss was between 2-4% of the yield (Havlín, 1974). House sparrows also impose threats to animal and human health as they act as agents in pathogen transmission or being the reservoir of numerous arboviruses that caused West Nile Virus, St. Louis encephalitis, Paramyxovirus2 and H5N1 (reviewed by Anderson (2006)). House sparrows in Europe serve as the primary reservoir of pathogenic bacteria such as *Salmonella* (reviewed by Anderson (2006)). In Britain, salmonellosis outbreaks mostly occurred within the vicinity of feeding stations where interactions with human are likely, and several reported cases of salmonellosis in human were caused by infections from garden birds *(Lawson et al., 2014)*.

The breeding season of house sparrows lasts from April until August (Anderson, 2006; Summers-Smith, 1988). Sparrow pairs can produce up to four clutches where each clutch may vary from one up to nine eggs, although in most populations it contains 3-6 eggs (Anderson, 2006; Summers-Smith, 1988). The incubation period fall between 11.4 to 12.2 days and chicks fledge around 14 days after eggs hatch (Anderson, 2006; Summers-Smith, 1988). The maximum nestling body mass is normally reached at the age of 10-12 days old whereas the tarsus obtains its final length at the age of 10 days (Anderson (2006) and the references therein).

After the breeding season during which house sparrows were born, natal dispersal may take place (Summers-Smith, 1988) where the first year house sparrows can move as far as 103-2350 m away from the breeding sites (Fleischer, Lowther, & Johnston, 1984; Paradis, Baillie, Sutherland, & Gregory, 1998). Breeding dispersal, which distanced between 73.5m (Paradis et al., 1998) up to 6550m in radius (Skjelseth, Ringsby, Tufto, Jensen, & Sæther, 2007), is rather uncommon in house sparrows where it occurs in only less than 6.7% of adults (Fleischer et al., 1984). Their core daily foraging movement is as small as 15-40m (Medeiros, 1998; Vangestel et al., 2010) with the max radius of 1600m (North, 1973). Overall house sparrows are considered highly sedentary (Anderson, 2006; Summers-Smith, 1988).

House sparrows are essentially granivores (Anderson, 2006). The adults from rural populations mainly feed on human-produced cereal grains (eg. maize, wheat, sunflower seeds, and weed seeds) while invertebrates make up 3%, 9%, 68% of their diet in autumn, spring, and summer, respectively (Anderson (2006) and references therein). On the other hand, 54% of the urban sparrow diet consists of wild bird seeds and human refuse (reviewed by Anderson (2006)) such as bread and peanuts (Gavett & Wakeley, 1986b; Summers-Smith, 1988). Calorimetric tests on the blood of house sparrows showed that the diet of urban individuals is higher in fat content than the those in rural areas (Gavett & Wakeley, 1986a). When comparing their nutritional stress, (Vangestel et al., 2010) found that urban house sparrows experience higher nutritional stress compared to their rural conspecifics. Nestlings feed exclusively on invertebrates and the important invertebrate groups vary with urbanization levels (Vincent, 2005). Diptera (tipulids) accounted a larger proportion of rural nestling diet, while urban nestlings mostly feed on Homoptera (aphids) (Vincent, 2005). The amount of plant materials increases in the nestling diet as the nestlings get older (Summers-Smith, 1988).

The urban populations of house sparrows in Europe show dramatic decline during recent decades but the rural populations have started to stabilize (Fig. 1) (De Laet & Summers-Smith, 2007). In 1950, house sparrows in Belgium occurred in 100-150 pairs per flock, but in 2000 only 20 pairs per flock were observed (De Laet & Summers-Smith, 2007). The reduction in the Belgian populations persists from an average of nine males per site in 2002 to only 6 males in 2011 (De Coster, De Laet, Vangestel, Adriaensen, & Lens, 2015). Numbers of factors have been proposed as the causes of the loss of urban house sparrows and one of them is insufficient food supplies (De Coster et al (2015) and the references therein). Nestling house sparrows are likely to experience nutritional inadequacy as invertebrates, the main food resources of nestlings, are low in abundance in more urbanized areas (Tryjanowski et al., 2015). When supplied with live invertebrate prey, their fledging success increased up to 55% (Peach et al 2014). There are also possibilities that adults in cities receive poor nutrition because they mainly depend on anthropogenic food (Anderson (2006) and the references therein) although the nutritional content probably insufficient to fulfill the nutritional requirements of house sparrows. As constrained nutrition, either in quantity and/or quality, may reduce survival rate of nestlings (Seress et al., 2012) and fitness of adults (Cyr & Romero 2007), therefore, the effects of nutrition as a potential factor that contributes to urban population decline cannot be neglected. In this study, the effects of urban diet and nutritional conditions are investigated to better understand the phenotypic response of house sparrows to urban environments.

In this study, I used the house sparrow as a model species to study bird response towards urban stressors. Although listed as one of the "least concern" species (IUCN, 2017) and is known as a classic example of urban exploiter species, the urban populations showed population decline in the European regions (De Laet & Summers-Smith, 2007). Data from years of bird census in Great Britain showed that the rural populations are apparently recovering whereas the urban populations continue to reduce in size (Fig. 1) (De Laet & Summers-Smith, 2007). In 1950, house sparrows in Belgium occurred in 100-150 pairs per flock, but in 2000 only 20 pairs per flock were observed (De Laet & Summers-Smith, 2007). The reduction in the Belgian populations persists from an average of nine males per site in 2002 to only 6 males in 2011 (De Coster, De Laet, Vangestel, Adriaensen, & Lens, 2015). As house sparrows are prevalent in urban, suburban and rural areas, it makes them an excellent model to compare environmental stress along the urbanization gradient. Moreover, the decline of house sparrow populations in the urban landscapes serves a good opportunity to study the effects of urbanization on the stress ecology in urban dwellers.

Study design

I applied dual (i.e. field sampling and experiment) approach to study the effects of urban-related environmental stress on house sparrows. Field samplings provide information on patterns that can be observed in nature which reflects the sum of effects of various factors that occur simultaneously. Meanwhile, experiments are advantageous in understanding ecological processes driven by those factors. By combining both approaches on the same individuals, I would be able to identify important factors that shape the observed patterns in nature and understand the mechanisms that generate the patterns. In this thesis, multiple indices of environmental stress (feather-based corticosterone level ($CORT_f$)), fluctuating asymmetry (FA), and ptilochronology) were used to compare the degree of stress exerted by urban and rural habitats and to assess the response of birds to the urban stressors. In addition to that, body condition, which is the proxy of individual quality, was also used to help in interpreting the effects of urban stress on birds. The integrations of multiple indices provide a more holistic view on how individual birds respond to urban stressors.

Field sampling

The environmental stress imposed by urban habitats was compared with that from rural areas. To do so, free-living house sparrows were captured using mist nets at urban and rural areas in Flanders, Belgium and in southern France. The field sampling in Belgium was conducted from Sept 2013 until March 2014 where a total of 373 birds were captured from 18 sites which comprised various degrees of urbanization level. In France, 115 house sparrows were captured between mid-Sept until early Oct 2014 from three urban areas and three rural areas. Upon capture, each individual was (i) ringed, aged (juvenile versus adult), sexed, and weighed, (ii) measured for their tarsus length, wing length, and bill dimension, and (iii) sampled for tail feathers. The tail feathers were used for the analysis of $CORT_f$, FA, and ptilochronology.

Field experiment

Unexplored data which was collected as part of a food supplementation experiment performed by Peach, Sheehan, and Kirby (2014) in the UK in 2008 was used to study the effects of urbanization on the phenotypic and physiologic responses in nestlings. The experiment was conducted between mid and late April on house sparrow nestlings at 10 gardens spread across all six study sites in rural and suburban areas. A total of 12 of 29 and five of 16 nests in rural and suburban areas, respectively, had access to the supplementary feeding which consisted of mealworm (*Tenebrio molitar*). The nests were examined weekly to determine the laying date and the number of eggs, chicks, and fledglings. At the age of 9-13 days, tail feathers were collected for $CORT_f$ quantification, tarsus length was measured for FA analysis, whereas body mass was taken for assessment of body condition. The degrees of environmental stress was compared between rural and suburban nestlings as interpreted by $CORT_f$, FA, body condition, and reproductive performance.

Aviary experiment

To study how adult birds respond towards urban stressors, I conducted a challenge experiment where the captured house sparrows in France were transferred from their natural habitats to aviaries to challenge them with habitat change. As urban areas undergo rapid changes, for example, due to building and road constructions, the habitat-change challenge would mimic the habitat change scenarios in urban areas. In the aviaries, they were challenged with changed food resources (diet composition and predictability). In the manipulation of diet composition, birds from each of the six sampling plots were randomly assigned to an urban diet or rural diet which resemble the food that commonly found in urban and rural areas (Chapter 2, Fig. 1). During the experiment, food was provided every two days in all groups and this represents the increase food predictability in urban habitats. Although high food predictability may not act as stressor as birds are likely to benefit from resource stability, this feature, which is unique to the urban environments, would potentially affect urban bird phenotype and physiology. Therefore food predictability was also tested in the experiment. Body mass was taken weekly and after six weeks the regrown tail feathers were collected for analyses of CORT_f, FA, and ptilochronology. By comparing these indices before and after the experiment, I was able to compare the potential differences in stress response between birds from urban and rural areas.

Indices of environmental stress

CORT gives information on the integrated signal of adrenocortical activity (i.e., both baseline and temporary acute elevations) in response to allostatic demands (Fairhurst et al., 2013; Romero & Fairhurst, 2016; Will et al., 2014). CORT has been widely used to study responses to urbanization because it is an essential mediator of energy metabolism and is released upon activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to changes in allostatic demands (Sapolsky, Romero, & Munck, 2000). Thus, CORT provides an integrated measurement of the challenges placed on organisms and their capacity to cope (Bonier, 2012; Dantzer, Fletcher, Boonstra, & Sheriff, 2014; Tarlow & Blumstein, 2007). CORT can be quantified from blood plasma and feathers. While plasma CORT gives a snapshot of hormone concentration at the time of plasma collections, CORT_f reflects the accumulated concentration of hormone secreted over the longer period of feather formations (Berk, McGettrick, Hansen, & Breuner, 2016). Since CORT level indicates birds' responses to different stressors, therefore, to facilitate results interpretations, CORT is ideally coupled with additional indices (McEwen & Wingfield, 2010; Romero, Dickens, & Cyr, 2009). Because of this reason, I also applied estimation of FA and ptilochronology in assessing environmental stress.

FA refers to small random deviations from perfect symmetry in bilateral traits (Ludwig, 2013). The mean of difference between the right and left values of a trait is zero and the variation is normally distributed around that mean (Palmer, 1994; Polak, 2003). Since the matching sides of each body trait are the expressions of the same genome and both experience the same environmental condition during development, therefore any deviations from left–right symmetry of a trait cannot be caused by genetic or environmental effects (Reeve, 1960). Instead, it corresponds to the inability of individuals to cope with stress during the growth of the trait (Auffray, Renaud, Alibert, & Nevo, 1999; Lens, Van Dongen, Kark, & Matthysen, 2002; Palmer, 1994). In specific, higher FA indicates lower ability to buffer against stressors during development (Auffray et al., 1999). The FA of adult house sparrows in this thesis was calculated from feather length while in nestlings it was estimated from tarsus length.

Ptilochronology was used to assess individual's nutritional status during the period of feather growth (Grubb Jr & Cimprich, 1990; Vangestel & Lens, 2011). Feathers grow a pair of light and dark growth bars within a 24-hours period where the light bars are formed from materials that deposited in the follicle during daytime while the dark bars are formed at night (Wood 1950). The width of the bars represents the nutritional status of the individual (Grubb, 2006). In specific, if an individual is in good nutritional state, more energy and nutrients can be supplied for feather generation, thus producing wider growth bars (Grubb, 2006). Nutritional stress occurs when body compartments which are controlled by nutrition are in an inadequate state caused by nutritional constraints (Grubb, 2006) such as reduced food quantity as indicated by narrower growth bars in feathers (Grubb, 1991). Other factors that may influence the width of growth bars are sex, age, and season where growth bars are wider in males and adults, and in feathers that grow in summer relative to winter (Grubb Jr, Waite, & Wiseman, 1991).

Body condition was used to represent individual quality. By using body condition, I can compare the relative size of energy reserves between individuals (Krebs & Singleton, 1993). Body condition is well represented by scaled body mass and in this thesis, it was calculated by using scaled mass index (Peig & Green, 2009). SMI 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-length (length from trait which showed the strongest correlation with body mass on a log-log scale) estimated by type-2 (standardized major axis; SMA) regression (Peig & Green, 2009).

Outline of the thesis

An overview of the characteristics of urban environments and how birds respond to the urban environmental changes are discussed in this first chapter. Based on this background knowledge, the following chapters (Chapter 2, 3, 4) will report the further investigations on the avian phenotypic responses towards urbanization through manipulation of nutritional conditions as a proxy to stressors. The focus is given to the responses at individual level. $CORT_f$ indicates the sum of CORT released during the period of feather generation but the stressors that induced the CORT secretion are not specified. As different species may respond differently to stressors, prior knowledge on how house sparrows respond to urban nutritional conditions is important to provide strong bases in explaining their CORT levels in natural environments. Therefore, in this thesis, I started by reporting studies on the mechanisms of CORT response (Chapter 2, 3, 4) and later followed by correlative study (Chapter 5) which uses findings from the previous chapters to elucidate the observed patterns.

In **Chapter 2**, I test whether body condition varies with urbanization levels, where urban areas are characterized by higher food predictability compared to rural areas. In specific, Starvation-Predation Risk hypothesis stated that individuals can be expected to maintain a lower body condition when food is abundant and predictable to optimise escape capacity from predators and to increase their fat reserves when food becomes unpredictable to reduce starvation risk. Alternatively - or simultaneously - improved winter survival in urban environments (e.g. Robb et al. (2008); Evans et al. (2015)) can cause resource overmatching due to increased population densities (Møller et al., 2012), and hence, may result in lower body reserves due to reduced individual foraging success and due to the persistence of "loser individuals" in the population (Resource-Matching hypothesis). If the Resource-Matching hypothesis is true, then I would predict that urban populations will be numerically dominated by birds with low body conditions. To test these hypotheses, I integrate both field sampling and aviary experiment to explore the variations in environmental stress levels between urban and rural habitats on free-living adult house sparrows in southern France and to study the phenotypic responses to urban stressors. The avian phenotypic responses towards stressors are discussed based on measurements of body condition, feather FA, and ptilochronology.

A similar dual approach as in Chapter 2 was also taken in **Chapter 3**, except that this chapter focuses on the physiological response ($CORT_f$). Here, I study whether urban birds are better at coping with urban stressors than their rural conspecifics. This question is addressed by challenging birds from both populations with novel stressors (habitat and dietary changes) during aviary experiment. Such novel stressors are predicted to cause stress (Bókony et al., 2012; Chavéz-Zichinelli et al., 2010; Fokidis, Orchinik, & Deviche, 2009a; Meillère et al., 2015). If urban and rural birds have similar coping abilities, then I would predict that post-experiment $CORT_f$ to be similar in both populations. If so, I also expected sparrows fed on their own (mimicked) diet to be less stressed than those fed on the alternative diet, irrespective of their urban or rural origin. Alternatively, if urban sparrows are better at coping with urban stress (indicated by developed desensitization and hence attenuated stress responses), the post-experiment $CORT_f$ of urban individuals is expected to be lower than $CORT_f$ of the rural ones. If this alternative hypothesis is true, then I would expect that rural birds will show higher post-experiment $CORT_f$ than urban birds when subjected to diet change.

While Chapter 2 and 3 concentrate on the effects of urbanization on adult birds, **Chapter 4** discusses the phenotypic and physiologic responses in nestlings. In particular, this chapter provides insight into how different scenarios of nutritional environment (presence vs. absence of supplementary feeding) may affect the phenotype (body condition and feather FA) and physiology ($CORT_f$) of nestlings in suburban and rural areas. I hypothesize that supplementary feeding allows house sparrows to achieve higher breeding success but at the cost of lower nestling quality. As abundant food supplies may permit both high and low-quality nestlings to survive, we also predict that within-brood variation in proxies of nestling quality would be larger for supplemental food broods than for unfed broods.

In **Chapter 5**, I applied my findings on how birds respond to urban stressors from Chapter 2 and 3 in explaining the observed stress levels in urban and rural habitats in Flanders, Belgium. In addition to that, this chapter emphasizes the importance of spatial scales in comparing bird responses. Specifically, the most suitable scale (either local scale, landscape scale, or the combination of both) to be used in urban avian ecology study is identified and discussed. Species persistence in environments depends on individual fitness (Maldonado-Chaparro et al 2017) and fitness is often the results of individual response to their environment (Sol et al 2013). Because species diversity at local scale is affected by the surrounding environments (Litteral & Shochat, 2017), therefore I predict that environments at landscape scale can influence phenotypic responses at local habitat.

I concluded this thesis with a synthesis of the main findings from Chapter 2, 3, 4, and 5 in **Chapter 6** where these results were placed in a wider context of the urban stress ecology. This chapter ends with a reflection on the application of dual approach and suggestions on some aspects of the study that still need further research.

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Predictable food supplies induce plastic shifts in avian scaled body mass

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Modified from:

Salleh Hudin, N., Strubbe, D., Teyssier, A., De Neve, L., White, J., Janssens, G. P. J., & Lens, L. (2016). Predictable food supplies induce plastic shifts in avian scaled body mass. *Behavioral Ecology* 27, 1833-1840.

NSH, AT, LDN, JW, LL designed the study; NSH, AT collected data; NSH performed ptilochronology analysis; NSH, LL performed FA analysis; GPJJ performed chemical analysis of nutrient composition; DS performed statistical analysis; NSH, DS, LDN, LL interpreted data; all authors drafted the manuscript.

Abstract

Urbanization constitutes one of the most profound forms of land-use change and strongly affects global biodiversity and ecosystem functioning. Expansion of urban areas typically leads to species loss but may also induce more subtle changes in species dynamics through selection or plasticity. Using a dual correlative (field) and experimental (aviary) approach, we here show that free-ranging urban house sparrows in southern France were smaller and lighter than their rural counterparts after allometric scaling, whereas two independent indices of nutritional (feather growth rates) and developmental (feather asymmetry) stress did not vary with urbanization. When subjecting these individuals to urban or rural diets in a highly predictable, controlled setting, rural but not urban sparrows decreased their body mass, independent of diet type, to the extent that initial scaled mass differences between urban and rural birds disappeared by the end of the captive period. By integrating field- and aviary measurements of body size and mass with indices of nutritional and developmental stress, we conclude that the lower scaled body masses of urban birds likely reflect a plastic response to predictable food supplies, possibly mediated through predation. Urban environments therefore do not necessarily constitute nutritionally stressful environments for species that typically cohabit with humans, such as house sparrows.

Introduction

Human activities strongly impact on biodiversity and ecosystem functioning, and for terrestrial ecosystems, urbanization represents one of the most profound forms of land-use alteration (Sala et al. 2000). For instance, under current land-use policies, urban land cover is predicted to be threefold higher in 2030 compared to 2000 (Seto et al. 2012) and such unprecedented urban sprawl will inevitably result in biodiversity loss (Sol et al. 2014; McKinney 2008). However, through selection, plasticity and eco-evolutionary feedbacks, urbanization may also induce more subtle changes in gene frequencies or phenotypic trait values that can ultimately affect demographic traits and population dynamics (reviewed in Alberti (2015)). While urbanization effects on species distribution patterns and community structuring are increasingly well documented, how individual organisms may successfully cope with - or adapt to - urban environments, remains less well understood (Evans et al. 2015; Sol et al. 2014; Shochat et al. 2004).

Building on an emerging body of empirical studies, urbanized areas are generally perceived as stressful environments for birds because of increased human activity (e.g. Lowry et al. (2013), noise, light and chemical pollution (Bichet et al. 2013; Tsipoura et al. 2011; Barber et al. 2010; Kempenaers et al.

2010) native predators and/or novel diseases (Brearley et al. 2013; Bradley and Altizer 2007). Yet, for some species, urbanisation may also result in improved ambient conditions, such as through reduced energy expenditure under the heat-island effect (e.g. Somers et al. (2013)) or increased access to anthropogenic food resources from feeders, leftovers or refuse that are typically more abundant and less prone to seasonal fluctuations than natural ones (Anderies et al. 2007; Shochat et al. 2004; Schoech and Bowman 2003).

While variation in food quality, availability and predictability can be expected to shape avian fitness through effects on individual development, survival and/or reproduction (Plummer et al. 2013; Chamberlain et al. 2009; Anderies et al. 2007), mechanistic relationships between food properties, nutritional condition and mass-regulation remain poorly understood (Meillère et al. 2015). For instance, birds are known to accumulate body reserves as a buffer against unpredictable food shortages and to prevent starvation (Cuthill et al. 2000), and individuals with a higher body condition have long been perceived to be in a better shape (Peig and Green 2009). However, evidence is growing that individual mass-regulation is not merely a response to food availability, but rather reflects complex trade-offs between food predictability and perceived predation risk (Cresswell et al. 2009; Macleod and Gosler 2006; Macleod et al. 2005a). Predation risk indeed constitutes an important constraint on foraging behavior of small passerines, as prolonged foraging to increase body condition may extend exposure time to predators, in addition to the fact that heavier birds may have a reduced escape capacity (Macleod et al. 2005b; Gentle and Gosler 2001; Gosler et al. 1995).

Building on the *Starvation-Predation Risk* hypothesis outlined above, individuals can be expected to maintain a lower body condition when food is abundant and predictable (such as in urban environments) to optimize escape capacity from predators, and to increase their fat reserves when food becomes unpredictable (such as during winter in natural environments) to outlive harsh conditions (Cresswell et al. 2009; Brodin 2007; McNamara et al. 2005). Alternatively - or simultaneously - improved winter survival in urban environments (e.g. Robb et al. (2008); Evans et al. (2015)) can cause resource overmatching due to increased population densities (Møller et al. 2012), and hence, may result in lower body reserves due to reduced individual foraging success and due to the persistence of “loser individuals” in the population (*Resource-Matching* hypothesis; Anderies et al. (2007); Shochat et al. (2004)). Apart from food availability and predictability, urban diets may also differ in nutritional value and therefore differentially fulfill nutritional and physiological needs, especially of nestlings (examples in Chamberlain et al. (2009); Heiss et al. (2009); Mennechez and Clergeau (2006); Peach et al. (2015); Sauter et al.

(2006); Seress et al. (2012); Sumasgutner et al. (2014)). To what extent qualitative differences in urban and rural diets may affect nutritional condition after fledging, however, has rarely been addressed in urban ecology studies (Auman et al. 2011; Reynolds et al. 2003; Schoech and Bowman 2003).

In this study, we experimentally test whether, and to what extent, food conditions associated with urban and rural habitats may affect nutritional condition in house sparrows [*Passer domesticus* (Linnaeus, 1758)]. Despite being a species that typically cohabits with humans, urban sparrow populations are currently undergoing consistent population declines across Europe, whereas rural populations seem to show recent signs of recovery after a past decline (De Laet and Summers-Smith 2007). Urban sparrows were earlier shown to be largely dependent on anthropogenic food sources (Anderson 2006) that are typically high in available carbohydrates and saturated fat, and low in fiber (De Filippo et al. 2010; Diamond 2002). Comparative studies on urban and rural sparrow populations in Hungary and France further showed that urban individuals are on average smaller than rural ones but do not have lower scaled mass indices (Meillère et al. 2015; Bókony et al. 2012), suggesting that urban and rural sparrows do not differ in body condition. Using a multi-component approach, both studies further inferred that adult sparrows do not experience the urban environment as "nutritionally stressful" (Meillère et al. 2015; Bókony et al. 2012).

We subjected house sparrows trapped in three urban and three rural populations in southern France to a common garden experiment in which individuals were provided with food that mimicked either an urban or a rural diet. In this way, we imposed a dietary change in some groups (urban birds provided with a rural diet and rural birds provided with an urban diet) while diets remained unchanged in an equal number of urban and rural control groups. In all treatments, food was provided ad libitum, hence mimicking a highly predictable (urban) environment. Before and after the treatment, we obtained detailed measurements of body condition and two independent measurements of (food) stress, i.e. fluctuating asymmetry (FA) and ptilochronology of original and induced (homologous) tail feathers. FA refers to small random deviations from perfect symmetry and reflects an organism's ability to cope with developmental stress (Lens et al. 2002), while ptilochronology uses daily feather growth rates to assess an individual's nutritional stress during the period of feather growth (Vangestel and Lens 2011; Grubb Jr and Cimprich 1990). First, we compared levels of phenotypic variation between wild-caught individuals from urban and rural populations. Individuals under increased stress were thereby predicted to show higher FA and smaller growth bars, and we used these patterns to interpret possible differences in body condition under natural conditions. Next, we assessed whether, and to what extent, the experimentally imposed dietary shift affected changes in body condition.

Methodology

Study area and sampling procedures

House sparrows were captured with mist-nets between September 18th and October 5th 2014 in three urban plots (Toulouse, Tarbes and Pau; latitude-longitude coordinates: 43°36'17"N, 1°26'50"E; 43°13'57"N, 0°4'41"E and 43°18'N, 0°22'42"W, resp.) and three rural plots (Caraman, Montégut-Savès and Cologne; 43°31'42"N, 1°44'11"E; 43°25'57"N, 0°58'11"E and 43°42'57"N, 0°55'39"E, resp.) in southern France (see Fig. 1a). All urban plots were located within the city centre and characterized by a high density of buildings and very limited vegetation, whereas rural plots were located near individual farms surrounded

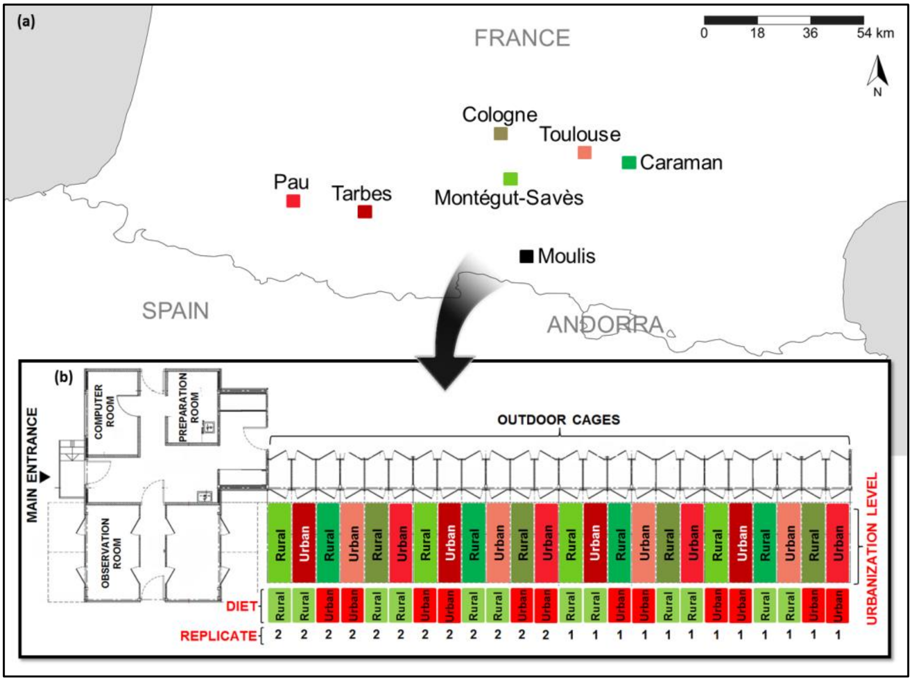

Fig. 1 (a) Sampling locations of three urban (Toulouse, Pau, Tarbes) and three rural (Cologne, Montégut-Savès, Caraman) house sparrow populations in southern France; (b) general design of the aviary experiment. Aviaries were located in Moulis.

by open agricultural fields. Urbanization levels at each trapping site were calculated as the percentage of built-up area within a 100 m radius (corresponding to the average home range of house sparrows during the non-breeding season, (Vangestel et al. 2010) using ArcMap 10. 2.2. MIPY Geo maps (MIPYGeo Grand Public) were available for all sites except Pau. For the latter location, we used a CORINE Land Cover map as urbanization levels calculated from both sets of maps were highly positively correlated (Pearson's $r = 0.96$, $p < 0.001$). Urbanization levels reached 100% in the three urban plots and were consistently low in the three rural plots (Caraman 3.77%; Montégut-Savès 5.03%; Cologne 3.95%). The nearest distance between two neighbouring sampling plots was 25 km, which was eight times larger than the average movement range of house sparrows (< 3.2 km, Anderson (2006) and references therein). Although house sparrows have occasionally been observed dispersing as far as 48 km (Tufto et al. 2005), such large-distance dispersal events are very rare (< 2% individuals, Altwegg et al. (2000)) and sampling plots can therefore be considered independent.

A total of 53 house sparrows were trapped in the three urban plots and 61 sparrows in the three rural ones (Table 1). Upon capture, (i) each individual was ringed, aged (juvenile versus adult), sexed, and weighed (to the nearest 0.01g using a digital balance), (ii) their tarsus length, wing length, bill length and bill height were measured (to the nearest 0.01 mm using a digital calliper), and (iii) the moulting stage of six tail feathers (left body side) was scored as "old" (score 0; based on colour pattern), "active moult" (score 1; < 50% of the fully-grown length), or "new" (score 2; > 50% of the fully-grown length) and a cumulative moult score was calculated for each individual. Next, the fifth (i.e. second outermost) right and left rectrix of each bird was plucked and stored in individually-labelled envelopes for analysis of fluctuating asymmetry and ptilochronology, after which birds were transferred to the experimental

Table 1. Number of house sparrows trapped in three urban and three rural plots in southern France, with indication of sex and age.

Plot	**Urban**			**Rural**		
	Toulouse	**Tarbes**	**Pau**	**Caraman**	**Montégut-Savès**	**Cologne**
Total	19	16	18	20	24	17
Male	12	10	12	12	11	9
Female	7	6	6	8	13	8
Adult	9	12	13	10	22	7
Juvenile	10	4	5	10	2	10

aviaries of the ‘Station d’Ecologie Expérimentale du CNRS’ (Moulis, France, 42°57'29"N, 1°5'11"E).

Experimental manipulation of food quality and predictability

During six weeks, transferred birds were kept in a modular outdoor aviary of which each module measured 4m x 1m with a height of 3m and was equipped with adequate numbers of roosting boxes and bamboo plants for perching (Fig. 1b). Birds from each of the six sampling plots were randomly assigned to an ‘urban diet’ treatment (30% corn, 25% bread, 25% cake, 20% potato chips) or a ‘rural diet’ treatment (49% corn, 24% wheat, 24% sunflower seed, 3% dried mealworm) (Table 2). These diets were selected according to literature review (Anderson (2006) and references therein) and a pilot field experiment in Flanders (Belgium) showing that urban individuals preferred the ‘urban diet’, and vice versa, when both diets were simultaneously provided to free-ranging individuals in a standardized design (De Neve, unpublished data). Diets were subjected to chemical analysis to determine the proximate components (dry matter, crude protein, crude fat, crude ash, crude fiber; Helrich et al. (1994)) as well as neutral and acid detergent fiber (Van Soest et al. 1991), and starch (polarimetry) and sugars (spectrophotometry). Both diets substantially differed in key nutrients such as a lower protein, energy and fiber content in the urban treatment (Table 2).

To avoid that dominance rank might influence access to food by juveniles and/or females and thus influence condition under experimental conditions, food was provided *ad libitum* in six feeders that were evenly distributed across each cage, and in a highly predictable way (i.e. presented every 48 hours). To prevent mineral or vitamin deficiencies, all treatment groups were equally provided with small complements of apple, lettuce and a soluble multi-vitamin supplement used for captive birds (Nekton™-S). Each bird origin*diet treatment combination was replicated twice to account for cage effects (Fig. 1b), hence birds from each of the six plots were assigned to one of four groups, resulting in 24 experimental groups that comprised all possible combinations of bird origin and diet (Fig. 1a,b). The average group size per cage was 4.75±0.74 (range: 3-5) individuals, and age and sex distributions were kept as constant as possible across groups. After assigning birds to the 24 cages, they were left to rest and acclimatize, and first measurements were conducted after two weeks. Overall mortality rates (22/114 ind; 19,3%) did not vary according to bird origin (urban 22,6%; rural 16,4%), sex (males 18,2%; females 20,8%), age (adults 17,8%; juveniles 22,0%) or diet (urban 17,9%; rural 21,1%) (Z-tests: all $p > 0.40$), and results can hence be considered unbiased by sparrow mortality. Body mass was measured once every week while regrown left and right fifth rectrices were collected after six weeks. Animal welfare, maintenance and experimental procedures followed the regulations and guidelines from the Directions Régionales de l'Environnement, de l'Aménagement et du Logement of France (permit number: 31-2014-09). All individuals were released at their original capture site after completion of the experiment.

Table 2. Nutrient composition of urban and rural diet used in the aviary experiment.

Parameters	**Fresh material**		**Dry matter**	
	Urban diet	**Rural diet**	**Urban diet**	**Rural diet**
Dry matter (%)	83.60	89.78	100.00	100.00
Moisture (%)	16.40	10.22	0.00	0.00
Ash (%)	1.85	1.59	2.22	1.77
Crude protein (%)	6.71	12.03	8.03	13.40
Crude fat (%)	11.76	15.06	14.07	16.77
Crude fiber (CF) (%)	0.80	1.00	0.96	1.12
Nitrogen-free extract (NFE) (%)	62.48	60.10	74.72	66.94
Acid detergent fiber (%)	1.35	3.73	1.62	4.16
Neutral detergent fiber (%)	13.74	26.33	16.44	29.33
Starch (%)	41.92	39.02	50.14	43.46
Sugars (%)	4.13	3.30	4.94	3.68
Gross energy (MJ/kg)*	17.30	19.51	20.70	21.73
Metabolisable energy (MJ/kg)**	12.61	13.98	15.09	15.57

*calculated according to Schiemann et al. (1971): GE (kJ/kg) = 23.9 CP + 39.7 EE + 20.0 CF + 17.4 NFE, with nutrient concentrations expressed in g/kg.

**calculated according to Larbier & Leclerq (1992): ME = 0.155 CP + 0.343 EE + 0.167 St + 0.130 Su.

CP is g of crude protein, EE is g of ether-extract, St is g of starch and Su is g of sugars (mono- and disaccharides) per kg of food.

Indices of sparrow body condition

While body condition is traditionally quantified using the residuals from an ordinary least squares analysis of body mass against body size, such procedure may violate various key assumptions (e. g. that functional relationships between body mass and size are linear, see Green (2001) for details). Therefore, here, we applied the Scaled Mass Index (SMI, Peig and Green (2009)), 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 and Green 2009). Following Bókony et al. (2012), we first determined that tarsus length was most strongly correlated with body mass on a log-log scale ($r = 0.667$, $p < 0.001$) and consequently applied that variable to scale body mass. After excluding 3 outliers (i.e. |standardized residual| > 3), the regression slope was 1.77, whereas average tarsus length was 18.4 mm. We thus calculated the scaled mass index as body mass x $(18.4/\text{tarsus length})^{1.77}$ (Peig and Green 2010; 2009). The slope of the SMA regression of log-mass on log-tarsus was similar for males and females ($p = 0.410$) and for juveniles and adults ($p = 0.253$). The scaled mass index was not correlated with wing length ($r = 0.056$, $p = 0.565$, $N = 114$) or bill length ($r = –0.11$, $p = 0.418$, $N = 114$). These two lines of results imply that by calculating the scaled mass index, we successfully controlled for body size differences among individuals. A similar tarsus-based scaling was applied to bill height, bill length and wing length.

Measurement of fluctuating asymmetry and ptilochronology

Levels of environmental and nutritional stress within/between urban and rural plots (prior to the experiment) and within/between dietary treatments (during the experiment) were assessed through measurements of fluctuating asymmetry (FA) and ptilochronology on original and induced feathers. All feathers were processed by a single person (NSH), as follows. First, each collected feather was pinned on a separate, white, polystyrene board and the total feather length (for FA analysis) was measured to the nearest 0.01 mm with a digital calliper. Second, 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. Third, each marked board was scanned (Océ OP1130) and growth bar widths (for ptilochronological analysis) were automatically measured with image analysis software (KS400 Zeiss). The entire procedure was repeated twice in time (i.e. starting with pinning the feather on the board), resulting in two independent measurements per feather. The level of repeatability among repeated measurements was calculated according to Lessells and Boag (1987), whereby the within-group and between-group mean squares were derived from one-way ANOVA analysis. Repeatability estimates were high, both for total length (original feathers: right trait side $r = 0.998$, $N = 59$, $p < 0.001$; left trait side $r = 0.999$, $N = 63$, $p < 0.001$; induced feathers: right trait side $r = 0.995$, $N = 83$, $p < 0.001$; left trait side $r = 0.928$, $N = 85$, $p < 0.001$) and for mean growth bar width (original feathers: right trait side $r = 0.78$; $N = 58$, $p < 0.001$; left trait side $r = 0.88$, $N = 62$, $p < 0.001$; induced feathers: right trait side $r = 0.93$, $N = 83$, $p < 0.001$; left trait side $r = 0.91$, $N = 85$, $p < 0.001$).

Because the degree of FA is often very small (typically in the order of 1% or less of the size of the trait, Møller and Swaddle (1997)) and because some traits cannot be measured with high accuracy, measurement error (ME) can be expected to cause an upward bias in the between-sides variance if not appropriately corrected for (Van Dongen et al. 1999; Merilä and Björklund 1995; Palmer and Strobeck 1986). Therefore, feather FA was analysed through mixed-regression analysis with Restricted Maximum Likelihood (REML) parameter estimation (Van Dongen et al. 1999). While yielding identical FA estimates as two-way mixed ANOVA models, the REML method allows to test for FA significance, to model heterogeneity in FA and measurement error among populations or treatments, to test for directional asymmetry (DA), and to obtain estimates of individual FA that are unbiased with respect to ME and DA. Fixed intercepts estimate overall trait size, fixed slopes estimate DA, and the random intercepts and slopes (both estimated *within* individuals) estimate the variation in individual trait value and individual FA, respectively (Van Dongen et al. 1999). Based on this analysis, variance due to ME was not heterogeneously distributed between sampling plots or treatment groups (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-ration test: $p < 0.0001$). FA measurements were not biased by directional asymmetry ($F_{1,55} = 0.16$; $p = 0.695$; denominator degrees of freedom by 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.

To account for possible confounding effects of variation in moulting stage on the properties of naturally-grown feathers, we calculated the residual values of feather FA and growth bar width based on a multiple regression model with cumulative moult score and total feather length as fixed factors, and sparrow ID as nested random effect (% variation explained: model with FA, $r^2 = 0.04$; model with growth bar width, $r^2 = 0.11$). These residuals were used for hypothesis testing.

Hypothesis testing

First, we tested for phenotypic differences between urban and rural sparrows prior to our experimental treatment. Therefore, we modelled both raw and scaled data on body mass, bill length, bill height and wing length as dependent variables and related these to urbanization (urban vs. rural origin), sex and age applying linear mixed models with capture location as a random effect (lmer function of R packages lme4 and lmerTest). Two-way interactions between fixed effects were modelled as well. For analyses of body mass and scaled mass index, time of capture was additionally included as covariate. Denominator degrees of freedom were computed by the default Satterthwaite's method. For all analyses on feather properties, multiple measurements on feathers from the same bird were accounted for by including sparrow identity as a random effect nested within capture location. Following Bókony et al. (2012), we adopted a frequentist approach whereby full models (i.e. models containing all explanatory variables considered here) were reduced in a stepwise manner, by excluding the variable with the highest p-value until only $p < 0.05$ predictors remained. Statistics and p-values mentioned in the text and tables are from the minimal model (all significant terms included), whereas statistics and p-values of non-significant terms were obtained by fitting each non-significant term separately into the minimal model.

Second, we tested experimental treatment effects on temporal variation in sparrow SMI under captivity. We therefore applied a linear mixed model with urbanization, sex, age, week of experiment and diet type as fixed factors. We first included the three-way interactions between week, urbanization and either diet or age, followed by the two-way interactions week*urbanization, week*diet and week*age. Cage, capture location and sparrow identity were included as random effects. FA and ptilochronological measurements on rectrices regrown after 6 weeks were analysed using linear mixed models. Urbanization, sex, age and diet treatment were coded as fixed effects, capture location and sparrow identity were specified as the random effects. All statistical analyses were performed in R statistical computing software (R Development Core Team 2015).

Results

Phenotypic differences between urban and rural sparrows

Sparrows captured in urban plots weighed less, were smaller, and had less heavy bills than their rural counterparts (Table 3, and see Table S1 in Supplementary Appendix S1). Body mass was also lower for juveniles than for adults. Males generally had larger wings while urban juvenile birds exhibited the smallest wing sizes. Urban birds had a significantly smaller SMI, as even after scaling, urban sparrows were on average about 3% lighter than rural birds. When scaling was applied to bill length and height, differences in beak morphology between rural and urban birds failed to reach significance, indicating that the differences found among the absolute values were due to allometric scaling. After scaling, urban birds also had significantly longer wings than rural birds, males had longer wings than females and juveniles were characterized by the shortest wing lengths. In contrast, unsigned FA values and mean growth bar widths did not differ between urban and rural birds (all $p > 0.94$, see Table S2 in Supplementary Appendix

S1), nor were they related to individual body mass or SMI (all p > 0.16).

Experiment

During the experimental treatment, SMI of rural sparrows decreased while those of urban ones remained stable (week*urbanization: 0.15±0.073, *d.f.* = 381, *t* = 2.03, p = 0.044; Fig. 2, see Table S3 in Supplementary Appendix S1). This effect was independent of diet or age (Fig. 2), as none of the relevant 2- or 3-way interactions were significant (Table S3 in Supplementary Appendix S1). At the end of the experiment, rural SMI values were only 1.5% higher than those of urban birds (compared to about 3% at capture; differences no longer significant: 0.45±0.43, *d.f.* = 83, *t* = 1.04, p = 0.30). Unsigned FA values measured from regrown feathers collected at the end of the experiment did not differ in relation to urbanization, diet or age (Table S4 in Supplementary Appendix S1) but tended to be higher than at capture, although not significantly (*t* = -1.21, *d.f.* = 41, p = 0.24). Likewise, growth bar widths of regrown feathers did not differ in relation to urbanization, diet or age (Table S4 in Supplementary Appendix S1) but were 19.6 % smaller compared to the original feathers (*t* = 36.8, *d.f.* = 739, p < 0.001), despite the fact that the total length of original and regrown feathers did not differ (*t* = 0. 367, *d.f.* = 109.99, p = 0.71).

Table 3. Statistical modelling of phenotypic variation among free-ranging urban and rural sparrows. Initial models included sparrow origin, sex and age (and trapping time for body mass and condition), non-significant variables are not shown. Estimates for "urbanization" refer to urban vs. rural birds; "age" compares juveniles vs. adults; "sex" compares females vs. males.

Dependent variable	**Predictor**	**Estimate/SD**	***d. f.***	***t***	**p**	**r^2**
Body mass (g)	urbanisation	-3.23±0.40	3. 85	-8.05	0.0015	0. 54
	age	1.25±0.35	92.	-3.52	<0.001	
Scaled Mass Index (SMI)	urbanisation	-0.79±0.35	102.	-2.26	0.026	0.17
	age	-1.31±0.37	102.	-3.57	<0.001	
Wing length (mm)	sex	2.06±0.51	100.0	4.05	<0.001	0. 35
	urbanisation*age	-2.88±1.05	100.0	-2.74	0.0072	
Wing length (scaled)	urbanisation	1.46±0. 54	101.	2.69	0.0083	0. 24
	sex	1.74±0. 55	101.	3.18	0.0020	
	age	-1.88±0. 57	101.	-3.33	0.0012	
Tarsus length (mm)	urbanisation	-0.98±0.17	3.97	-5.88	0.0043	0. 28
Bill height (mm)	urbanisation	-0.39±0.064	103.	-6.10	<0.001	0. 28
Bill length (mm)	urbanisation	-0.47±0.12	4. 46	-3.96	0.014	0.15

Discussion

Using a two-pronged (i.e. correlative and experimental) approach, we show that free-ranging house sparrows from urban populations in southern France were smaller than their rural counterparts for all traits except wing length. Urban birds were also lighter than rural ones, even when accounting for absolute size differences by scaling body mass against tarsus length. While such results are often interpreted as a signature of lower body condition, individual-based indices of nutritional (feather growth) and developmental (feather asymmetry) stress were not higher in free-ranging urban birds. When temporarily subjecting sparrows trapped in urban and rural populations to experimental feeding treatments in captivity, birds originating from rural populations decreased their body mass whereas body masses from urban birds remained stable, to such an extent that initial (scaled) mass differences disappeared by the end of the experiment. The fact that during the food experiment, bar widths and FA of newly-grown feathers did not vary with origin of the birds nor diet treatment, indicates that birds originating from rural populations did not suffer from higher captive or nutritional stress than those originating from urban populations, and further supports the hypothesis that the lower scaled masses observed in free-ranging urban sparrows reflect a plastic response to predictable food supplies, possibly mediated through predation.

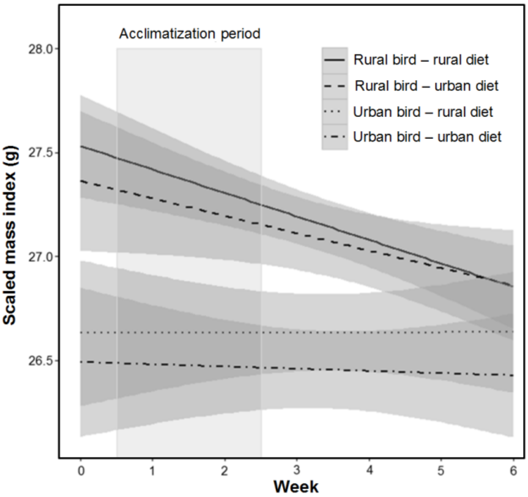

Fig. 2 Temporal shifts in scaled body mass (SMI) during a common-garden experiment in which house sparrows originating from urban and rural populations were provided with highly predictable food mimicking either an urban or rural diet. Week 0 represents data during capture. See text for details.

The fact that free-ranging urban birds were smaller than rural ones most likely reflects conditions during the nestling phase, given that sparrow tarsi are full-grown at fledging (Anderson 2006). Nestlings of multiple bird species have indeed been found to be smaller and lighter in urban areas, suggesting that for many species, natural nestling food may be restricted and that anthropogenic food sources do not constitute adequate alternatives (reviewed by Chamberlain et al. (2009)). Invertebrates constitute an important component of sparrow nestling diets, and supplemental feeding of invertebrate prey was earlier shown to increase reproductive success of this species in urban areas (Peach et al. 2015). Likewise, (Vincent 2005) showed that sparrow fledgling mass was positively related to the proportion of Diptera in their diet. Yet, in our study, free-ranging urban sparrows weighed less than rural ones also after scaling for body size, which appears to be in line with the "*Resource Overmatching*" hypothesis (Shochat et al. 2004) predicting lower intake rates due to high population densities with the persistence of "loser" individuals. However, the distribution of scaled body masses did not support the prediction that urban populations were numerically dominated by birds with a low body condition. Instead, SMI data showed a platykurtic distribution (kurtosis < 3; 2.54 and 2.67 respectively) with rural birds exhibiting a stronger positive skewness than urban ones (0.055 versus 0.21), hence indicating that rural populations hosted more (rather than less) individuals with a relatively lower SMI than urban ones. Finally, two independent phenotypic stress markers (feather growth and feather FA) did not provide evidence either that urban sparrows would be in worse condition than rural ones.

Sparrows originating from rural populations decreased their body masses to "urban levels" under predictable food conditions in captivity, while no mass change was observed in birds from urban origin, irrespective of the diet provided. This finding suggests a strategic mass regulation in response to the lower risk of starvation associated with a predictable food regimen (*Starvation-Predation risk hypothesis*), rather than a signature of nutritional stress. Several other studies, too, refuted the assumption that smaller and leaner urban sparrows would be inferior to their larger and heavier rural conspecifics. For example, (Chávez-Zichinelli et al. 2010) found no consistent differences in corticosterone (as stress indicator) or immunoglobulin concentrations (as indicator of immune function) between rural and urban house sparrows, while (Meillère et al. 2015) failed to find differences in muscle score, haematocrit, and both baseline and stress-induced corticosterone levels. To fully test the *Starvation-Predation risk* hypothesis and understand why body mass regulation or nutritional stress indicators did not vary with captive diet, it would have been preferable to experimentally subject urban and rural birds to an unpredictable food regime as well. However, minimal nutrient requirements need to be known before such experiment can be conducted in an ethical way, which is very difficult given that metabolic rates, and therefore also nutrient requirements, have been shown to vary with body condition (Van Weyenberg et al. 2008).

By integrating the correlative and experimental results outlined above, we conclude that the leaner sparrows found in urban areas are most probably not in poorer condition compared to their heavier rural counterparts, but rather regulate their body masses in a plastic response to predictable food supplies, possibly mediated through predation. Predation pressure is likely to vary locally, and although we do not have quantitative data on actual or perceived predation pressure in our study area, Seress et al. (2011) experimentally showed that Hungarian urban sparrows (in particular juveniles) responded more strongly to simulated predator attacks, which was interpreted as evidence for higher exposure rates to predation. If so, lowering body mass through strategic mass regulation (facilitated by high food predictability) may indeed be advantageous for urban birds through increased take-off ability and flight performance (Witter and Cuthill (1993) and references therein). In line with this, urban birds in our study had both a lower SMI and longer wings after scaling (Table 2), and as such, showed larger wing loads compared to urban birds. Still, the assumption of differential predation pressure along urban gradients remains a contested and hotly-debated issue in urban ecology (Newsome et al. 2015; Sorace 2002; Gering and Blair 1999), and more experimental studies are therefore needed to test the relative importance of predation and starvation risk in influencing avian.

Acknowledgements

We are indebted to H. Matheve, P. Vantieghem and H. De Rycke for field- and lab assistance, and to A. Chaine for logistic support with the aviary experiments. The field sampling protocol was approved by the Ethical Committee of Ghent University. Sparrows were caught under capture and ringing permit n°15038 (French National Natural History Museum). The aviary experiment was conducted in the experimental aviaries of the Station d'Ecologie Expérimentale du CNRS at Moulis (France).

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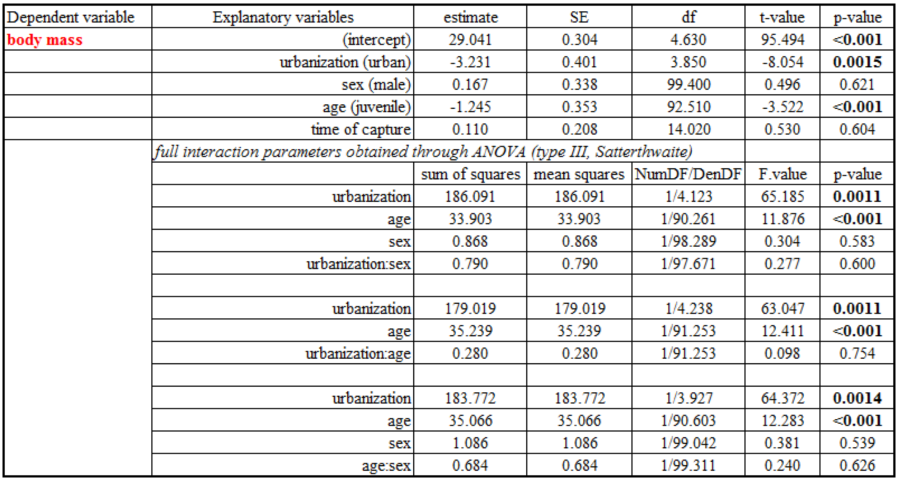
Supplementary Materials

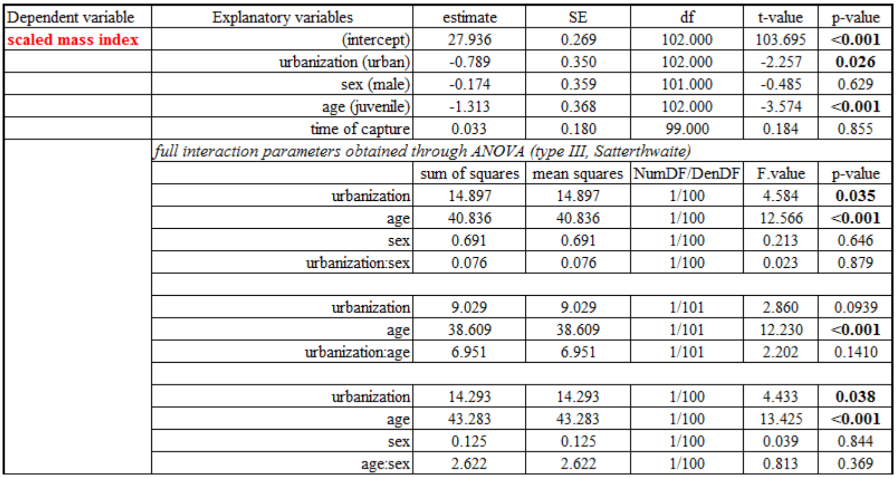
Table S1. Statistical modelling of phenotypic variation among free-ranging urban and rural sparrows. Model selection started with full models (i.e. models containing all explanatory variables considered), and variables were removed in a stepwise manner, by excluding the variable with the highest p-value until only $P < 0.05$ predictors remained. Statistics and p-values mentioned in the text and tables are from the minimal model (all significant terms included), whereas statistics and P-values of non-significant terms were obtained by fitting each non-significant term separately into the minimal model.

Table S1a: body mass



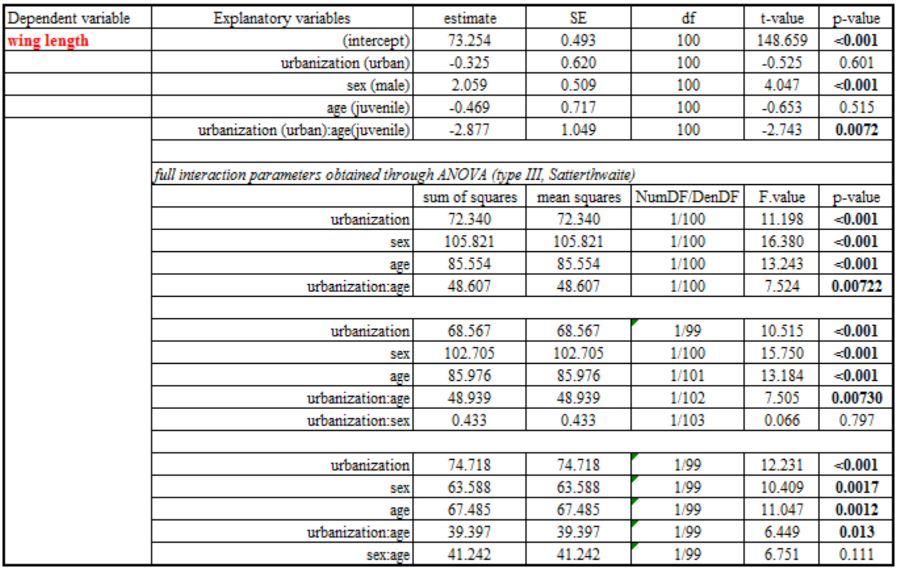
Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
body mass	(intercept)	29.041	0.304	4.630	95.494	**<0.001**
	urbanization (urban)	-3.231	0.401	3.850	-8.054	**0.0015**
	sex (male)	0.167	0.338	99.400	0.496	0.621
	age (juvenile)	-1.245	0.353	92.510	-3.522	**<0.001**
	time of capture	0.110	0.208	14.020	0.530	0.604
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	186.091	186.091	1/4.123	65.185	**0.0011**
	age	33.903	33.903	1/90.261	11.876	**<0.001**
	sex	0.868	0.868	1/98.289	0.304	0.583
	urbanization:sex	0.790	0.790	1/97.671	0.277	0.600
	urbanization	179.019	179.019	1/4.238	63.047	**0.0011**
	age	35.239	35.239	1/91.253	12.411	**<0.001**
	urbanization:age	0.280	0.280	1/91.253	0.098	0.754
	urbanization	183.772	183.772	1/3.927	64.372	**0.0014**
	age	35.066	35.066	1/90.603	12.283	**<0.001**
	sex	1.086	1.086	1/99.042	0.381	0.539
	age:sex	0.684	0.684	1/99.311	0.240	0.626

Table S1b: body condition



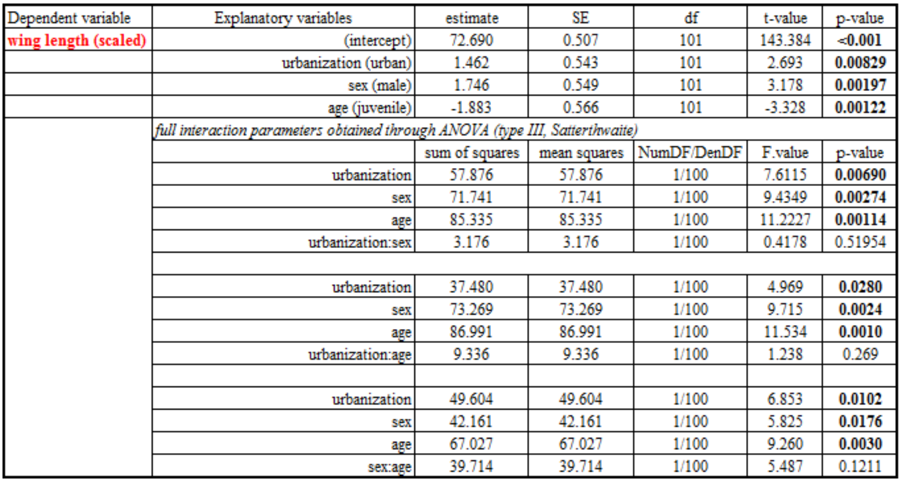
Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
scaled mass index	(intercept)	27.936	0.269	102.000	103.695	**<0.001**
	urbanization (urban)	-0.789	0.350	102.000	-2.257	**0.026**
	sex (male)	-0.174	0.359	101.000	-0.485	0.629
	age (juvenile)	-1.313	0.368	102.000	-3.574	**<0.001**
	time of capture	0.033	0.180	99.000	0.184	0.855
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	14.897	14.897	1/100	4.584	**0.035**
	age	40.836	40.836	1/100	12.566	**<0.001**
	sex	0.691	0.691	1/100	0.213	0.646
	urbanization:sex	0.076	0.076	1/100	0.023	0.879
	urbanization	9.029	9.029	1/101	2.860	0.0939
	age	38.609	38.609	1/101	12.230	**<0.001**
	urbanization:age	6.951	6.951	1/101	2.202	0.1410
	urbanization	14.293	14.293	1/100	4.433	**0.038**
	age	43.283	43.283	1/100	13.425	**<0.001**
	sex	0.125	0.125	1/100	0.039	0.844
	age:sex	2.622	2.622	1/100	0.813	0.369

Table S1c: wing length



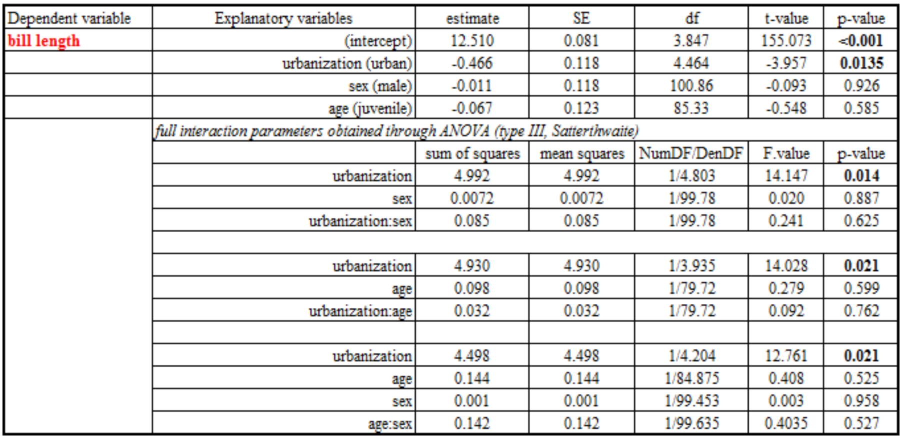
Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
wing length	(intercept)	73.254	0.493	100	148.659	**<0.001**
	urbanization (urban)	-0.325	0.620	100	-0.525	0.601
	sex (male)	2.059	0.509	100	4.047	**<0.001**
	age (juvenile)	-0.469	0.717	100	-0.653	0.515
	urbanization (urban):age(juvenile)	-2.877	1.049	100	-2.743	**0.0072**
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	72.340	72.340	1/100	11.198	**<0.001**
	sex	105.821	105.821	1/100	16.380	**<0.001**
	age	85.554	85.554	1/100	13.243	**<0.001**
	urbanization:age	48.607	48.607	1/100	7.524	**0.00722**
	urbanization	68.567	68.567	1/99	10.515	**<0.001**
	sex	102.705	102.705	1/100	15.750	**<0.001**
	age	85.976	85.976	1/101	13.184	**<0.001**
	urbanization:age	48.939	48.939	1/102	7.505	**0.00730**
	urbanization:sex	0.433	0.433	1/103	0.066	0.797
	urbanization	74.718	74.718	1/99	12.231	**<0.001**
	sex	63.588	63.588	1/99	10.409	**0.0017**
	age	67.485	67.485	1/99	11.047	**0.0012**
	urbanization:age	39.397	39.397	1/99	6.449	**0.013**
	sex:age	41.242	41.242	1/99	6.751	0.111

Table S1d: wing length (scaled)



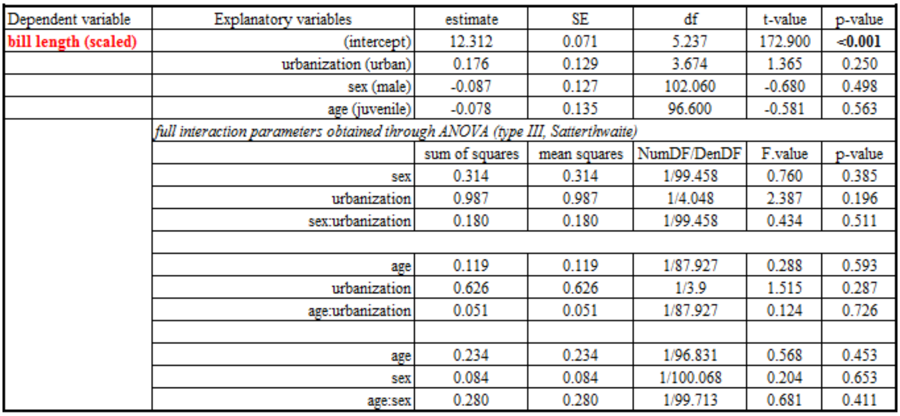
Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
wing length (scaled)	(intercept)	72.690	0.507	101	143.384	**<0.001**
	urbanization (urban)	1.462	0.543	101	2.693	**0.00829**
	sex (male)	1.746	0.549	101	3.178	**0.00197**
	age (juvenile)	-1.883	0.566	101	-3.328	**0.00122**
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	57.876	57.876	1/100	7.6115	**0.00690**
	sex	71.741	71.741	1/100	9.4349	**0.00274**
	age	85.335	85.335	1/100	11.2227	**0.00114**
	urbanization:sex	3.176	3.176	1/100	0.4178	0.51954
	urbanization	37.480	37.480	1/100	4.969	**0.0280**
	sex	73.269	73.269	1/100	9.715	**0.0024**
	age	86.991	86.991	1/100	11.534	**0.0010**
	urbanization:age	9.336	9.336	1/100	1.238	0.269
	urbanization	49.604	49.604	1/100	6.853	**0.0102**
	sex	42.161	42.161	1/100	5.825	**0.0176**
	age	67.027	67.027	1/100	9.260	**0.0030**
	sex:age	39.714	39.714	1/100	5.487	0.1211

Table S1e: bill length



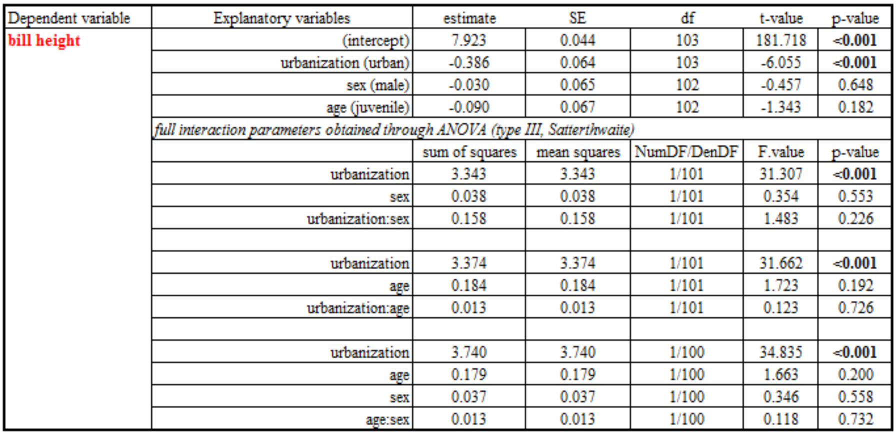
Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
bill length	(intercept)	12.510	0.081	3.847	155.073	**<0.001**
	urbanization (urban)	-0.466	0.118	4.464	-3.957	**0.0135**
	sex (male)	-0.011	0.118	100.86	-0.093	0.926
	age (juvenile)	-0.067	0.123	85.33	-0.548	0.585
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	4.992	4.992	1/4.803	14.147	**0.014**
	sex	0.0072	0.0072	1/99.78	0.020	0.887
	urbanization:sex	0.085	0.085	1/99.78	0.241	0.625
	urbanization	4.930	4.930	1/3.935	14.028	**0.021**
	age	0.098	0.098	1/79.72	0.279	0.599
	urbanization:age	0.032	0.032	1/79.72	0.092	0.762
	urbanization	4.498	4.498	1/4.204	12.761	**0.021**
	age	0.144	0.144	1/84.875	0.408	0.525
	sex	0.001	0.001	1/99.453	0.003	0.958
	age:sex	0.142	0.142	1/99.635	0.4035	0.527

Table S1f: bill length (scaled)



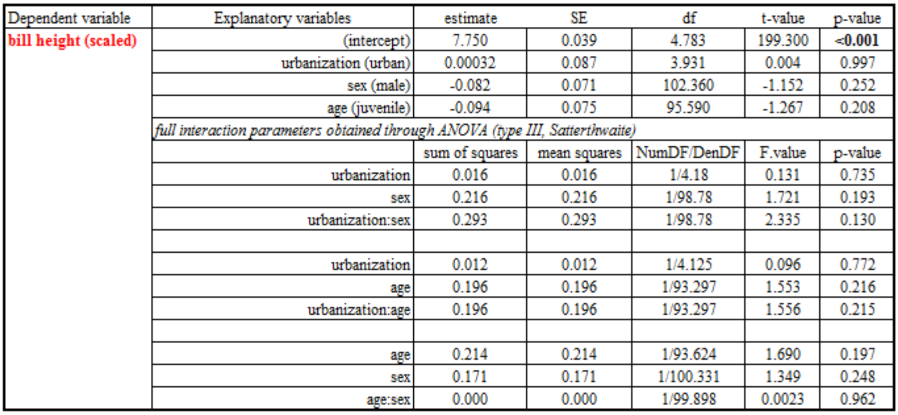
Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
bill length (scaled)	(intercept)	12.312	0.071	5.237	172.900	**<0.001**
	urbanization (urban)	0.176	0.129	3.674	1.365	0.250
	sex (male)	-0.087	0.127	102.060	-0.680	0.498
	age (juvenile)	-0.078	0.135	96.600	-0.581	0.563
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	sex	0.314	0.314	1/99.458	0.760	0.385
	urbanization	0.987	0.987	1/4.048	2.387	0.196
	sex:urbanization	0.180	0.180	1/99.458	0.434	0.511
	age	0.119	0.119	1/87.927	0.288	0.593
	urbanization	0.626	0.626	1/3.9	1.515	0.287
	age:urbanization	0.051	0.051	1/87.927	0.124	0.726
	age	0.234	0.234	1/96.831	0.568	0.453
	sex	0.084	0.084	1/100.068	0.204	0.653
	age:sex	0.280	0.280	1/99.713	0.681	0.411

Table S1g: bill height



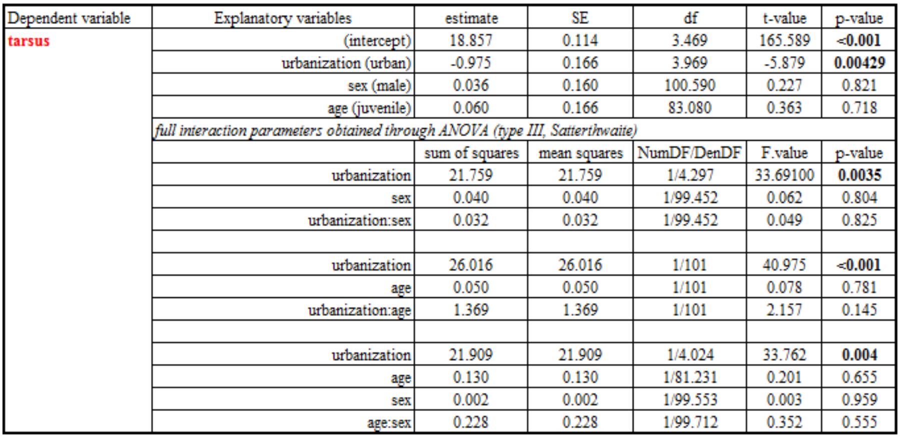
Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
bill height	(intercept)	7.923	0.044	103	181.718	**<0.001**
	urbanization (urban)	-0.386	0.064	103	-6.055	**<0.001**
	sex (male)	-0.030	0.065	102	-0.457	0.648
	age (juvenile)	-0.090	0.067	102	-1.343	0.182
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	3.343	3.343	1/101	31.307	**<0.001**
	sex	0.038	0.038	1/101	0.354	0.553
	urbanization:sex	0.158	0.158	1/101	1.483	0.226
	urbanization	3.374	3.374	1/101	31.662	**<0.001**
	age	0.184	0.184	1/101	1.723	0.192
	urbanization:age	0.013	0.013	1/101	0.123	0.726
	urbanization	3.740	3.740	1/100	34.835	**<0.001**
	age	0.179	0.179	1/100	1.663	0.200
	sex	0.037	0.037	1/100	0.346	0.558
	age:sex	0.013	0.013	1/100	0.118	0.732

Table S1h: bill height (scaled)



Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
bill height (scaled)	(intercept)	7.750	0.039	4.783	199.300	**<0.001**
	urbanization (urban)	0.00032	0.087	3.931	0.004	0.997
	sex (male)	-0.082	0.071	102.360	-1.152	0.252
	age (juvenile)	-0.094	0.075	95.590	-1.267	0.208
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	0.016	0.016	1/4.18	0.131	0.735
	sex	0.216	0.216	1/98.78	1.721	0.193
	urbanization:sex	0.293	0.293	1/98.78	2.335	0.130
	urbanization	0.012	0.012	1/4.125	0.096	0.772
	age	0.196	0.196	1/93.297	1.553	0.216
	urbanization:age	0.196	0.196	1/93.297	1.556	0.215
	age	0.214	0.214	1/93.624	1.690	0.197
	sex	0.171	0.171	1/100.331	1.349	0.248
	age:sex	0.000	0.000	1/99.898	0.0023	0.962

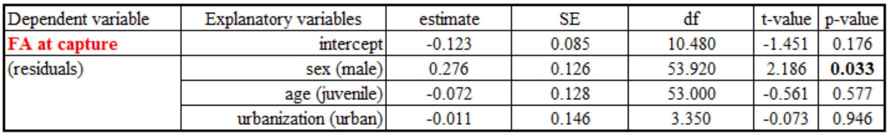
Table S1i: tarsus



Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
tarsus	(intercept)	18.857	0.114	3.469	165.589	**<0.001**
	urbanization (urban)	-0.975	0.166	3.969	-5.879	**0.00429**
	sex (male)	0.036	0.160	100.590	0.227	0.821
	age (juvenile)	0.060	0.166	83.080	0.363	0.718
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	21.759	21.759	1/4.297	33.69100	**0.0035**
	sex	0.040	0.040	1/99.452	0.062	0.804
	urbanization:sex	0.032	0.032	1/99.452	0.049	0.825
	urbanization	26.016	26.016	1/101	40.975	**<0.001**
	age	0.050	0.050	1/101	0.078	0.781
	urbanization:age	1.369	1.369	1/101	2.157	0.145
	urbanization	21.909	21.909	1/4.024	33.762	**0.004**
	age	0.130	0.130	1/81.231	0.201	0.655
	sex	0.002	0.002	1/99.553	0.003	0.959
	age:sex	0.228	0.228	1/99.712	0.352	0.555

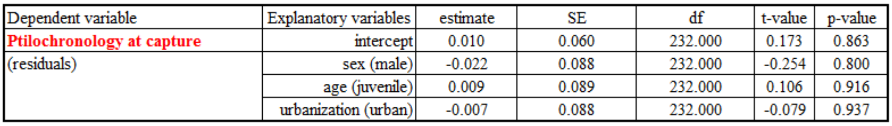
Table S2. Statistical modelling of fluctuating asymmetry (FA) and ptilochronological growth bars of free-ranging urban and rural sparrows.

Table S2a: FA at capture



Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
FA at capture	intercept	-0.123	0.085	10.480	-1.451	0.176
(residuals)	sex (male)	0.276	0.126	53.920	2.186	**0.033**
	age (juvenile)	-0.072	0.128	53.000	-0.561	0.577
	urbanization (urban)	-0.011	0.146	3.350	-0.073	0.946

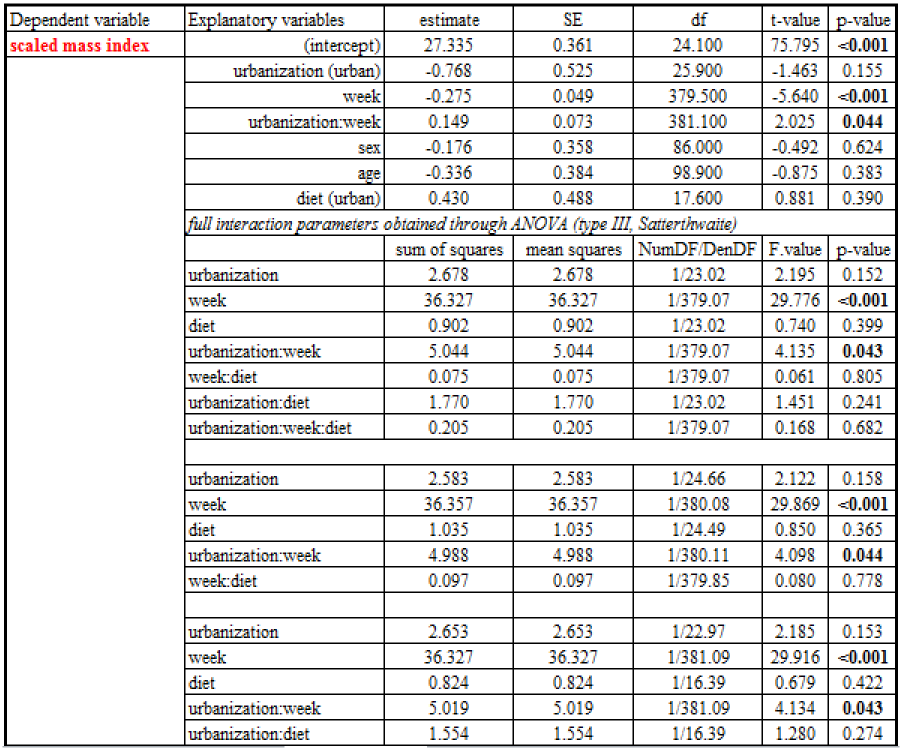
Table S2b: Ptilochronology at capture



Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
Ptilochronology at capture	intercept	0.010	0.060	232.000	0.173	0.863
(residuals)	sex (male)	-0.022	0.088	232.000	-0.254	0.800
	age (juvenile)	0.009	0.089	232.000	0.106	0.916
	urbanization (urban)	-0.007	0.088	232.000	-0.079	0.937

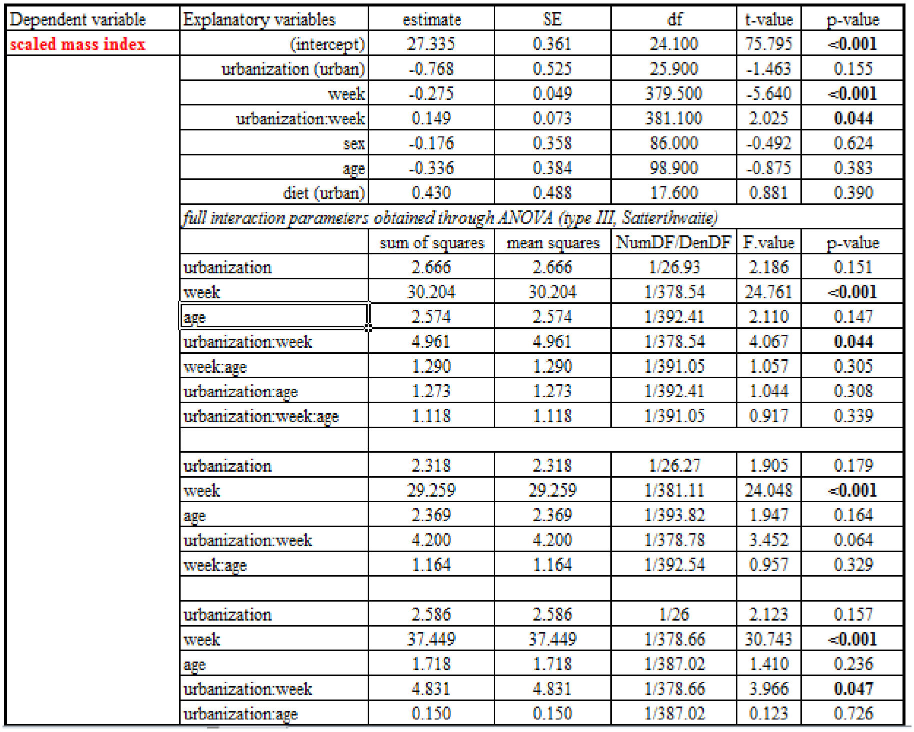
Table S3. Temporal shifts in scaled body mass (SMI) during a common-garden experiment in which house sparrows originating from urban and rural populations were provided with highly predictable food mimicking either an urban or rural diet.

Table S3a: model including three-way interaction between week, urbanization and diet



Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
scaled mass index	(intercept)	27.335	0.361	24.100	75.795	**<0.001**
	urbanization (urban)	-0.768	0.525	25.900	-1.463	0.155
	week	-0.275	0.049	379.500	-5.640	**<0.001**
	urbanization:week	0.149	0.073	381.100	2.025	**0.044**
	sex	-0.176	0.358	86.000	-0.492	0.624
	age	-0.336	0.384	98.900	-0.875	0.383
	diet (urban)	0.430	0.488	17.600	0.881	0.390
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	2.678	2.678	1/23.02	2.195	0.152
	week	36.327	36.327	1/379.07	29.776	**<0.001**
	diet	0.902	0.902	1/23.02	0.740	0.399
	urbanization:week	5.044	5.044	1/379.07	4.135	**0.043**
	week:diet	0.075	0.075	1/379.07	0.061	0.805
	urbanization:diet	1.770	1.770	1/23.02	1.451	0.241
	urbanization:week:diet	0.205	0.205	1/379.07	0.168	0.682
	urbanization	2.583	2.583	1/24.66	2.122	0.158
	week	36.357	36.357	1/380.08	29.869	**<0.001**
	diet	1.035	1.035	1/24.49	0.850	0.365
	urbanization:week	4.988	4.988	1/380.11	4.098	**0.044**
	week:diet	0.097	0.097	1/379.85	0.080	0.778
	urbanization	2.653	2.653	1/22.97	2.185	0.153
	week	36.327	36.327	1/381.09	29.916	**<0.001**
	diet	0.824	0.824	1/16.39	0.679	0.422
	urbanization:week	5.019	5.019	1/381.09	4.134	**0.043**
	urbanization:diet	1.554	1.554	1/16.39	1.280	0.274

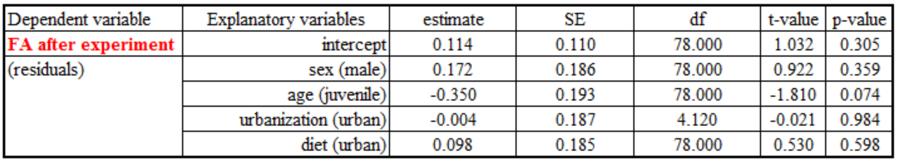
Table S3b: model including three-way interaction between week, urbanization and age



Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
scaled mass index	(intercept)	27.335	0.361	24.100	75.795	<**0.001**
	urbanization (urban)	-0.768	0.525	25.900	-1.463	0.155
	week	-0.275	0.049	379.500	-5.640	<**0.001**
	urbanization:week	0.149	0.073	381.100	2.025	**0.044**
	sex	-0.176	0.358	86.000	-0.492	0.624
	age	-0.336	0.384	98.900	-0.875	0.383
	diet (urban)	0.430	0.488	17.600	0.881	0.390
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F.value	p-value
	urbanization	2.666	2.666	1/26.93	2.186	0.151
	week	30.204	30.204	1/378.54	24.761	<**0.001**
	age	2.574	2.574	1/392.41	2.110	0.147
	urbanization:week	4.961	4.961	1/378.54	4.067	**0.044**
	week:age	1.290	1.290	1/391.05	1.057	0.305
	urbanization:age	1.273	1.273	1/392.41	1.044	0.308
	urbanization:week:age	1.118	1.118	1/391.05	0.917	0.339
	urbanization	2.318	2.318	1/26.27	1.905	0.179
	week	29.259	29.259	1/381.11	24.048	<**0.001**
	age	2.369	2.369	1/393.82	1.947	0.164
	urbanization:week	4.200	4.200	1/378.78	3.452	0.064
	week:age	1.164	1.164	1/392.54	0.957	0.329
	urbanization	2.586	2.586	1/26	2.123	0.157
	week	37.449	37.449	1/378.66	30.743	<**0.001**
	age	1.718	1.718	1/387.02	1.410	0.236
	urbanization:week	4.831	4.831	1/378.66	3.966	**0.047**
	urbanization:age	0.150	0.150	1/387.02	0.123	0.726

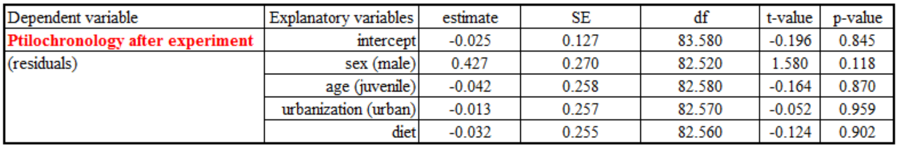
Table S4. Statistical modelling of fluctuating asymmetry (FA) and ptilochronological growth bars of urban and rural sparrows at the end of the common-garden experiment.

Table S4a: FA at the end of the common-garden experiment



Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
FA after experiment	intercept	0.114	0.110	78.000	1.032	0.305
(residuals)	sex (male)	0.172	0.186	78.000	0.922	0.359
	age (juvenile)	-0.350	0.193	78.000	-1.810	0.074
	urbanization (urban)	-0.004	0.187	4.120	-0.021	0.984
	diet (urban)	0.098	0.185	78.000	0.530	0.598

Table S4b: Ptilochronology at the end of the common-garden experiment



Dependent variable	Explanatory variables	estimate	SE	df	t-value	p-value
Ptilochronology after experiment	intercept	-0.025	0.127	83.580	-0.196	0.845
(residuals)	sex (male)	0.427	0.270	82.520	1.580	0.118
	age (juvenile)	-0.042	0.258	82.580	-0.164	0.870
	urbanization (urban)	-0.013	0.257	82.570	-0.052	0.959
	diet	-0.032	0.255	82.560	-0.124	0.902

Are Urban Birds Better At Coping With Chronic Stress? An Experimental Study

3

Modified from:

Salleh Hudin, N., De Neve, L., Strubbe, D., Teyssier, A., Aerts, J., Fairhurst, G. D., White, J., & Lens, L.. (2017). Are urban birds better at coping with chronic stress? An experimental study (manuscript).

NSH, AT, LDN, JW, LL designed the study; NSH, AT collected data; NSH performed ptilochronology analysis; LL, NSH performed FA analysis; JA, NSH performed CORT analysis; DS performed statistical analysis; NSH, DS, LDN, LL interpreted data; all authors drafted the manuscript.

CHAPTER 3

Abstract

The release of corticoid hormones allows animals to cope with environmental challenges that cause fluctuations in energetic demands (allostatic state) and so to restore energy balance. Urban environments expose individuals to abiotic and biotic challenges but may also constitute stable environments that buffer seasonal fluctuations in resource availability and the stochasticity of natural environments. In line with these ideas, urban and rural bird populations were often found to have similar baseline and stress induced plasma corticosterone levels. Here we aimed at disentangling possible underlying mechanisms that could explain these patterns: (i) urban environments are not more stressful, (ii) urban birds are habituated to known stressors, (iii) urban birds developed dampened stress responses. Wild-caught urban and rural house sparrows (*Passer domesticus*) were exposed to a combined captivity and diet treatment. We measured corticosterone in natural moulted feathers plucked prior to the experiment ($CORT_f$) and induced feathers grown during the experiment ($CORT_{f\_ind}$) as a long-term integrated measure of stress physiology. Urban and rural house sparrows had similar corticosterone levels under natural conditions, but also in response to novel stressors caused by the experiment, supporting the growing notion that urban environments may not constitute stressful environments during the non-breeding season. Yet, $CORT_{f\_ind}$ did vary according to the age and sex of the birds, and the strength of these intrinsic effects also varied with urbanisation. Juveniles and males originating from urban populations had the highest $CORT_{f\_ind.}$ We could not find evidence that $CORT_f$ was consistent within individuals across different moults. Complex mechanisms behind CORT deposition in feathers could partially explain these results, and our study stresses the need of incorporating both intrinsic and environmental factors for the interpretation of variation in $CORT_f$ between populations.

Introduction

All organisms experience numerous daily routines, seasonal life-history cycles and environmental challenges that cause fluctuations in their energetic demands, also referred to as 'allostatic state' (McEwen & Wingfield, 2003). One of the central mechanisms by which organisms respond to these challenges in order to maintain their energetic balance is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which releases glucocorticoid hormones (Sapolsky, Romero, & Munck, 2000). In birds, for example, corticosterone levels in blood plasma have been widely used to study stress responses as an integrated measure of the challenges placed on an organism and their capacity to cope (Bonier, 2012; Breuner, Patterson, & Hahn, 2008; McEwen & Wingfield, 2003; McEwen & Wingfield, 2010; Sapolsky et al., 2000);(Angelier, Weimerskirch, Dano, & Chastel, 2007; Kitaysky, Piatt, Wingfield, & Romano, 1999; Schoech, Bowman, & Reynolds, 2004). The acute release of corticosterone is often associated with an adaptive short-term survival response, as it re-allocates resources to support immediate vital processes (Breuner et al., 2008; Romero, 2004; Wingfield & Kitaysky, 2002). However, chronic stress may lead to prolonged elevated corticosterone levels due to the frequent and/or long-term exposure to stressors (Cyr, Earle, Tam, & Romero, 2007; Ellis, McWhorter, & Maron, 2012), resulting in damage of health, reproduction and survival, rather than protection (Breuner et al., 2008; Kitaysky, Kitaiskaia, Piatt, & Wingfield, 2003; Koren et al., 2012; Lendvai, Giraudeau, & Chastel, 2007; McEwen, 1998; Wingfield & Romero, 2001). Yet, organisms may habituate to repeated stressors by learning that these are harmless and can be ignored. If so, the HPA axis becomes no longer activated when exposed to these particular stressors, but the individual maintains the full ability to respond to novel ones (Cyr & Romero, 2009). Alternatively, repeated challenges may cause alterations in HPA activity and attenuate the release of stress hormones without the perception that a challenge is no longer threatening (desensitization, (Cyr & Romero, 2009; Rich & Romero, 2005)). In such case, the stressful environment affects the organism-wide stress physiology which can incur costs as it may comprise an individual’s ability to respond to ecologically-relevant stressors (Cyr & Romero, 2009). The most extreme response to severe stressors is exhaustion in which the physiological system breaks down, and stress hormones decrease because the stress response cannot longer be maintained (Cyr & Romero, 2009).

As urban environments continue to expand and encroach into natural ones, understanding how organisms cope with anthropogenic disturbances becomes increasingly important in view of sustainable conservation and management strategies (Alberti, 2015; Ellis et al., 2012; Magle, Hunt, Vernon, & Crooks, 2012; Marzluff, 2001; Shochat, Warren, Faeth, McIntyre, & Hope, 2006). Owing to the expanding body of literature on urban birds, general patterns have emerged on characteristics that enable some species to colonize and thrive in cities over others (Bonier, Martin, & Wingfield, 2007; Davey, Chamberlain, Newson, Noble, & Johnston, 2012; Evans, Chamberlain, Hatchwell, Gregory, & Gaston, 2011; Kark, Iwaniuk, Schalimtzek, & Banker, 2007). However, even in successful species, urbanisation continues to expose individuals to abiotic and biotic challenges, such as high human activity, noise and light pollution, toxin levels and non-native predators/diseases (e.g. (Bichet et al., 2013; Bradley & Altizer, 2007; Grimm et al., 2008; Lowry, Lill, & Wong, 2013)). Yet, for other populations, urban habitats may as well constitute stable environments that buffer seasonal fluctuations in resource availability and the stochasticity of natural environments (Anderies, Katti, & Shochat, 2007; Fokidis, Greiner, & Deviche, 2008), and hence, may cause lower - rather than higher - allostatic fluctuations. Thus, the net allostatic load of urban individuals can either be expected to be higher or lower than of conspecifics living in more natural habitats, depending on how individuals experience urban challenges versus opportunities (Bonier, 2012). In line with these contradicting predictions, patterns of variation in baseline and stress-induced plasma corticosterone concentrations among urban and rural bird populations are highly heterogeneous (reviewed by (Bonier, 2012)). For example, in house sparrows (*Passer domesticus*), a common urban-dwelling passerine, several studies found similar baseline corticosterone levels in blood plasma between urban and adjacent rural populations, at least during the non-breeding season (Bókony, Seress, Nagy, Lendvai, & Liker, 2012; Chavéz-Zichinelli et al., 2010; Fokidis, Orchinik, & Deviche, 2009; Meillère, Brischoux, Parenteau, & Angelier, 2015). Such pattern could either result from the fact that (i) urban environments are not experienced as stressful as commonly thought, (ii) urban individuals have habituated to known stressors in their environment, maintaining a similar stress physiology as rural individuals (Bonier, 2012; Cyr & Romero, 2009), and/or (iii) urban birds exhibit desensitization and have thereby developed a changed stress physiology with dampened stress-responses to novel stimuli (Atwell et al., 2012; Cyr & Romero, 2009).

In order to disentangle these different mechanisms, we exposed wild-caught urban and rural house sparrows to a double challenge experiment by (i) translocating them to an outdoor aviary and (ii) supplying them with food that either mimicked an urban or rural diet, in a full-factorial design. Earlier studies found evidence for increased baseline and stress-induced plasma glucocorticoid concentrations due to captivity, even when sampled after weeks (Cyr & Romero, 2008; Marra, Kevin, & Bruce, 1995), while captive individuals can be predicted to experience the highest chronic stress when supplied with a diet that deviates most strongly from their natural one. Because corticosterone is deposited continuously in feathers as they grow, feather corticosterone ($\mathrm{CORT_f}$) is believed to provide a long-term integrated measure of both baseline and acute levels in response to allostatic demands during feather synthesis, and hence, a more holistic signal of stress-physiology compared to the “snap-shot” of a blood sample (reviewed in Romero and Fairhurst (2016). To quantify corticosterone concentrations in sparrow tail feathers ($\mathrm{CORT_f}$), we applied a recent UPLC-MS/MS method that was earlier shown to reduce the problem of cross-reactivity due to its high specificity. Prior to sacrificing tail feathers for $\mathrm{CORT_f}$ analysis, we also measured their levels of ptilochronology (growth bar width; (Grubb & Cimprich, 1990)) and fluctuating asymmetry (FA; Lens, Van Dongen, Kark, and Matthysen (2002)) as complementary indices of nutritional and developmental stress, respectively (Grubb & Cimprich, 1990; Vangestel & Lens, 2011).

We first compared $CORT_f$ from original tail feathers (i.e. regrown after natural moult) that reflected the natural stress levels to which individuals had been exposed prior to trapping. Next we quantified $CORT_f$ from the homologous feathers grown during experimental treatment (i.e. regrown after induced moult) to quantify the extent to which urban and rural house sparrows differed in their ability to cope with, and habituate to, novel stressors (i.e. captivity and diet treatments; Cyr and Romero (2009)). Since existing literature found no consistent patterns in the effects of urbanization on avian physiological response (Bókony et al., 2012; Chavéz-Zichinelli et al., 2010; Fokidis et al., 2009; Meillère et al., 2015), we thereby predicted $CORT_f$ in induced feathers to be similar between urban and rural sparrows. If so, we expected sparrows fed on their own (mimicked) diet to be less stressed than those fed on the alternative diet, irrespective of their urban or rural origin. Alternatively, if urban sparrows developed desensitisation (and hence attenuated stress responses), $CORT_f$ in induced feathers of urban individuals was expected to be lower compared to induced feathers of rural ones, while a diet change was predicted to affect the latter individuals more strongly than the former.

Methodology

Study area and sampling procedures

House sparrows were captured with mist-nets between September 18th and October 5th 2014 in three urban plots (Toulouse, Tarbes and Pau; latitude-longitude coordinates: 43°36'17"N, 1°26'50"E; 43°13'57"N, 0°4'41"E and 43°18'N, 0°22'42"W, resp.) and three rural plots (Caraman, Montégut-Savès and Cologne; 43°31'42"N, 1°44'11"E; 43°25'57"N, 0°58'11"E and 43°42'57"N, 0°55'39"E, resp.) in southern France. Urbanization levels at each trapping site were calculated as the percentage of built-up area within a 100 m radius (corresponding to the average home range size of house sparrows during the non-breeding season (Vangestel, Braeckman, Matheve, & Lens, 2010) using ArcMap 10. 2.2. MIPY Geo maps (MIPYGeo Grand Public) were available for all sites except Pau. For the latter location, we used a CORINE Land Cover map as urbanization levels calculated from both sets of maps were highly positively correlated (Pearson's $r = 0.96$, $p < 0.001$). Urbanization levels reached 100% in the three urban plots and were consistently low in the three rural plots (Caraman 3.77%; Montégut-Savès 5.03%; Cologne 3.95%). The nearest distance between two neighbouring sampling plots was 25 km, which was eight times larger than the average movement range of house sparrows (< 3.2 km, Anderson (2006) and references therein). Although house sparrows have occasionally been observed dispersing as far as 48 km (Tufto, Ringsby, Dhondt, Adriaensen, & Matthysen, 2005), such large-distance dispersal events are very rare (< 2% individuals, Altwegg, Ringsby, and SÆther (2000)) and sampling plots can therefore be considered statistically independent.

A total of 53 house sparrows were trapped in the three urban plots and 61 sparrows in the three rural ones. Upon capture, each individual was ringed, aged (juvenile versus adult) and sexed, and the moult stage of six tail feathers (left body side) was scored as “old” (score 1; based on color and wear), “active moult” (score 2; < 50% of the full-grown feather length), or “new” (score 3; > 50% of the full-grown feather length). In some rare cases “no feather” was observed, which could be due to lost during capture or when a moulting feather was not yet visible; these cases were assigned a score of two (i.e. the average score). Cumulative moult scores of all six rectrices were calculated for each bird. Next, the fifth (i.e. second outermost) right and left rectrix of each bird was plucked and stored in individually-labeled envelopes. For each of the plucked feathers, a “moult state” category was assigned based on the categories outlined above but with the following additional subdivisions: (i) “Old feather” (**O**), (ii) “Moulting feather”, which were either too small to pluck (**M$_1$** – moulting but not plucked) or too small for corticosterone analysis (**M$_2$** – moulting plucked). (iii) “New feather” (> 50% of the full-grown feather length) which were either still growing (**N$_1$**) or fully grown (**N$_2$**). All new feathers were large enough for corticosterone analyses. (iv) “Lost feather” of which the moult state was not known (excluded from further analyses). The age-related distribution of feather states across populations is shown in Figure S1. After sampling, all birds were transferred to the experimental aviaries of the ‘Station d’Ecologie Expérimentale du CNRS’ (Moulis, France, 42°57'29"N, 1°5'11"E).

Challenge experiment

Transferred birds were kept in a modular outdoor aviary, of which each module (further referred to as ‘cage’) measured 4m x 1m with a height of 3m and was equipped with adequate numbers of roosting boxes and bamboo plants for perching. Birds from each of the six sampling plots were randomly assigned to a diet mimicking either an urban (‘urban diet’ treatment; 30% corn, 25% bread, 25% cake, 20% potato chips) or rural diet (‘rural diet’ treatment; 49% corn, 24% wheat, 24% sunflower seed, 3% dried mealworm) which differed in nutrient composition (for further details see (Salleh Hudin et al., 2016)).

Each bird origin*diet treatment combination was replicated twice to account for cage effects, hence birds from each of the six plots were assigned to one of four groups, resulting in 24 experimental groups that comprised all possible combinations of bird origin and diet. The average group size per cage was 4.75±0.74 (range: 3-5) individuals, and age and sex distributions were kept as constant as possible across groups (Salleh Hudin et al., 2016). The length of the induced feathers was measured once every week and after six weeks, before release of the birds, the fully grown induced left and right fifth rectrices were collected. Growth time of the induced feather was defined as the number of weeks it took for the feather to complete growth (range 3 – 6 weeks, accuracy 0.5). This value was derived from the correlation between week and feather length, for each feather separately. For example, if a feather showed linear growth until week 4 and then stopped growing, the growth time received a score of 4. Alternatively, when growth in feather length was linear until week 3 and slowed down between week 3 and 4, after which it did not increase any more, then growth time got a score of 3.5.

Animal welfare, maintenance and experimental procedures followed the regulations and guidelines from the Directions Régionales de l'Environnement, de l'Aménagement et du Logement of France (permit number: 31-2014-09). All individuals were released at their original capture site after completion of the experiment. Sample sizes of natural and induced feathers in relation to population of origin, age and sex can be found in Table S1.

Quantification of $CORT_f$ level

A single feather, weighing on average 10 mg, was sampled and dirt (*e.g.* feces, mud, etc.) was manually removed by using tweezers. Next, the 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 was determined on an analytical balance (g). By 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 ethanol followed by ultrapure water and dried with a paper tissue to avoid cross-contamination between samples. Of the homogenized sample 0.0100 g ± 0.0005 g was weighed into a 10 ml test tube. Subsequently, 8 ml of methanol was added as extraction solvent and 10 µL of a corticosterone-d_8 solution of 0.5 µg L^{-1} was added as internal standard. When smaller amounts of feather tissue were used, the volume of corticosterone-d_8 was adapted accordingly. The sample was vortex-mixed for 30 s, placed on an overhead shaker at 60 rpm for 1 h at room temperature, and centrifuged for 10 min at 3500 g at 7 °C. All supernatant was taken, evaporated to dryness under nitrogen at 60 °C using a nitrogen evaporator, and reconstituted in 5 ml H_2O/MeOH (80:20; v/v). After conditioning a C_{18} SPE column with 3 ml of methanol followed by 3 ml of ultrapure water, the sample was loaded. The column was washed with 4.5 ml H_2O/MeOH (65:35; v/v) and retained compounds were eluted with 2.5 ml H_2O/MeOH (20:80; v/v) into a 10 ml test tube and evaporated to dryness under nitrogen at 60 °C using a nitrogen evaporator. The sample was finally reconstituted in 50 µL H_2O/MeOH (80:20; v/v) in a vial and analyzed by means of UPLC-MS/MS. Since in future research matrix-matched calibration curves are not practically feasible, calibration curves were made in diluent and a factor of 10000 was used to calculate the corresponding GC concentration in a standardized sample weight of 10 mg of feather.

Feather ptilochronology and fluctuating asymmetry (FA)

Total feather length, feather mass and growth bar widths (ptilochronology) were measured by a single person (NSH) following the detailed procedures outlined in (Salleh Hudin et al., 2016). Measurements were repeated resulting in two independent measurements per feather, which were highly repeatable (Salleh Hudin et al., 2016). Feather quality was estimated as the residuals from the regression between feather mass and feather length ($r^2 = 0.78$, N=113 feathers; De La Hera, Pérez-Tris, and Tellería (2010)).

Because the degree of FA is often very small (typically in the order of 1% or less of the size of the trait (Møller & Swaddle, 1997), and because some traits cannot be measured with high accuracy, measurement error (ME) can be expected to cause an upward bias in the between-sides variance if not appropriately corrected for (Merilä & Björklund, 1995; Palmer & Strobeck, 1986; Van Dongen, Molenberghs, & Matthysen, 1999). Therefore, feather FA was analyzed through mixed-regression analysis with restricted maximum likelihood (REML) parameter estimation (Van Dongen et al., 1999). While yielding identical FA estimates as 2-way mixed ANOVA models, the REML method allows to test for FA significance, to model heterogeneity in FA and ME among populations or treatments, to test for directional asymmetry (DA), and to obtain estimates of individual FA that are unbiased with respect to ME and DA. Fixed intercepts estimate overall trait size, fixed slopes estimate DA, and the random intercepts and slopes (both estimated *within* individuals) estimate the variation in individual trait value and individual FA, respectively (Van Dongen et al., 1999). Based on this analysis, variance due to ME was not heterogeneously distributed between sampling plots or treatment groups (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 (δ^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 by Satterthwaite's formula following (Verbeke & 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.

Statistical analysis

In order to explore if $CORT_f$ in natural feathers varied according to urbanisation level (urban *vs.* rural), Linear Mixed Models (LMM) were fitted, including the population of origin as a random intercept. We also added the intrinsic factors sex and age (juvenile *vs* adult) to the model, as it is known that stress physiology can vary between ages and sexes (Bonier, 2012; Bonier, Martin, Sheldon, et al., 2007; Dantzer, Fletcher, Boonstra, & Sheriff, 2014). Feather length was included as a covariate, because $CORT_f$ is influenced by the length of the feather (Patterson, Kitaysky, Lyons, & Roby, 2015) as it is passively deposited during feather formation (Bortolotti, 2010; Jenni-Eiermann, Helfenstein, Vallat, Glauser, & Jenni, 2015; Romero & Fairhurst, 2016). In addition moult state (Old *vs* New feather) was added as a fixed factor (see Results for the rationale behind this).

To test if $CORT_f$ in naturally-moulted feathers was related to proxies of nutritional and developmental stress (ptilochronology and feather FA, respectively), we built two separate LMM's, which allowed testing if correlations between $CORT_f$ and each proxy varied between sexes, ages (adult *vs.* juvenile) or urbanization levels (urban *vs.* rural). Origin was always included as random factor and feather length and moult state as covariates.

From 85 birds, we succeeded in extracting a full-grown induced tail feather. Sample sizes were fairly evenly distributed among treatments, ages and sexes (Fig. S2A) and each treatment included induced feathers proceeding from old, moulting and new feathers (Fig. S2B). Previous work showed that variation in ptilochronology and fluctuating asymmetry of induced feathers was not significantly explained by urbanisation, diet, sex or age (Salleh Hudin et al., 2016). Here we additionally tested if variation in these measurements were affected by moult state of the natural feather as well as growth time and feather length of the induced feather. We also analysed by means of a LMM if induced feather quality (mass corrected for length) was affected by urbanisation level (urban *vs* rural origin), diet treatment, sex or age, including all 2-way interactions and the 3-way interactions urbanisation*diet*sex and urbanisation*diet*age. We also included moult state of the natural feather and growth time of the induced feather to test if these variables explained additional variation in induced feather quality. Cage nested in origin was included as random effect.

For 55 individuals of which we obtained $\mathrm{CORT_f}$ levels from both natural and induced feathers, we tested if CORT levels between both feather sets were correlated, and to what extent these correlations varied with urbanisation level, diet treatment, moult state of the natural feather, age and/or sex. In this analysis, natural feathers were either New or Old as moulting feathers were not large enough to analyse CORT levels (see above), hence explaining the reduced sample size.

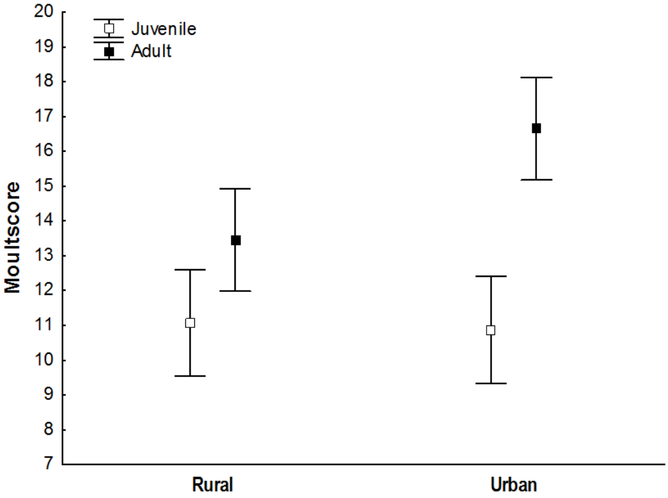
To test for differences in stress physiology ($\mathrm{CORT_{f\_ind}}$) between urban and rural birds in response to our challenge experiment, we applied a LMM with urbanisation, diet treatment, sex, age and all two-way and three-way interactions as fixed factors. Moult state of the natural feather and length of the induced feather were included as covariates (Jenni-Eiermann et al., 2015; Romero & Fairhurst, 2016), while cage nested within population of origin was included as random factor. Because $\mathrm{CORT_f}$ of natural feathers was not significantly related to $\mathrm{CORT_{f\_ind}}$ (see Results), we did not include the former as a covariate, hence allowing a sample size of 85 individuals. Results were very similar when we did include $\mathrm{CORT_f}$ of natural feathers as a covariate in the model (not shown). To examine if variation in $\mathrm{CORT_{f\_ind}}$ was explained by ptilochronology and FA of induced feathers, two separate LMM's were fitted including urbanisation, diet, sex and age, as well as their interactions with FA or ptilochronology as predictors.

During hypothesis testing, full models (i.e. models containing all explanatory variables considered above) were reduced by excluding effects with the highest P-value until AIC of reduced models did not decrease compared to the previous less parsimonious model. Interactions were always removed before main effects. Significant statistics and P-values mentioned in the text and tables refer to the final minimal model, whereas statistics and P-values of non-significant terms were obtained by fitting each non-significant term separately into the minimal model.

Results

Moult scores of natural feathers did not differ between sexes (LMM, $F_{1,105} = 0.06$, $p = 0.81$), but significantly differed between urban and rural populations, depending on age (LMM, urbanization*age, $F_{1,108} = 8.51$, $p = 0.0043$, Fig. 1). Adults from urban populations initiated their moult earlier than those from rural ones, while most juvenile birds were not yet moulting at the time of the study (Fig. 1). As a consequence, moult state of extracted tail feathers in which CORT was measured was unevenly distributed among populations and ages (see Fig. S1). To verify whether $\mathrm{CORT_f}$ was confounded with

Fig 1. Average moultscore (± SE) in juveniles and adults from urban and rural populations. Moultscore ranges from 6 to 18, and higher scores indicate advanced moult (see Material and Methods).



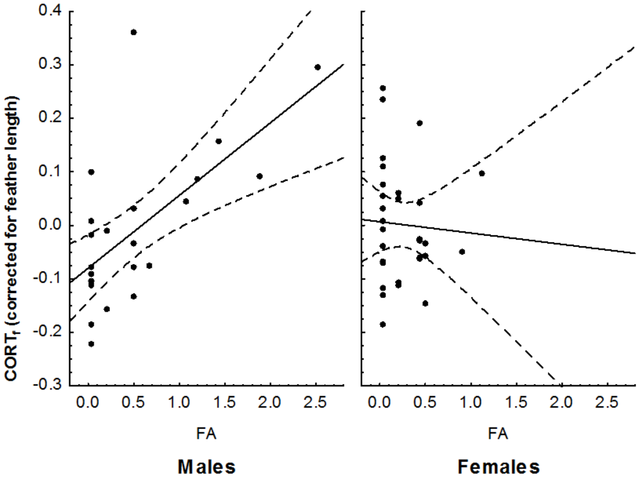
moult state (e.g. between-year variation in environmental condition may result in differences in feather quality and stress physiology of old and new feathers (Patterson et al., 2015; Romero & Fairhurst, 2016)), we modeled proxies of adult feather quality (length, quality, ptilochronology) in relation to moult state, urbanisation and sex by means of a GLMM's with population of origin included as a random factor. Females had shorter ($F_{1,29.9}$=12.92, P=0.0012) and lower quality feathers ($F_{1,31}$=4.34, P=0.045) than males, but none of the other relationships were significant (all P>0.29). Hence, environmental conditions during moult were apparently quite similar between years, even though moult status did vary with population of origin and age and was therefore included as covariate during hypothesis testing (similar results were obtained if old and new feathers were modeled separately; results not shown). Under natural conditions, $CORT_f$ did not significantly differ between rural and urban areas, nor between sexes or ages (all P>0.11, Table 1). Feather FA was positively related to $CORT_f$ in males but not in females (LMM, sex*FA: $F_{1,48}$=3.63, P=0.063, Table S2, Fig. 2), while ptilochronology and interactions with urbanisation, sex or age did not explain significant variation in $CORT_f$ (LMM, all P>0.14).

Variation in ptilochronology of induced feathers was marginally explained by the moult state of the natural feather ($F_{4,68.2}$ = 2.43, P=0.055, Fig. 3), after controlling for length ($F_{1,70.3}$ = 10.08, P=0.0022, estimate 1.24 ± 0.39) and growth time ($F_{1,67.4}$ = 21.35, P<0.001, estimate -0.76 ± 0.16) of the induced

Table 1. Results from the LMM testing $\mathrm{CORT_f}$ in natural feathers in relation to urbanization, sex and age. Significant effects from the minimal model are shown in bold. Statistics and P-values of non-significant terms were obtained by fitting each non-significant term separately into the minimal model. Estimates ± SE are shown for New feathers versus Old feathers, Female versus Male, Adult versus Juvenile, Rural versus Urban. N= 60 feathers. Similar results were obtained when including 12 new growing feathers.

	Estimate	**DF**	**F**	**P**
Main effects/covariates				
Feather Length	**0.326 ± 0.041**	**1.58**	**65.51**	**<0.0001**
Moult state	-0.011 ± 0.045	1.57	0.06	0.805
Sex	-0.0013 ± 0.035	1.57	0.00	0.971
Age	0.053 ± 0.045	1.57	1.33	0.253
Urbanization	0.014 ± 0.034	1.57	0.17	0.679
Interactions				
Urbanization*Sex		1.55	0.01	0.909
Urbanization*Age		1.55	0.11	0.745
Age*Sex		1.55	2.73	0.110
Urbanisation*Sex*Age		4.51	0.83	0.512

Fig 2. $\mathrm{CORT_f}$ (corrected for feather length) in relation to FA in males and females. Full model results can be found in Table S3.

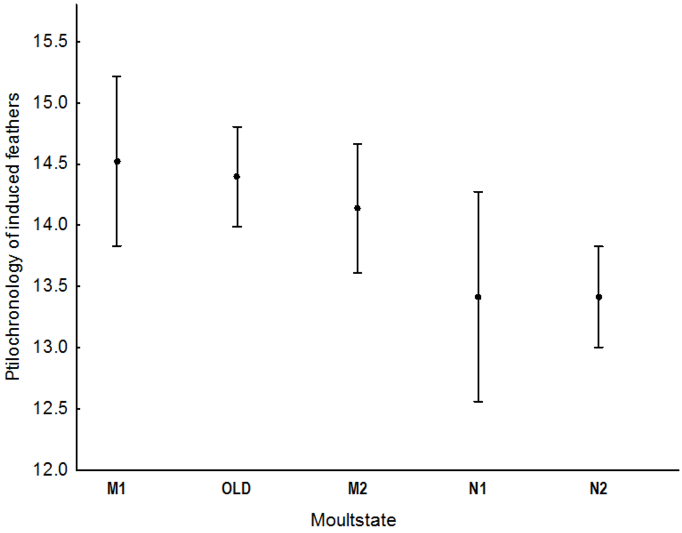


feather. The largest growth bar widths and fastest growth rates were measured in feathers of which regrowth was not experimentally induced but rather resulted from natural moult (category M_1, see Material and Methods) and in old feathers. In contrast, feathers induced by plucking naturally-moulted new feathers, showed the slowest growth rate (Fig. 3). Furthermore, FA of induced feathers was not affected by moult state (LMM $F_{4,65.8} = 1.41$, P=0.24), but was positively correlated with the growth time of the induced feather (LMM $F_{1,74} = 6.32$, P=0.014, estimate 0.28 ± 0.11).

When comparing the subset of induced feathers proceeding from extracted naturally-moulted fullgrown new feathers, then induced feathers were shorter, lighter and had lower ptilochronology measurements (Repeated measurements, both $P < 0.0001$).

Variation in induced feather quality (mass corrected for length) was not significantly affected by moult state (LMM $F_{4,74.5} = 2.00$, P=0.103), but was significantly explained by diet treatment (LMM

Fig 3. Average (±95% CI) ptilochronology of induced feathers according to moult state of the extracted feather, after considering variation explained by feather length and growth time (see text). Moult states are presented in order of increasing costs that already had been invested in moult: $\mathbf{M_1}$ – Old feather is dropped, but moulting feather is too small to be plucked **O -** Old feather, $\mathbf{M_2}$ – moulting feather, <50% grown and large enough to be plucked, $\mathbf{N_1}$ – new feather, >50% but not full grown, $\mathbf{N_2}$ **-** new feather full grown.



$F_{1,14.9}$ = 4.91, P=0.042), sex (LMM $F_{1,70.1}$ = 12.64, P=0.0007) and marginally explained by urbanisation (LMM $F_{1,15.1}$ = 3.93, P=0.066, see Table S3 for full model results). Males grew feathers of higher quality than females, and birds from urban origin and kept on an urban diet grew higher quality feathers (Fig. 4).

On average, $CORT_f$ did not differ between natural and induced feathers of the same individual (Repeated Measurements, $F_{1,54}$ = 1.016, P = 0.318). Furthermore, $CORT_{f\_ind}$ was not correlated to $CORT_f$ in natural feathers (R^2 = 0.0004, P=0.88), nor did this relationship vary with moult state of the natural feather (New *vs* Old), age or sex (GLM, all P>0.54). Variation in CORT of induced feathers was mainly explained by two-way interactions between urbanisation and age/sex, as well as the interaction between diet and age (Table 2, Fig. 5). Overall, juveniles showed higher stress responses to captivity than adults, in particular those originating from urban populations and exposed to an 'urban diet' (Fig. 5A, 5C, Table 2). Likewise, urban males showed higher stress responses to captivity than females (Fig. 5B, Table 2).

Ptilochronology and FA measurements on induced feathers were not related to $CORT_{f\_ind}$, neither did any of the two-way interactions with sex, age, urbanisation or diet significantly explain variation in $CORT_{f\_ind}$ (FA model, all P>0.31, Ptilochronology model, all P>0.13).

Fig 4. Induced feather quality in males and females from rural and urban sites in relation diet treatment

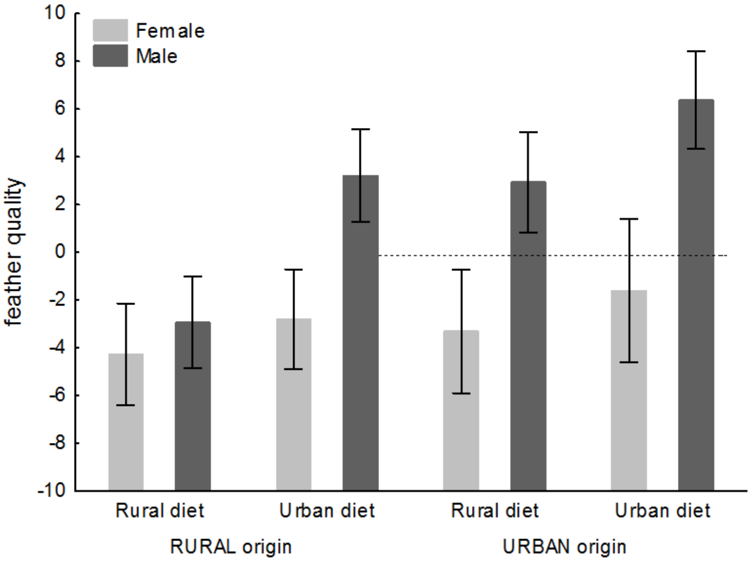


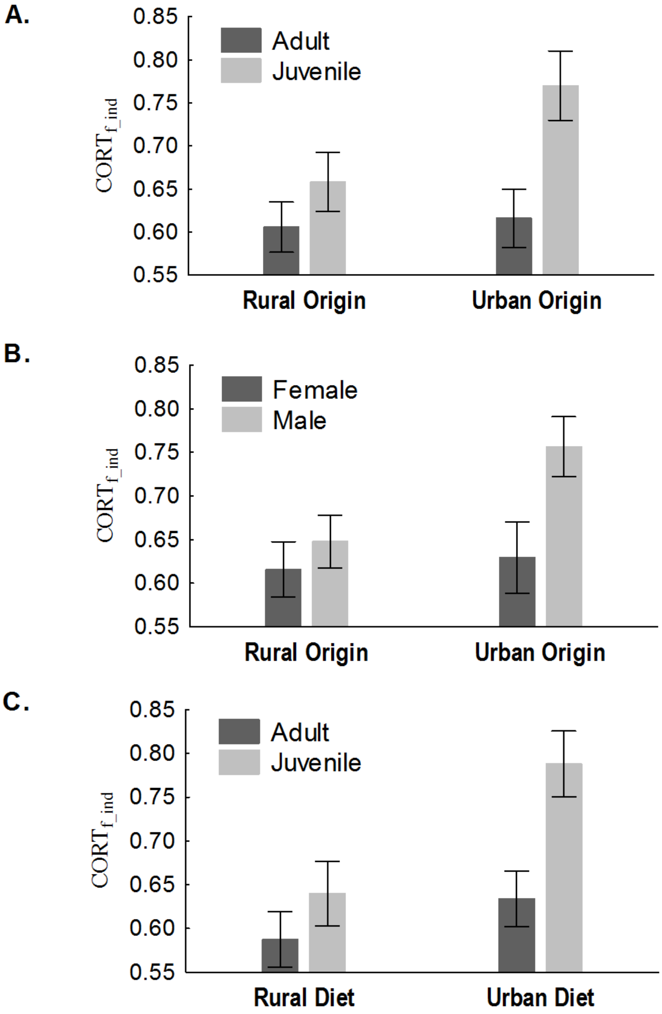
Table 2. Results from LMM explaining variation in CORT$_{f\_ind}$ (CORT in induced feathers). The minimal model is shown in bold. Statistics and P-values of non-significant effects were obtained by fitting each non-significant term separately into the minimal model. Estimates ± SE are given for Rural versus Urban sites, Females versus Males and Adults versus juveniles.

	Estimate	**DF**	**F**	**P**
Main effects/covariates				
Induced feather length	**0.131 ± 0.046**	**1.672**	**8.31**	**0.0053**
Moult state		4.636	0.67	0.617
Age	**-0.205 ± 0.047**	**1.66**	**17.27**	**<0.0001**
Sex	**-0.127 ± 0.038**	**1.601**	**9.85**	**0.0026**
Urbanization	**-0.159 ± 0.055**	**1.141**	**2.08**	**0.171**
Diet	**-0.148 ± 0.052**	**1.136**	**5.49**	**0.035**
Interactions				
Sex*Age		1.65	0.11	0.739
Urbanization*Diet		1.122	1.01	0.334
Urbanization*Sex		**1.598**	**4.36**	**0.041**
Urbanization*Age		**1.656**	**4.24**	**0.043**
Diet*Sex		1.593	2.53	0.117
Diet*Age		**1.666**	**4.04**	**0.048**
Urbanisation*Diet*Sex		3.386	1.50	0.231
Urbanisation*Diet*Age		2.246	0.77	0.473
Urbanisation*Sex*Age		2.645	2.24	0.115

Discussion

Neither descriptive nor experimental literature so far provided concluding evidence that urban sparrows show a different stress physiology compared to their rural counterparts (Bókony et al., 2012; Chavéz-Zichinelli et al., 2010; Fokidis et al., 2009; Meillère et al., 2015), while evidence is growing that stress physiology is plastic and variable among ages and sexes (Aharon-Rotman, Buchanan, Klaassen, & Buttemer, 2017; Dantzer et al., 2014). Building on these emerging patterns, we predicted that a novel stressor, simulated by a combined captivity and diet treatment, would affect the allostatic load in urban and rural individual in similar ways. Results of our study indeed confirm that urban and rural sparrows had comparable levels of corticosterone in feathers generated under natural moulting conditions, suggesting that allostatic load during moult did not differ in relation to urbanisation. In response to a novel

Fig 5. Variation in CORT$_{f\_ind}$ (CORT of induced feathers) in relation to significant interactions by A) Urbanisation and Age, B) Urbanisation and Sex, and C) Diet and Age. Full model results can be found in Table 2.



stressor, however, urban birds tended to have slightly higher CORT levels in induced feathers than rural birds, although not significantly so and confounded by age and sex. This non-significant trend was also in the opposite direction of what would be expected if urban birds habituate sooner to captivity or developed a changed stress physiology (desensitization with reduced capacity to react to a novel stressor; Cyr and Romero (2009)). Hence, our study of longer-term (i.e. during feather growth) HPA activity supports the growing notion that urban environments do not necessarily constitute stressful environments during the non-breeding season, as was earlier also concluded from studies of short-term baseline plasma CORT levels (Bókony et al., 2012; Chavéz-Zichinelli et al., 2010; Fokidis et al., 2009; Meillère et al., 2015).

Decreased food availability is one environmental factor that could affect allostatic load and CORT levels (Angelier et al., 2007; Kitaysky et al., 1999; Schoech et al., 2004). However, urban environments may actually provide species such as house sparrows with favourable foraging opportunities, at least for adults. While this appears to contract the common finding that urban sparrows are often leaner than rural ones (Bókony et al., 2012; Meillère et al., 2015; Salleh Hudin et al., 2016), experimental studies earlier suggested that the lower body condition of urban birds likely reflects a plastic response to more predictable food supplies, trading-off with changed predation risk (Dulisz, Nowakowski, & Gornik, 2016; Salleh Hudin et al., 2016). In line with this, no differences between urban and rural populations could be found in adult feather ptilochronology (Salleh Hudin et al., 2016) or adult feather quality (Chapter 3; Meillere et al. (2017)), a trait that is known to be affected by diet quality (Pap, Vagasi, Czirjak, & Barta, 2008). Also, studies on behavioural coping styles predicted urban sparrows to be bolder than rural ones (Atwell et al., 2012; Lendvai, Bókony, & Chastel, 2011), however, a recent experimental study could not confirm this (Vincze et al., 2016). Irrespective of the underlying mechanism(s) involved, urban habitats thus appear to fulfil the energetic requirements of house sparrows quite well, which may explain why such urban exploiters do not change their stress physiology in response to urbanization, unlike some urban adaptor species (Dantzer et al., 2014). Adult house sparrows did however initiate moult earlier in urban populations compared to rural populations, which is in agreement with results from a recent study on the Carolina chickadees (*Poecile carolinensis*; Hope, Stabile, and Butler (2016)). An earlier onset of moult in urban environments is likely a carry-over effect from an earlier onset of breeding in urban songbirds (Chamberlain et al., 2009), because the onset of moult is correlated with the end of reproductive activities (Siikamaki, Hovi, & Ratti, 1994; Vega Rivera, McShea, Rappole, & Haas, 1998).

The few studies that implicitly model intrinsic factors in stress physiology (reviewed in Dantzer et al. (2014) revealed age- and sex-related variation in phenotypic plasticity in glucorticoid levels, highlighting the value of taking these factors into account when comparing patterns across studies (Aharon-Rotman et al., 2017). Significant differences in CORT levels of experimentally induced feathers between sexes and ages (but not in naturally moulted ones; see also Koren et al., 2012) found in our study confirm the latter: when challenged by captivity and diet treatments, juveniles showed stronger stress responses than adults, and males showed stronger stress responses than females (Fig. 5). Adding to the level of complexity, the strength of these intrinsic effects also varied with urbanisation, as juveniles and males originating from urban populations had the highest CORT concentrations in their regrown feathers (Fig. 5). It is possible that higher CORT responses are adaptive for less experienced individuals (such as juveniles), as a higher HPA activity may increase their reactivity, and hence, success in escaping from life-threatening events. In support of this, juvenile house sparrow males were the most difficult ones to trap in an aviary experiment on sex- and age-related differences in escape response, and escape ability related to stress response, which was overall higher in juvenile birds (De Neve, Ibañez-Alamo, & Soler, 2010). Similarly, Rivers, Liebl, Owen, Martin, and Betts (2012) found that a higher baseline CORT level in fledgling Swainson's trushes (*Catharus ustulatus*) was related to increased post-fledging survival rates, which was suggested to be mediated by higher locomotor activity allowing better foraging and effective escape from predators. Why in our study juveniles originating from urban populations showed a stronger stress response than those from rural areas, however, remains puzzling. A possible, yet still speculative, explanation is that the stress response of the former was mediated by a higher perceived predation risk due to the high abundance of cats and corvids in urban areas.

With respect to gender, most studies so far reported higher effect sizes of increased glucorticoid levels in males compared to females (Dantzer et al. (2014), but see Aharon-Rotman et al. (2017) for an opposite trend). Here, we also found that males, on average, responded more strongly to captive stress than females, in particular in individuals from urban populations (Fig. 5). To the best of our knowledge, no consistent explanation exists for such sex-specific differences in responsiveness during the non-reproductive state (Dantzer et al., 2014). Yet the complex mechanisms behind CORT deposition into feathers (Romero & Fairhurst, 2016) may offer a possible explanation. CORT is passively deposited during feather formation (Bortolotti, 2010; Jenni-Eiermann et al., 2015; Romero & Fairhurst, 2016), and is therefore likely influenced by the amount of material incorporated into growing feathers (Patterson et al., 2015). High plasma CORT and low-quality diet can both have negative effects on feather growth and structure (DesRochers et al., 2009; Lattin, Reed, DesRochers, & Romero, 2011; Pap et al., 2008; Strochlic & Romero, 2008) and thereby influence CORT deposition. In addition, because induced feathers do not emerge through natural moult, we can expect that different physiological mechanisms might be at play that could affect feather development, and hence the deposition of CORT. We indeed found that feather ptilochronology of induced feathers (related to growth time) was related to moult state, i.e. the costs already invested in natural moult. Individuals that had invested less in moult had the fastest growing induced feathers. However, neither moult state nor growth time did significantly affect induced feather quality, but sex and urbanisation did. Induced tail feathers were of lower quality in females than males, which is consistent with a sexual difference in tail feather quality of natural moulted feathers (Chapter 3; (Meillere et al., 2017)). In addition, males from urban populations and those on an urban diet produced induced feathers of highest quality (Fig. 4), which is a similar pattern found as with $CORT_{f\_ind}$. So, even if we corrected the CORT models for feather length (very similar results were obtained when corrected for feather quality), potential differences in physiology and feather properties between the sexes may still affect CORT deposition in feathers, and hence, partially explain sexual differences in $CORT_{f\_ind}$. A recent experimental study using CORT implants in house sparrows revealed that $CORT_{f\_ind}$ indeed reflected plasma CORT during feather synthesis, but not in a direct quantitative manner (Aharon-Rotman et al., 2017), as was also found previously by Jenni-Eiermann et al. (2015). Intrinsic factors such as sex and inter individual variation in $CORT_f$ pre-treatment ($CORT_f$ of feathers plucked prior to the experiment) also correlated with $CORT_f$ of feathers grown during experimentation, irrespective of treatment (Aharon-Rotman et al., 2017). However, we could not confirm this individual persistence in $CORT_f$ across different moults as CORT in natural moulted feathers (pre-treatment) was not correlated to CORT in induced feathers, and this relationship was not affected by sex, age, urbanisation of moult state of the plucked feather. So, results of our study strongly plead in favour of incorporating both intrinsic and environmental factors when trying to explain variation in feather CORT.

Acknowledgements

We are indebted to H. Matheve, P. Vantieghem and H. De Rycke for field and lab assistance, and to A. Chaine for logistic support with the aviary experiments. The field sampling protocol was approved by the Ethical Committee of Ghent University. Sparrows were caught under capture and ringing permit n°15038 (French National Natural History Museum). The aviary experiment was conducted in the experimental aviaries of the Station d'Ecologie Expérimentale du CNRS at Moulis (France).

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Supplementary Materials

Table S1. The number of captured birds (Males *vs* Females; Adults *vs* Juveniles) in each population and the number of induced feathers that were collected after 6 weeks in captivity. A total of 113 birds were captured, of which 59.3% were males and 63.7% were adults. Induced feathers could be collected from 87 birds.

Origin	**Urbanization**	**Sex**		**Age**	
		Male	Female	Adult	Juvenile
Caraman	Rural	12/8	7/6	9/8	10/6
Cologne	Rural	9/9	8/7	7/7	10/9
Montégut-Savès	Rural	12/10	12/10	22/18	2/2
Pau	Urban	12/6	6/4	13/8	5/2
Tarbes	Urban	10/6	6/3	12/8	4/1
Toulouse	Urban	12/12	7/6	9/9	10/9
TOTAL		**67/51**	**46/36**	**72/58**	**41/29**

Table S2. Results from the LMM testing relationships between CORT_f in natural feathers and fluctuating asymmetry (FA) according to sex, age and urbanisation level. Effects that remained in the minimal model are shown in bold. Statistics and P-values of non-significant terms were obtained by fitting each non-significant term separately into the minimal model.

	DF	**F**	**P**
Main effects/covariates			
Feather Length	**1.48**	**70.49**	**<0.0001**
Moult state	1.47	0.54	0.465
FA	**1.48**	**1.92**	**0.173**
Sex	**1.48**	**4.45**	**0.040**
Age	1.46	0.16	0.690
Urbanization	1.231	0.01	0.949
Interactions			
Urbanization*FA	1.426	0.04	0.836
Sex*FA	**1.48**	**3.63**	**0.063**
Age*FA	1.44	0.02	0.899

Table S3. Results from LMM explaining variation in induced feather quality. The minimal model is shown in bold. Statistics and P-values of non-significant effects were obtained by fitting each non-significant term separately into the minimal model. Estimates ± SE are given for Adults versus Juveniles, Females versus Males, Rural versus Urban sites and Rural versus Urban diet

	Estimate	**DF**	**F**	**P**
<u>Main effects/covariates</u>				
Growth time	-1.096 ± 0.841	1.747	1.70	0.196
Moult state		4.745	2.00	0.103
Age	-0.182 ± 1.519	1.801	0.01	0.905
Sex	**-4.956 ± 1.394**	**1.701**	**12.64**	**0.0007**
Urbanization	**-3.293 ± 1.660**	**1.151**	**3.93**	**0.066**
Diet	**-3.625 ± 1.636**	**1.149**	**4.91**	**0.043**
<u>Interactions</u>				
Sex*Age		1.791	0.06	0.813
Urbanization*Diet		1.14	0.09	0.770
Urbanization*Sex		1.689	1.40	0.241
Urbanization*Age		1.789	0.30	0.588
Diet*Sex		1.705	1.38	0.244
Diet*Age		1.799	1.41	0.523
Urbanisation*Diet*Sex		1.666	0.26	0.611
Urbanisation*Diet*Age		1.767	0.00	0.985

Fig S1. Distribution of feather state of the plucked tail feathers prior to the experiment, according to populations of origin and age. Bourgezes, Cox and Mont are rural areas, while Pau, Tarbes and Toulouse are urban areas.

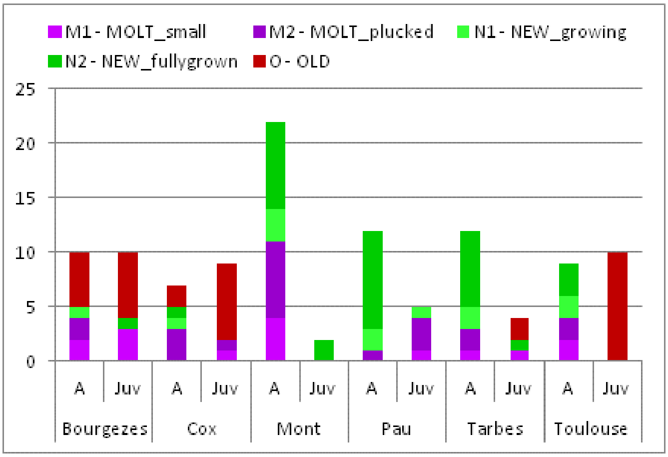
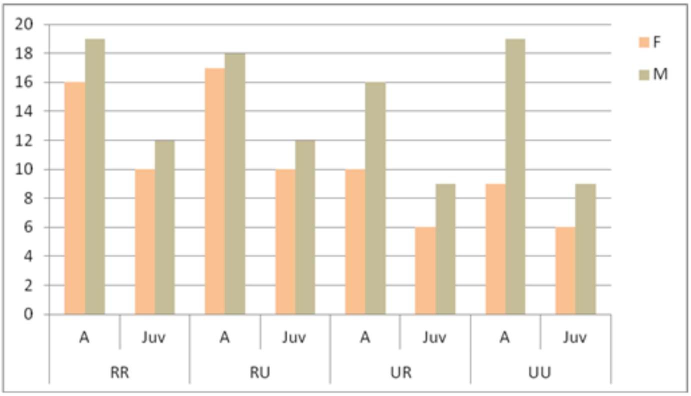
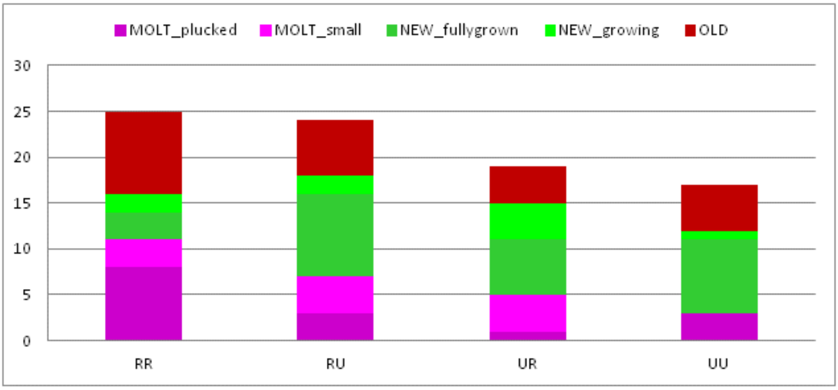


Figure S2.

A. The number of induced feathers in relation to treatment, age and sex.



B. The number of induced feathers proceeding from each moult state of the natural extracted feather.



Supplementary feeding increases nestling feather corticosterone early in the breeding season in house sparrows

4

Modified from:

Salleh Hudin, N., De Neve, L., Strubbe, D., Fairhurst, G. D., Vangestel, C., Peach, W. J., & Lens, L. Supplementary feeding increases nestling feather corticosterone early in the breeding season in house sparrows. *Ecology and Evolution*. 00: 1-9

LL, LDN, DS, WJP, VC designed the study; NSH, GDF performed CORT analysis; NSH, LL performed FA analysis; DS performed statistical analysis; NSH, DS, LDN, LL interpreted data; all authors drafted the manuscript.

Abstract

Several studies on birds have proposed that a lack of invertebrate prey in urbanized areas could be the main cause for generally lower levels of breeding success compared to rural habitats. Previous work on house sparrows *Passer domesticus* found that supplemental feeding in urbanized areas increased breeding success but did not contribute to population growth. Here we hypothesize that supplementary feeding allows house sparrows to achieve higher breeding success but at the cost of lower nestling quality. As abundant food supplies may permit both high and low quality nestlings to survive, we also predict that within-brood variation in proxies of nestling quality would be larger for supplemental food broods than for unfed broods. As proxies of nestling quality we considered feather corticosterone ($CORT_f$), body condition (Scaled Mass Index, SMI) and tarsus based Fluctuating Asymmetry (FA). Our hypothesis was only partially supported as we did not find an overall effect of food supplementation on FA or SMI. Rather, food supplementation affected nestling phenotype only early in the breeding season in terms of elevated $CORT_f$ levels and a tendency for more variable within-brood $CORT_f$ and FA. Early food supplemented nests therefore seemed to include at least some nestlings that faced increased stressors during development, possibly due to harsher environmental (e.g. related to food and temperature) conditions early in the breeding season that would increase sibling competition, especially in larger broods. The fact that $CORT_f$ was positively, rather than inversely, related to nestling SMI further suggests that factors influencing $CORT_f$ and SMI are likely operating over different periods or, alternatively, that nestlings in good nutritional condition also invest in high quality feathers.

Introduction

Coping with environmental perturbations, such as bad weather, predators or food shortages, is a primary challenge faced by organisms. These unpredictable stressors often create energetically demanding conditions that may trigger an acute response in the form of short-term elevations of the hormone corticosterone (CORT; the end product of activation of the hypothalamic-pituitary-adrenal axis). CORT redirects energy expenditure by changing physiology and behaviour in order to meet such demands and to return to normal activities (referred to as 'allostatic load' (McEwen and Wingfield, 2003; Romero, 2004)). Acute stress responses are considered adaptive as they enable individuals to cope with perturbations and increase immediate survival, as well as to prepare them to successfully respond to the reoccurrence of stressors (Blas et al., 2007; Romero, 2004; Sapolsky et al., 2000). Along these lines, Egyptian vultures *Neophron percnopterus* elevated their CORT levels when faced with limited food resources, which increased their foraging activity to avoid starvation (Carrete et al., 2013). However, when individuals coping with stressors fail to improve their condition through behavioural and/or physiological modifications, they may experience chronically elevated CORT levels (Kitaysky et al., 2003; Sapolsky et al., 2000) which can lead to detrimental effects on fitness (Hayward and Wingfield, 2004; Kitaysky et al., 2003; Pravosudov and Kitaysky, 2006; Rubolini et al., 2005). Chronic stress may be particularly harmful during early life stages since young animals are typically vulnerable to adverse conditions. A substantial body of evidence suggests that elevated CORT levels during prenatal or nestling development affect growth and can weaken immune function, with long-term consequences for physiology, morphology and behaviour (Bebus et al., 2016; Blas et al., 2007; Kitaysky et al., 2003; Love and Williams, 2008; Rubolini et al., 2005; Saino et al., 2005; Schmidt et al., 2013; Schoech et al., 2011; Spencer and Verhulst, 2007; Spencer et al., 2009). Hence, exposure to periods of stress at the nestling stage can have an impact on fitness, and may therefore translate into population level effects.

Limited food availability is an important early-life stressor known to cause elevated CORT levels (Boonstra, 2013; Herring et al., 2011; Pravosudov and Kitaysky, 2006; Saino et al., 2003), especially in altricial bird species in which starvation is known to be the main cause of nestling mortality (Martin, 1987). Nestlings may respond to nutritional restriction by increasing begging displays (Kitaysky et al., 2001a; Loiseau et al., 2008b) to elicit an increase in parental provisioning rates (Roulin and Dreiss, 2012). Prolonged periods of limited food supplies may also increase sibling competition and result in brood reduction where high quality nestlings survive and gain more food from their parents (Roulin and Dreiss, 2012).

Reduced food provisioning of nestlings may arise when environmental conditions are unfavourable for invertebrates, which constitute the most important source of proteins in the diet of passerine nestlings (White, 2008). A reduction of invertebrate abundance and diversity may, for example, occur due to urbanization (Vergnes et al., 2014), and studies of birds have already proposed a lack of invertebrate prey as a primary cause of lower observed breeding success in urban areas compared to less urbanized areas (Chamberlain et al., 2009; Mennechez and Clergeau, 2006). House sparrows (*Passer domesticus*) have been associated with humans for hundreds of years and are a classical example of an urban exploiter (e.g. Kark et al., 2007). Yet, sparrow populations in urban centres across Europe started to decline in the late 1980s (De Laet and Summers-Smith, 2007; Shaw et al., 2008). Several hypotheses have been proposed to explain this decline, among which are a loss of nesting sites and adequate food sources

(Summers-Smith, 2003). In support of this hypothesis, fledging production was increased when house sparrow nests were supplemented with mealworms in domestic gardens in the city of Leicester, UK (Table S1 *sensu* Peach et al., 2014). Similarly, a significant positive influence of mealworm supplementation on per-capita fledgling counts was shown in house sparrows from suburban areas in London (Peach et al., 2015), where a population decline of 60% was observed during the decade preceding the study (Raven et al., 2007). However, despite the improved reproductive success resulting from the experiment, house sparrows did not show population growth or recovery (Peach et al., 2015). These observations suggest that post-fledging mortality may have been high, which could be linked to reduced phenotypic and/or physiological quality arising during the nestling period.

So far, avian food supplementation studies during the breeding season mainly evaluated outcomes on maternal investment (e.g. laying date, incubation, clutch and egg size) and reproductive success (number of fledglings) (Ruffino et al., 2014), but there is a surprising lack of information on the potential effects on the quality of fledglings. In this study, we explore multiple proxies of fledgling quality collected as part of the food supplementation experiment performed by Peach et al. (2014). This study demonstrated that daily provision of live invertebrate prey increased reproductive success (fledgling production) by 55% but had no significant impact on average nestling tarsus length or body mass (Table S1 *sensu* Peach et al., 2014). In line with these findings, we hypothesise that under experimental food supplementation, parents could fulfil the basic nutritional requirements of more nestlings, allowing low quality nestlings to survive instead of dying from starvation in the completion for food with higher quality nestlings. If so, food supplemented nests are expected to contain both high and low quality nestlings at the end of the nestling period, resulting in no effect on average nestling quality, but showing larger within-brood variation in proxies of nestling quality compared to nests without access to supplemental food.

To test our hypothesis, we used three indices of nestling quality collected from the birds in the study reported by Peach et al. (2014): CORT levels in tail feather samples (feather corticosterone, $CORT_f$), fluctuating asymmetry (FA) of tarsus length, and scaled mass index (SMI). Given that feathers start to emerge soon during nestling development (i.e. day 4, Anderson 2006) and newly grown feathers incorporate circulating CORT (Fairhurst et al., 2013; Jenni-Eiermann et al., 2015), $CORT_f$ provides an integrated signal of adrenocortical activity (i.e. both baseline and temporary acute elevations) in response to allostatic demands during the nestling period (Fairhurst et al., 2013; Will et al., 2014; see Romero and Fairhurst, 2016 for a review). FA is the within-individual difference in size of bilaterally symmetrical traits and, as an indicator of developmental stress, higher FA are indicative for a lower ability to buffer against stressors during development (Auffray et al., 1999). SMI is a good proxy of nestling body condition (Peig and Green, 2009), which typically shows a positive correlation with recruitment probability in passerine bird species (Cleasby et al., 2010; Monrós et al., 2002).

Methodology

Species and study sites

House sparrows breed from April until August, during which pairs can produce up to four clutches (Summers-Smith, 1988). House sparrows lay clutches of typically 3-5 eggs, with incubation lasting approximately 11 days, and with chicks fledging around 14 days after eggs hatch (Summers-Smith, 1988). Wing feathers typically first emerge in 4-day-old chicks after which feather growth is approximately linear until fledging (Anderson, 2006). Sparrow nestlings are fed almost exclusively on invertebrates such as beetles, caterpillars and aphids, but as nestlings get older, more vegetable material is added to their diet (Anderson, 2006). Nutritional stress affecting nestling house sparrows is probably caused by a lack of suitable invertebrate prey especially larger items such as caterpillars and beetles, resulting in low sparrow nestling body mass (Peach et al., 2008) and smaller body size in adult house sparrows in urban areas (Seress et al., 2012).

We conducted this study on house sparrow nestlings raised in nest boxes in three rural (Houghton-on-the-Hill, Hungarton and Keyham) and three suburban (Braunstone, Thurmaston and Western Park) sites in Leicester, UK, in 2008 (Table A1 in Peach et al., 2014). Nest boxes (all single chamber) were fixed to the sides of mainly residential buildings (usually 2 – 4 boxes per property), 4 – 5 m above ground level. Each occupied nest box was categorized as being situated in either suburban or rural localities depending on the presence of farmland within 100 m of the box (present = rural, absent = suburban). Data for this study were obtained from 29 nests situated in rural areas and from 16 nests in suburban areas.

Supplementary feeding experiment

Supplementary feeding of mealworms (*Tenebrio molitar*) was initiated at 10 garden locations spread across all six study sites between mid- and late-April. Feeding always began after nesting had started (a full clutch was usually laid) but before any eggs hatched, and continued on a daily basis until early August when nesting ceased. The supplementary feeding, therefore, had the potential to influence a range of reproductive parameters but not the size or timing of first clutches. In total, 12 out of 29 and five out of 16 nests in rural and suburban areas, respectively, had access to the supplementary feeding. At each feeding location, a total of 33 g of live mealworms (comprising approximately 300 mixed-size worms) were provided each day, split evenly between early morning and late afternoon feeds. Worms were provided in a single metal feeder comprising a holding tray, a protective rainfall cover and a surrounding metal cage to exclude larger birds like European starlings (*Sturnus vulgaris*).

All feeders were placed in private gardens within 30 m of a nest box occupied by breeding house sparrows. Nest box observations were carried out to determine if mealworms were being provided to the chicks (see Peach et al., 2014 for further details). Although we did not measure casual provisioning of supplementary food by local people, no residents with occupied nest boxes (or their immediate neighbours) provided mealworms or any other form of protein. There were also no retail outlets or fast-food outlets within 100 m of any nest so that potential food sources of waste were not available. Provisioning of seed and vegetable material (mainly bread) was widespread and probably ubiquitous across our study areas but is unlikely to have been confounded with our feeding treatment. Habitat composition was similar in fed and unfed localities (Peach et al. 2014) suggesting that the availability of key foraging habitats was also unlikely to be confounded with our feeding treatment.

The contents of all nest boxes containing fresh nesting material were checked at least once each week between April until late mid-August to determine the laying date (LD; 1st March equals to LD = 1) and number of eggs and chicks. The number of live chicks present at 9 –13 days after hatching was presumed to be the number of fledglings. At that time, nestlings were weighed (mass to the nearest 0.1 g) and three independent measurements of the left and right tarsus length were taken of every nestling (to the nearest 0.1 mm) for analysis of FA. From each nestling, two tail feathers were collected and kept in individual envelopes for $\mathrm{CORT_f}$ analysis.

CORT analysis

We used a methanol-based procedure (Bortolotti et al., 2008) to extract CORT from feathers in two batches. After removing the calamus from each feather, we cut the remaining samples into small pieces ($<5\ \mathrm{mm}^2$) and added 10 mL of methanol (HPLC grade, VWR International, Mississauga, Ontario, Canada) to each sample. We then sonicated samples in a water bath at room temperature for 30 min, followed by overnight incubation in a water bath at 50 °C. We separated methanol from the feather pieces using vacuum filtration, and placed the methanol extracts in a 50 °C water bath to evaporate in a fume hood. Once extracts were dry, we reconstituted them in a small volume of phosphate buffered saline (PBS; 0.05M, pH 7.6) and stored them at –20 °C until analysis by radioimmunoassay (RIA). We assessed the efficiency of the extraction procedure by including three feather samples spiked with a small amount (approximately 5000 CPM) of ^{3}H-labeled corticosterone (see Appendix S1 in Bortolotti et al. (2008) for more details). On average, 91 % (SD = 1) of the radioactivity was recoverable in the reconstituted samples, and CORT values were adjusted for recoveries.

We analyzed CORT by RIA as in previous studies (Bortolotti et al., 2008; Fairhurst et al., 2013), and this technique has been replicated in house sparrows (Treen et al., 2015). We assayed duplicates of reconstituted methanol extracts in three assays using a commercial antiserum (Sigma-Aldrich, St. Louis, MO, USA; product# C8784). Serial dilutions of sample extracts were parallel to the standard curve, indicating no interference with the antibody. We computed assay variation using three aliquots, each measured in duplicate, of the same standard CORT solution, created from purified CORT (Sigma-Aldrich, St. Louis, MO, USA), in each assay. Average intra-assay coefficient of variation (CV) was 8.5 % (SD = 1.5) and inter-assay CV was 11.1 %. The average detection limit of our assays was 14.20 pg CORT/100 µL sample (SD = 4.01) and all values were above detection limits. Data values were normalized by length (i.e., pg CORT/mm of feather) to correct for the time-dependent deposition of CORT (Bortolotti, 2010; Jenni-Eiermann et al., 2015; see Romero and Fairhurst, 2016 for a discussion). CORT$_f$ assays were performed at the University of Saskatchewan, Canada.

Measurement of fluctuating asymmetry (FA)

Levels of environmental and nutritional stress between nestlings of broods with and without access to supplementary feeding were assessed through measurements of FA of tarsus length. As FA estimation was based on repeated measurements of left and right trait sides, we first estimated the level of repeatability among repeated measurements within each side. Based on a one-way ANOVA analysis of the within- and between-side mean squares (Lessells and Boag, 1987), the repeated measurements of tarsus length showed very high statistical repeatability (right tarsus: $r = 0.997$, $n = 83$, $p < 0.001$; left tarsus: $r = 0.992$, $n = 83$, $p < 0.001$). Despite this high repeatability, the fact that the degree of FA is often very small (typically on the order of $\leq$1% of the size of the trait; Møller and Swaddle, 1997), measurement error (ME) may still cause an upward bias in the between-sides variance if not appropriately corrected for (Merilä and Björklund, 1995; Palmer and Strobeck, 1986; Van Dongen et al., 1999). Therefore, tarsus FA was analysed through mixed-regression analysis with Restricted Maximum Likelihood (REML) parameter estimation (Van Dongen et al., 1999). While yielding identical FA estimates as two-way mixed ANOVA models, the REML method allows to test for FA significance, to model heterogeneity in FA and measurement error among populations or treatments, to test for directional asymmetry (DA), and to obtain estimates of individual FA that are unbiased with respect to ME and DA. Fixed intercepts estimate overall trait size, fixed slopes estimate DA, and the random intercepts and slopes (both estimated *within* individuals) estimate the variation in individual trait value and individual FA, respectively (Van Dongen et al., 1999). Variance in signed FA was much larger than variance in ME, and was highly significant (likelihood-ratio test: $p < 0.0001$). FA measurements were not biased by directional asymmetry after correcting the denominator degrees of freedom by Satterthwaite's formula (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.

Scaled mass index

As an estimate of body condition, we used the Scaled Mass Index (SMI; Peig and Green, 2009), which adjusts the mass of all individuals to that which they would have obtained if they had the same body size. SMI was calculated 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 and Green, 2009). Following Bókony et al. (2012), we first verified whether tarsus length was most strongly correlated with body mass on a log-log scale ($r = 0.78$, $P < 0.001$) and consequently applied that variable to scale body mass. No outliers were present in the data (i.e. all |standardized residual| < 3, max value was 2.73), the regression slope was 2.95 and average tarsus length was 20.2 mm. We thus calculated the SMI as body mass x $(20.2/\text{tarsus length})^{2.95}$ (Peig and Green, 2009; Peig and Green, 2010).

Statistical analysis

We first used univariate tests to explore how our three indicators of nestling quality (i.e. CORT_f levels, tarsus-based FA, and SMI) related to each other, before building three separate linear mixed models (lmer function of R library 'lme4'; Bates et al., 2015) to assess how these indicators relate to the experimental provisioning of food (fed or not), taking into account factors that could affect our proxies of nestling quality, such as laying date, brood size, urbanization (rural vs. suburban), nestling age (days) and brood reduction. Brood reduction was calculated as the difference between the number of hatched eggs and fledged young. Hence, it measures the number of young that died in the nest and is interpreted as a measure of intensity of food competition among siblings before brood reduction takes place (Mock and Parker, 1997; Soler and Aviles, 2010). We started with models that included all variables mentioned above. SMI, $CORT_f$ and FA are three physically unrelated metrics that may relate to stressful conditions during the nestling period via different mechanisms. In order to explain the variation that did not co-vary between these parameters, we added the remaining metrics as covariates to each model. Correlations between fixed continuous predictors were weak (all $r < 0.44$). To test whether effects of experimental food provisioning changed over the breeding season or differed between urban and rural sites, the two-way and three-way interactions between laying date, urbanization and experimental food provisioning were also added to the models. A nested random effect (i.e. nest box within study site) was included to account for the non-independence of nestlings from the same nest and of nest boxes within the same study site, respectively.

During hypothesis testing, we adopted a frequentist approach whereby full models (i.e. models containing all explanatory variables considered above) were reduced in a stepwise manner, by excluding the variable with the highest P-value until only predictors with $P < 0.05$ remained. Statistics and P-values mentioned in the text and table are invariably referring to the final model (i.e., only significant terms included), whereas statistics and P-values of non-significant terms were obtained by fitting each non-significant term separately into the minimal model. As Larsen et al. (2015) showed a U-shaped relationship of CORT levels with timing of breeding, $CORT_f$ models were run with laying date both as a linear and a quadratic effect. Likelihood-ratio tests were used to assess whether $CORT_f$ levels were best explained by quadratic or by linear terms, using a χ^2 test (R function 'anova') to select the best fitting minimal model. When treating laying date as a categorical variable (early versus late breeding), however, similar results were obtained (data not shown).

Lastly, to test whether nests provided with supplemental food exhibited a higher variation in stress-levels among nestlings, we first calculated the difference between the young with the highest and the lowest value of $CORT_f$, tarsus-based FA and SMI in each nest. This range value was then used as the dependent variable in linear mixed model with the same fixed effects as explained above (study site was included as a random effect). For this analysis, we could only include nests for which all mentioned variables could be collected for at least two juveniles, reducing the sample size from 45 to 33 nests.

Results

The three indicators of nestling quality were positively correlated with one another ($CORT_f$ levels vs. tarsus-based FA: $r = 0.23$, p = 0.038; $CORT_f$ levels vs. SMI: $r = 0.27$, p = 0.016; SMI vs. tarsus-based FA: $r = 0.34$, p = 0.0017).

When assessing variation in $CORT_f$ levels among nestlings, a model including a quadratic laying date term provided a better fit than a linear model ($\chi^2(2)=7.42$, p = 0.024). The quadratic model included a significant interaction between laying date and access to experimentally provided supplemental food, as well as significant positive effects of brood reduction and SMI (Table 1). $CORT_f$ levels were highest early and late in the breeding season ($F_{2,72} = 5.70$, p = 0.0051; Fig. 1), and early in the breeding season nestlings with access to supplemental food were characterized by larger $CORT_f$ concentrations than unsupplemented nestlings (laying date * food provisioning: $F_{2,72} = 5.34$, p = 0.0069; Table S2, Fig. 1). In addition, $CORT_f$ levels were higher for nestlings with larger SMI and for those originating from nests where brood reduction took place (estimate and standard error = 0.60±0.27, t = 2.26, p = 0.027

Table 1. Results from reduced General Linear Mixed Models explaining variation in feather corticosterone ($CORT_f$) levels, tarsus-based FA, and SMI among house sparrow nestlings. Initial models included food provisioning (fed vs. unfed), laying date (linear and quadratic terms), brood size, brood reduction, nestling age (days) and urbanization (rural vs. suburban). Only significant terms are listed here and full model results are given in Tables S1-S3.

Dependent	**Predictor variable**	**Estimate**	**SE**	***d. f.***	***t***	**p**
$CORT_f$	Laying date (linear term)	-25.140	8.004	72	-3.141	0.0020
	Laying date (quadratic term)	28.781	12.497	72	2.303	0.0240
	Brood reduction	1.658	0.640	72	2.590	0.0120
	SMI	0.604	0.267	72	2.261	0.0270
	Food provisioning	-0.919	-0.919	72	-0.781	0.437
	Laying date (linear term)*food provisioning	29.398	10.855	72	2.708	0.0080
FA	SMI	0.024	0.0073	73.850	3.330	0.0014
SMI	Laying date (linear term)	-0.036	0.0150	41.980	-2.582	0.0130
	FA	4.192	1.2430	50.850	3.373	0.0014

1.66±0.64, t = 2.59, p = 0.012, respectively; Table 1). Other included variables failed to reach statistical significance (i.e. all p > 0.29, see Table S2). FA was higher for heavier nestlings (0.024±0.0073, t = 3.33, p = 0.0014), and tended to be lower later in the breeding season (-0.0017±0.0009, t = 1.88, p = 0.063; Table S3). Besides the relationship with FA (4.19±1.24, t = 3.37, p = 0.0014), nestling SMI declined throughout the breeding season (-0.039±0.015, t = -2.58, p = 0.013; Table S4).

Supplemented nests exhibited higher among-nestling variation in both $CORT_f$ and FA at the beginning of the breeding season (Tables S5 and S6), while the range in indicators of nestling quality did not vary with laying date for control nests. However, these patterns failed to reach statistical significance, possibly because of the limited sample size ($CORT_f$: interaction between laying date and food provisioning $F_{1,27} = 1.862$, p = 0.074; tarsus-based FA: $F_{1,27} = 2.298$, p = 0.14; see Table S5 and S6). No such seasonal pattern was apparent for SMI measurements ($F_{1,27.35} = 0.170$, p = 0.684; see Table S7).

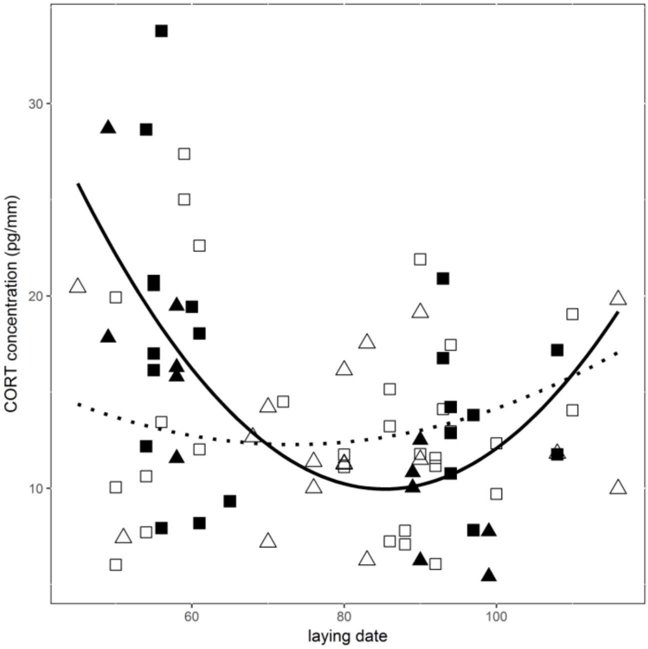


Figure 1. Relationship between laying date and feather corticosterone ($CORT_f$) levels measured in sparrow nestlings from rural (rectangles) and suburban (triangles) areas. Nest boxes with access to supplemental food are filled black. The solid line indicates the trend in $CORT_f$ levels throughout the

breeding season for nests with access to supplementary food, while the dotted line depicts the trend for nests without such access (full model information in Table S1).

Discussion

Given that our experimental food supplementation resulted in higher fledging production (Table S1 *sensu* Peach et al. 2014) but not, in a separate study, enhanced population growth (Peach et al., 2015), we hypothesized here that food supplementation allows parents to fulfil the requirements of both low and high quality nestlings. As such, we predicted an increase in within-nest variation in nestling quality, but not in average quality, in supplemented nests. These predictions were partially supported by our results, as we did not find an overall effect of food supplementation on $CORT_f$, tarsus-based FA or nestling body condition, while there was a slight, not significant, tendency of higher within-nest variation in $CORT_f$ and tarsus-based FA early in the breeding season, but not SMI.

Yet, we also found that food supplementation affected nestling phenotype in terms of elevated $CORT_f$ levels (controlled for SMI, Table 1), but only early in the breeding season. So early food supplemented nests seemed to include at least some nestlings that faced increased stressors during development. A possible explanation for this early breeding season effect could be that the harsher weather conditions of early spring produced suboptimal conditions, in terms of microclimate and food availability (i.e. insects), which could increase sibling competition (e.g. for food or for a warm place in the nest box), as both of these factors have been shown to affect allostatic load resulting in higher CORT concentrations (Braasch et al., 2014; Fairhurst et al., 2012; Kitaysky et al., 2001b; Lopez-Jimenez et al., 2016). Such an effect of sibling competition, if present, should be most apparent in food supplemented nests, because more nestlings survived (Table S1 *sensu* Peach et al., 2014), and elevated $CORT_f$ levels are expected to be most marked in lowest ranked nestlings (e.g. López-Jiménez et al. 2016). This may explain the tendency for within-nest variation in $CORT_f$ and tarsus-based FA to be greater in supplementary fed nests early in the breeding season. The fact that nestlings also exhibited higher average $CORT_f$ levels in nests that suffered brood reduction further supports the idea that sibling competition may increase allostatic load in house sparrows.

Unexpectedly, the three indices used in this study to measure nestling quality were positively correlated, regardless of the experimental treatment. Nestlings in good body condition (SMI) were also those with higher levels of $CORT_f$ and higher tarsus-based FA. Rather, we expected that elevated levels of glucocorticoids during development, reflected in high levels of $\mathrm{CORT_f}$ (Bortolotti et al., 2008; Fairhurst et al., 2013), would be associated with reduced body condition (Fairhurst et al., 2013; Rubolini et al., 2005; Wada and Breuner, 2010). Indeed, more optimal parental provisioning rates have earlier been suggested to reduce circulating CORT levels (Kitaysky et al., 2001b), which explains the general finding that nestlings in good nutritional condition had lower $\mathrm{CORT_f}$ levels (e.g. Lopez-Jimenez et al., 2016; Will et al., 2014). However, in our study, factors that influenced $\mathrm{CORT_f}$ and SMI likely operated over different periods. $\mathrm{CORT_f}$ values reflect circulating CORT levels over the entire feather growth period. Hence, especially in nests with large broods and/or those that suffered brood reduction, this includes the period of strong nestling competition and the period following brood reduction. By contrast, SMI was computed from metrics scored at the end of the nestling period, and may therefore provide information over a relatively limited period of time compared to feather growth. Early in the nestling period, in a competitive brood environment, higher CORT levels may actually have benefitted those nestlings in terms of begging and/or positioning close to the nest hole to obtain more food (e.g. Loiseau et al., 2008a; Ruppli et al., 2012), and allowed them to survive the brood reduction process. After brood reduction, sibling competition for food was probably relaxed among the remaining nestlings, resulting in a high body condition at the end of the nestling period, but concurrent reductions in circulating CORT may not have been reflected in $\mathrm{CORT_f}$ because levels were low for a short period in time relative to the entire feather growth period (Romero and Fairhurst, 2016). Such a scenario could explain higher $\mathrm{CORT_f}$ in nests that suffered brood reduction and also explain a positive association between $\mathrm{CORT_f}$ and SMI. Increased CORT levels early in the nestling period could, however, come at the cost of an unstable hormonal/developmental setting and thus increased levels of FA.

In addition, recent studies suggested that interpreting $\mathrm{CORT_f}$ levels can be complex because variations in feather density may exert an important effect on CORT deposition (Patterson et al., 2015). Given that CORT is passively deposited during feather formation (Bortolotti, 2010; Jenni-Eiermann et al., 2015; Romero and Fairhurst, 2016), it is possible that $\mathrm{CORT_f}$ is influenced by the amount of material incorporated into growing feathers, resulting in lower $\mathrm{CORT_f}$ in less dense feathers, regardless of circulating CORT levels (Patterson et al., 2015). Both high plasma CORT concentrations and food limitation can interfere with feather development and feather quality, resulting in feathers that are lighter, weaker and with an altered micro-structure (DesRochers et al., 2009; Lattin et al., 2011). So, even if nutritional stress and decreased body condition typically increase plasma CORT (e.g. Kitaysky et al., 2001b; Müller et al., 2010; Poisbleau et al., 2010), concurrent $\mathrm{CORT_f}$ elevations should only be expected if feather quality remains unaffected by nutritional status (Lopez-Jimenez et al., 2016; Patterson et al., 2015; Will et al., 2014; see Romero and Fairhurst, 2016) for a review). To our knowledge, no study has examined how nutritional condition affects CORT_f in relation to feather development and quality in passerine species, and so experimental validation is needed to reliably interpret CORT in passerine nestling feathers.

In conclusion, it seems that food supplementation did not affect indices of nestling quality directly. However, early in the breeding season, nestlings from food supplemented nests did seem to suffer from more stressors, potentially from increased sibling competition, that were absent in control nests or in nests initiated later in the breeding season. Although experimental validation is needed to know how nutritional status affects development and quality of house sparrow traits, our study provides support for the hypothesis that food supply during early life has implications for nestling quality. Yet, the observed inter-relationships among nestling SMI, tarsus-based FA and CORT_f all suggest that any mechanism linking food supply to population growth in this species remains highly complex.

Acknowledgements

We are grateful to the residents of Leicester who allowed access to their gardens and particularly to those who put out mealworms each day. Danielo Ojeda, Danaë K. Sheehan, Ian Dillon, and Josh Murfitt conducted the field work, while T. Marchant kindly allowed us to use her endocrine lab. The wider Leicester study was jointly funded by the RSPB and Natural England through the Action for Birds in England Partnership. Analyses were funded by the Interuniversity Attraction Poles Program SPEEDY (initiated by the Belgian Science Policy Office) and the Ministry of Higher Education of Malaysia.

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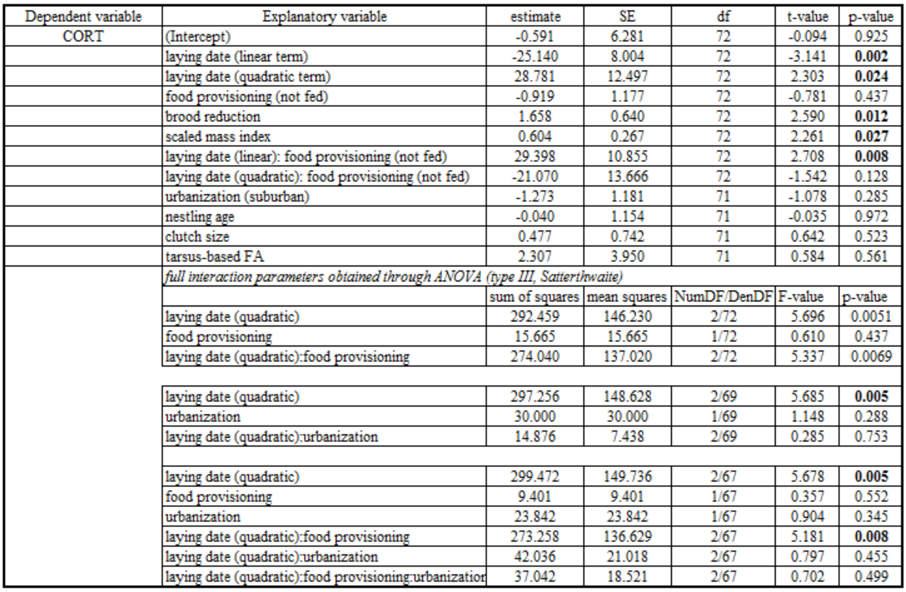
Supplementary Materials

Table S1. Effect of mealworm provision on the average number of fledglings produced from individual nesting attempts of house sparrows in suburban-rural Leicester in 2008. The difference in fledgling production between fed and unfed nests was statistically significant (Wald test: $F_{1,60} = 9.68$, $P<0.003$) after allowing for any effects of study area (six localities), landscape (suburban, rural) and month when first egg was laid (May, June, July). Adapted from Peach et al. (2014)

Treatment	Number of nests	Mean (SE) number of fledglings per nesting attempt
Fed	**34**	2.79 (0.230)
Unfed	**91**	1.80 (0.148)

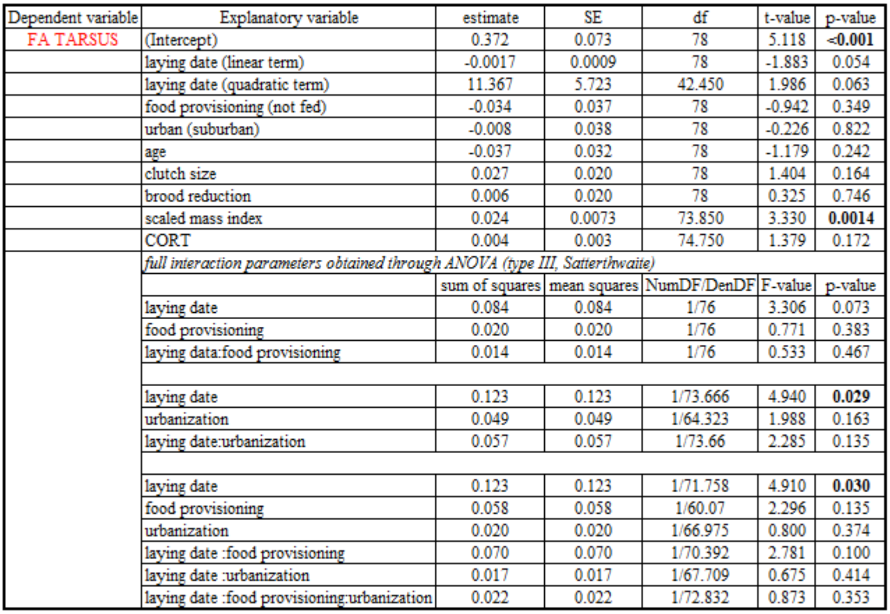
Table S2. Results from all explanatory variables tested to explain variation in CORT$_f$ levels. P-values of the reduced model are shown in bold, while results from all other variables were obtained by adding them one at a time onto the reduced model.



Dependent variable	Explanatory variable	estimate	SE	df	t-value	p-value
CORT	(Intercept)	-0.591	6.281	72	-0.094	0.925
	laying date (linear term)	-25.140	8.004	72	-3.141	**0.002**
	laying date (quadratic term)	28.781	12.497	72	2.303	**0.024**
	food provisioning (not fed)	-0.919	1.177	72	-0.781	0.437
	brood reduction	1.658	0.640	72	2.590	**0.012**
	scaled mass index	0.604	0.267	72	2.261	**0.027**
	laying date (linear): food provisioning (not fed)	29.398	10.855	72	2.708	**0.008**
	laying date (quadratic): food provisioning (not fed)	-21.070	13.666	72	-1.542	0.128
	urbanization (suburban)	-1.273	1.181	71	-1.078	0.285
	nestling age	-0.040	1.154	71	-0.035	0.972
	clutch size	0.477	0.742	71	0.642	0.523
	tarsus-based FA	2.307	3.950	71	0.584	0.561
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F-value	p-value
	laying date (quadratic)	292.459	146.230	2/72	5.696	0.0051
	food provisioning	15.665	15.665	1/72	0.610	0.437
	laying date (quadratic):food provisioning	274.040	137.020	2/72	5.337	0.0069
	laying date (quadratic)	297.256	148.628	2/69	5.685	**0.005**
	urbanization	30.000	30.000	1/69	1.148	0.288
	laying date (quadratic):urbanization	14.876	7.438	2/69	0.285	0.753
	laying date (quadratic)	299.472	149.736	2/67	5.678	**0.005**
	food provisioning	9.401	9.401	1/67	0.357	0.552
	urbanization	23.842	23.842	1/67	0.904	0.345
	laying date (quadratic):food provisioning	273.258	136.629	2/67	5.181	**0.008**
	laying date (quadratic):urbanization	42.036	21.018	2/67	0.797	0.455
	laying date (quadratic):food provisioning:urbanization	37.042	18.521	2/67	0.702	0.499

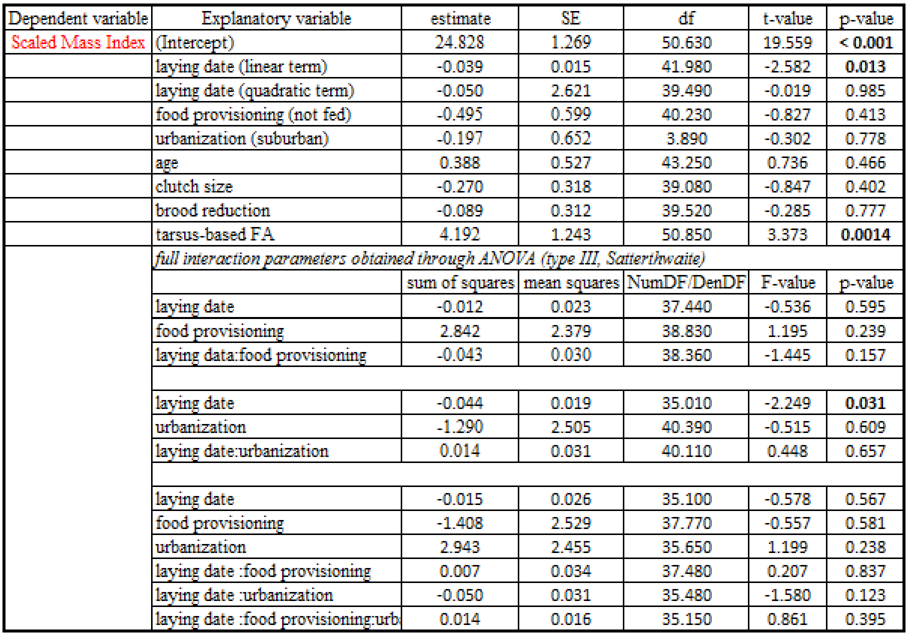
Note that for the three stress indicators (CORT-levels, tarsus-based FA and SMI), models were run with laying date as a linear or a quadratic term (see main text). For CORT-levels, the model with laying date as quadratic term performed best (see main text), and this quadratic model is therefore presented here.

Table S3. Results from all explanatory variables tested to explain variation in Tarsus-based FA. P-values of the reduced model are shown in bold, while results from all other variables were obtained by adding them one at a time onto the reduced model.



Dependent variable	Explanatory variable	estimate	SE	df	t-value	p-value
FA TARSUS	(Intercept)	0.372	0.073	78	5.118	<**0.001**
	laying date (linear term)	-0.0017	0.0009	78	-1.883	0.054
	laying date (quadratic term)	11.367	5.723	42.450	1.986	0.063
	food provisioning (not fed)	-0.034	0.037	78	-0.942	0.349
	urban (suburban)	-0.008	0.038	78	-0.226	0.822
	age	-0.037	0.032	78	-1.179	0.242
	clutch size	0.027	0.020	78	1.404	0.164
	brood reduction	0.006	0.020	78	0.325	0.746
	scaled mass index	0.024	0.0073	73.850	3.330	**0.0014**
	CORT	0.004	0.003	74.750	1.379	0.172
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F-value	p-value
	laying date	0.084	0.084	1/76	3.306	0.073
	food provisioning	0.020	0.020	1/76	0.771	0.383
	laying data:food provisioning	0.014	0.014	1/76	0.533	0.467
	laying date	0.123	0.123	1/73.666	4.940	**0.029**
	urbanization	0.049	0.049	1/64.323	1.988	0.163
	laying date:urbanization	0.057	0.057	1/73.66	2.285	0.135
	laying date	0.123	0.123	1/71.758	4.910	**0.030**
	food provisioning	0.058	0.058	1/60.07	2.296	0.135
	urbanization	0.020	0.020	1/66.975	0.800	0.374
	laying date :food provisioning	0.070	0.070	1/70.392	2.781	0.100
	laying date :urbanization	0.017	0.017	1/67.709	0.675	0.414
	laying date :food provisioning:urbanization	0.022	0.022	1/72.832	0.873	0.353

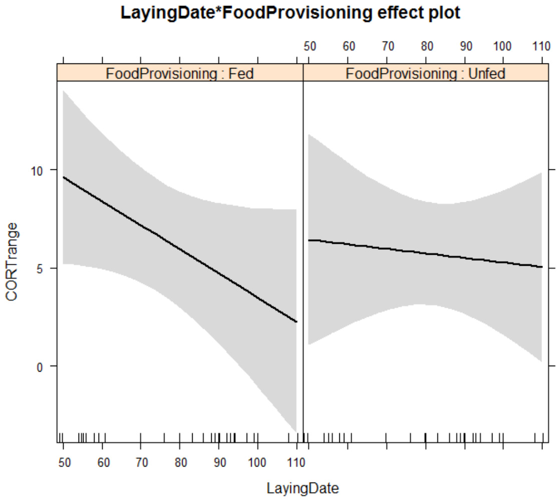
Table S4. Results from all explanatory variables tested to explain variation in SMI (Scaled Mass Index). P-values of the reduced model are shown in bold, while results from all other variables were obtained by adding them one at a time onto the reduced model.



Dependent variable	Explanatory variable	estimate	SE	df	t-value	p-value
Scaled Mass Index	(Intercept)	24.828	1.269	50.630	19.559	**< 0.001**
	laying date (linear term)	-0.039	0.015	41.980	-2.582	**0.013**
	laying date (quadratic term)	-0.050	2.621	39.490	-0.019	0.985
	food provisioning (not fed)	-0.495	0.599	40.230	-0.827	0.413
	urbanization (suburban)	-0.197	0.652	3.890	-0.302	0.778
	age	0.388	0.527	43.250	0.736	0.466
	clutch size	-0.270	0.318	39.080	-0.847	0.402
	brood reduction	-0.089	0.312	39.520	-0.285	0.777
	tarsus-based FA	4.192	1.243	50.850	3.373	**0.0014**
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F-value	p-value
	laying date	-0.012	0.023	37.440	-0.536	0.595
	food provisioning	2.842	2.379	38.830	1.195	0.239
	laying data:food provisioning	-0.043	0.030	38.360	-1.445	0.157
	laying date	-0.044	0.019	35.010	-2.249	**0.031**
	urbanization	-1.290	2.505	40.390	-0.515	0.609
	laying date:urbanization	0.014	0.031	40.110	0.448	0.657
	laying date	-0.015	0.026	35.100	-0.578	0.567
	food provisioning	-1.408	2.529	37.770	-0.557	0.581
	urbanization	2.943	2.455	35.650	1.199	0.238
	laying date :food provisioning	0.007	0.034	37.480	0.207	0.837
	laying date :urbanization	-0.050	0.031	35.480	-1.580	0.123
	laying date :food provisioning:urb	0.014	0.016	35.150	0.861	0.395

Table S5. Results from all explanatory variables tested to explain variation in the range of CORT$_f$ levels per house sparrow nest. P-values of the reduced model are shown in bold, while results from all other variables were obtained by adding them one at a time onto the reduced model.

Dependent variable	Explanatory variable	estimate	SE	df	t-value	p-value
CORT: range per nest	(Intercept)	-28.934	9.334	30.000	-3.100	**0.00419**
	laying date (linear term)	0.010	0.045	29.000	0.214	0.832
	laying data (quadratic term)	6.519	4.483	28.000	1.454	0.157
	food provisioning (not fed)	0.312	1.584	29.000	0.197	0.845
	brood reduction	-0.825	0.837	29.000	-0.985	0.333
	scaled mass index	1.381	0.425	30.000	3.250	**0.00285**
	laying date (linear):food provisioning (not fed)	0.154	0.083	27.000	1.862	0.074
	laying date (quadratic): food provisioning (not fed)	2.944	11.919	25.000	0.247	0.807
	urbanization (suburban)	-0.473	1.770	-2.796	0.267	0.808
	nestling age	0.196	1.375	29.000	0.143	0.887
	clutch size	0.104	0.938	29.000	0.111	0.912
	tarsus-based FA	15.919	7.599	30.000	2.095	**0.045**
	full interaction parameters obtained through ANOVA (type III, Satterthwaite)					
		sum of squares	mean squares	NumDF/DenDF	F-value	p-value
	laying date	-0.060	0.058	27.000	-1.036	0.309
	food provisioning	-11.880	6.710	27.000	-1.770	0.088
	laying date:food provisioning	0.154	0.083	27.000	1.862	0.074
	laying date	-0.064	0.049	26.124	-1.320	0.198
	urbanization	-17.420	6.995	26.161	-2.490	**0.019**
	laying date:urbanization	0.215	0.085	26.902	2.527	**0.018**
	laying date	-0.123	0.060	22.422	-2.035	0.054
	food provisioning	-11.907	6.263	20.917	-1.901	0.071
	urbanization	-16.819	7.099	23.455	-2.369	**0.026**
	laying date :food provisioning	0.146	0.079	21.181	1.837	0.080
	laying date :urbanization	0.197	0.095	23.992	2.074	**0.049**
	laying date :food provisioning:urbanization	0.012	0.038	21.883	0.322	0.750



Do phenotypic responses to local urbanisation differ between habitats embedded in urban or rural landscapes?

5

Modified from:

Salleh Hudin, N., De Neve, L., Teyssier, A., Rouffaer, L., Aerts, J., & Lens, L.. (2017). Do phenotypic responses to local urbanisation differ between habitats embedded in urban or rural landscapes? (manuscript).

LL, LDN designed the study; NSH, AT, LR collected data; AT performed ptilochronology analysis; LL, NSH performed FA analysis; JA, NSH performed CORT analysis; LDN, NSH performed statistical analysis; NSH, LDN, LL interpreted data; all authors drafted the manuscript.

Abstract

The use of different spatial scales to quantify urbanization levels is known to affect the resulting patterns on species diversity in relation to urban environments, where diversity at local scale showed contrasting patterns with diversity measured at the landscape scale. More interestingly, the combination of both scales explains patterns in diversity better than when used separately. Based on these findings, we predicted that local conditions are influenced by the landscape context and so that both scales interact to explain variation in individual phenotypic responses. Therefore, we here explored if the association between different phenotypic traits and urbanization levels at the local scale interacted with the urbanization level at the landscape scale in house sparrows (*Passer domesticus*). We used the average foraging range (400 m, local scale) and the maximum daily foraging range (1600 m, landscape scale) as biologically relevant spatial scales. Our hypothesis was partially confirmed as relationships between body condition (scaled body mass) and local urbanisation levels were affected by urbanisation at the landscape scale. These patterns are likely explained by a combination of changing conditions in food availability and predation pressure at local and landscape scales. In addition, morphological traits (body condition, tarsus length) in relation to local urbanisations varied with sex, and wing length overall decreased with local urbanisation levels. In accordance with previous studies, our results of levels in feather corticosterone, fluctuating asymmetry and ptilochronology did not reveal any evidence that urban environments would be stressful to house sparrows.

Introduction

One of the major anthropogenic forces that change world land cover is urbanization (Grimm et al., 2008). Humans replace natural landscapes into impervious surfaces, buildings, and transportation systems which indirectly cause substitutions of natural reserves by artificial resources (Grimm et al., 2008; Marzluff, Bowman, & Donnelly, 2001; McKinney, 2002). This change in environmental conditions often results in reductions of species richness and evenness in local habitats and the declines of native species worldwide (Chace & Walsh, 2006; Grimm et al., 2008; Paul & Meyer, 2001). The persistence of organisms in these environments depends on their ability to modify their morphology, physiology, and behavior to cope with the changing environmental conditions within their lifetime (McDonnell & Hahs, 2015). The altered phenotypes may only remain in short term, known as phenotypic plasticity, or they may lead to microevolution if shifts in genotypes occurred (McDonnell & Hahs, 2015). An example of microevolution is shown by Cheptou, Carrue, Rouifed, and Cantarel (2008) where weed populations, *Crepis sancta*, which inhabit small patches surrounded by an unsuitable matrix of buildings and asphalt in cities are under the pressure of competition, inbreeding, and extinction. While having high dispersal ability could possibly reduce such pressures, the combination of common garden experiment and genetic modelling revealed that dispersal is costly in this species and that urban-induced habitat fragmentation caused evolution towards lower dispersal that occurred only within 5-12 generations (Cheptou et al., 2008). Such microevolutionary changes can leave ecological signatures which in turn may result in cascading effects at phenotype, population, community and ecosystem levels; and thus, lead to eco-evolutionary dynamics (Alberti, 2015; Pelletier, Garant, & Hendry, 2009).

Given the potential influence of urbanization on organisms, studies on urban ecology, particularly on birds, have increased substantially during the last decades (Marlzuff, Bowman, & Donnelly, 2001). Many of these studies focused on the comparison of bird populations living in areas that differ in urbanization level. Previous studies employed various descriptive criteria to distinguish the different urbanization levels, however these criteria were often unclear and arbitrary (reviewed by Marzluff et al. (2001)). Because of this ambiguity, urban ecological studies nowadays determine urbanization levels by using quantitative methods; for example the built-up percentage is among the most common parameters used (eg. Bókony, Seress, Nagy, Lendvai, and Liker (2012); Chávez-Zichinelli et al. (2010); Foltz et al. (2015); Liker, Papp, Bókony, and Lendvai (2008); Meillère et al. (2017); Zhang et al. (2011)). Nevertheless, there are large variations between studies on how much built-up percentage is necessary to classify an area as urbanized. The calculations of built-up percentage are also dependent on the size of areas, henceforth called scales. The scales used to quantify urbanization levels differ greatly among studies and these variations will affect the resulting urbanization level of an area. Standardization of these criteria and scales is important as it allows appropriate replications and comparisons between studies and generalization of predictions and conclusions.

Urban ecology studies often use the terms local and landscape scales. The local scale describes small ecological habitats ranging from few meter squares to thousands of hectares (Poiani, Richter, Anderson, & Richter, 2000). The environmental components (biotic and abiotic) of the local habitat such as food resources, nesting sites, microclimates, competition, and predation are important in determining whether individuals will live there or not (Litteral & Shochat, 2017). The larger area surrounding the local habitat is classified as the landscape scale. The areas within the landscape scale may comprise several habitat types, and therefore, should show more variation in structure and composition than the areas within the local scale (Poiani et al., 2000). The current body of knowledge showed that the use of different scales in studies on species diversity yield inconsistent results. For example, bird species richness reduced with increasing urbanization when measured at local scale across taxa, but at a coarser scale, the opposite trend was found (Pautasso (2007) and the references therein). Despite the attempts to understand the roles of the local and landscape scales and their relative importance, there is also evidence that the combination of both scales is best at predicting species diversity (Litteral & Shochat, 2017). It is not surprising that the combination of local and landscape scales can better describe patterns of species diversity in relation to their environment because birds select habitats according to environmental conditions at multiple spatial scales; such as territories within habitats, habitats within landscapes, and so on (Litteral & Shochat, 2017).

Species persistence in an environments depends on individual fitness (Maldonado-Chaparro, Read, & Blumstein, 2017) and fitness is often the results of individual response to their environment (Sol, Lapiedra, & González-Lagos, 2013). Because species diversity at local scale is affected by the surrounding environments (Litteral & Shochat, 2017), we predicted that environments at landscape scale can influence phenotypic responses at local habitat. In the case of species interactions, for instance, every species has its own niche and species may differ in their interaction with urbanization (eg. some species prefer urbanized areas while others do not). We can expect that all species that have overlapping niches will affect one another, and species that have a space-use that ranges up to the landscape scale are likely to affect species that are present at smaller scales. As an example, a predator species may need large foraging areas to fulfil its requirements. If the predator is restricted to forest habitats, then we can expect that a forest patch surrounded by larger forested areas will house more predators than a forest patch located within a city. Therefore, the predation pressure in the continuous forest should be higher than in the urban forest patch. These differences in predation pressures are likely to cause differences in the phenotypic responses of the prey species.

To the best of our knowledge, there is no general consensuses on what scale sizes are appropriate in defining the different urbanization levels. For avian species diversity studies, Marzluff et al. (2001) proposed the use of 100 ha area to define urbanization level. However, appropriate scales might be different when focusing on phenotypic responses of one species because species differ in their home ranges (reviewed by Jetz, Carbone, Fulford, and Brown (2004)). For example, the home range of house sparrows (*Passer domesticus*) is 0.0032-0.49 ha (Vangestel, Braeckman, Matheve, & Lens, 2010) whereas the rock doves (*Columba livia*), which are two times larger than the house sparrows, have a home range of 1.34-4.96 ha (Sol & Senar, 1995). Therefore, the scales used to quantify urbanization should correspond to the biologically relevant space-use of the species of interest (Wu & Li, 2006).

The aim of our study is to explore if the association between phenotypic traits and urbanization level at local scale interact with the urbanization level at the landscape scale in house sparrows, a species that is distributed along a wide range of urbanisation gradients. We assigned 400 m and 1600 m in radius as the local and the landscape scale, respectively. These spatial boundaries correspond to the average and maximum foraging ranges of house sparrows, and hence, are ecologically meaningful for this species (Anderson, 2006; Heij & Moeliker, 1990). We measured phenotypic traits that correspond to physiological stress responses (feather corticosterone, CORT_f; fluctuating asymmetry, FA; ptilochronology) and morphological responses (scaled body mass (SMI), tarsus length, wing length, bill height).

Methodology

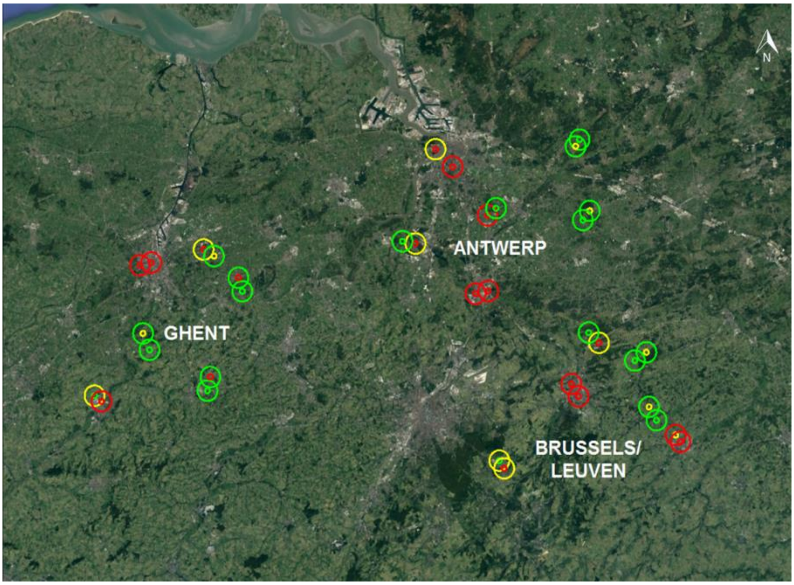
Study area and sampling procedures

House sparrows were trapped in locations that varied in level of urbanization at two spatial scales (see further), within a 4655 km2 polygon demarcated by the cities of Gent, Antwerp, Brussels, and Leuven (Flanders, Belgium). First, we identified 18 plots (each measuring 3 by 3 km) located within the polygon boundary and varying in the extent of urbanization as calculated with ArcGIS v9.2 and its spatial analyst extension. These 18 plots were a subset of a larger set of 27 plots chosen for a larger project including a wide array of taxa, including the house sparrows (Rouffaer et al. (2016); Fig. 1). As a base layer we used the vectorial Large-scale Reference Database (GRB; Vlaanderen (2013)). Cut-off points for the percentage of built-up areas were set at 0-5% for “rural” plots (lowest level of urbanization), 5-15% for “suburban” plots (intermediate level), and >15% for “urban” plots (highest level). To ensure a more natural environment for the lowest urbanization class, we only selected plots comprising >20% of ecologically valuable areas, as described by the vectorial ‘Biologische Waarderingskaart’ (AGIV, 2015).

Next, we used all available population information (i.e. own inventories conducted during winter 2012-13 complemented with available data from various citizen science projects) to select 36 sparrow populations within these 18 plots (18 in rural plots, 9 in suburban plots and 9 in urban plots) that were located at least 1 km apart (to allow statistical independence) and were characterized by contrasting levels of urbanization at a local scale (Table S1). For each of these populations, we recalculated the level of urbanization at two spatial scales (see higher for ecological rationale): “local” scale (400 m radius around the center of the main capture site) and “landscape” scale (1600 m radius around the center of the main capture site, thereby excluding the 400 m radius of the local scale). Therefore, each site may have different urbanization levels at the different scales.

The 400 m radius corresponds to the “normal daily foraging range” of sparrows, as 89% of the individuals were observed to stay within this range for foraging (Heij and Moeliker (1990). At this scale, urbanization level was considered as a continuous variable, and is hereafter referred to as urbanization at "local scale". The 1600 m radius corresponds to the "maximum daily foraging range" because house sparrows have been observed to sometimes fly as far as this distance for foraging (Anderson (2006) and the references therein) and will hereafter be referred to as urbanization at "landscape scale". Moreover, review of existing literature revealed that the majority of urban bird studies, excluding corvids, found that scales of 500 m or larger is considered as landscape scale (reviewed by Litteral and Shochat (2017)).

Fig 1. Sampling sites across urban gradients in Belgium. Small circles represent the local scale (400 m in radius) while the large circles represent the landscape scale (1600 m in radius). Urbanization level is indicated by colors where red, yellow, and green where indicate urban, suburban, and rural environments, respectively.



Each sparrow population was sampled twice (Sep-Dec 2013 and Jan-Mar 2014). A total of 405 house sparrows were trapped by mist-netting. Upon capture, each individual was (i) ringed, sexed, and weighed (to the nearest 0.01g using a digital balance); (ii) their tarsus length, wing length, and bill height were measured (to the nearest 0.01 mm using a digital calliper); (iii) the fifth (i.e. second outermost) right and left rectrices of each bird were plucked and stored in individually-labeled envelopes for analysis of feather corticosterone, fluctuating asymmetry and ptilochronology. Afterward, the birds were released back at their capture sites. After excluding data on recaptured individuals, juveniles, and individuals without feather samples that reached the standard for $\mathrm{CORT_f}$ analysis (see below), we have a final dataset that comprises of 315 individuals, of which 135 females and 180 males. Individuals were captured in each population (range 3 – 25, mean 8.75 ± 4.32), and in most populations both males and females were captured, except for one site with only males and one site with only females.

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 through oral permission by the respective land owners. The sampling protocols used were approved by the Ethical Committee VIB Ghent site (EC2013-027).

Quantification of $CORT_f$ level

A single feather, weighing on average 10 mg, was sampled and dirt (*e.g.* feces, mud, etc.) was manually removed by using tweezers. Next, the feather was flattened on a polystyrene board along with 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 was determined on an analytical balance (g). By 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 ethanol followed by ultrapure water and dried with a paper tissue to avoid cross-contamination between samples. Of the homogenized sample 0.0100 g ± 0.0005 g was weighed into a 10 ml test tube. Subsequently, 8 ml of methanol was added as extraction solvent and 10 µL of a corticosterone-d_8 solution of 0.5 µg L^{-1} was added as internal standard. When lower amounts of feather were used, the volume of corticosterone-d_8 was adapted accordingly. The sample was vortex-mixed for 30 s, placed on an overhead shaker at 60 rpm for 1 h at room temperature, and centrifuged for 10 min at 3500 g at 7 °C. All supernatant was taken, evaporated to dryness under nitrogen at 60 °C using a nitrogen evaporator, and reconstituted in 5 ml

H_2O/MeOH (80:20; v/v). After conditioning a C_{18} SPE column with 3 ml of methanol followed by 3 ml of ultrapure water, the sample was loaded. The column was washed with 4.5 ml H_2O/MeOH (65:35; v/v) and retained compounds were eluted with 2.5 ml H_2O/MeOH (20:80; v/v) into a 10 ml test tube and evaporated to dryness under nitrogen at 60 °C using a nitrogen evaporator. The sample was finally reconstituted in 50 µL H_2O/MeOH (80:20; v/v) in a vial and analyzed by means of UPLC-MS/MS. Since in future research matrix-matched calibration curves are not practically feasible, calibration curves were made in diluent and a factor of 10000 was used to calculate the corresponding GC concentration in a standardized sample weight of 10 mg of feather.

Measurement of ptilochronology

All feathers ($N = 320$) were processed by a single person (AT), as follows. First, each collected feather was pinned on a separate, white, polystyrene board and the total feather length (also for FA analysis) was measured to the nearest 0.01 mm with a digital calliper. Second, 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. Third, each marked board was scanned (Océ OP1130) and growth bar widths (for ptilochronological analysis) were automatically measured with image analysis software (KS400 Zeiss).

Measurement of fluctuating asymmetry

A total of 335 pairs of rectrices were used in the FA analysis. Two times measurements of rectrix length from each side yielded high repeatability values (right side $r = 0.932$, $N = 315$, $P < 0.001$; left side $r = 0.937$, $N = 315$, $P < 0.001$) following equation by Lessells and Boag (1987). Because the degree of FA is often very small (typically in the order of 1% or less of the size of the trait, Møller and Swaddle (1997)) and because some traits cannot be measured with high accuracy, measurement error (ME) can be expected to cause an upward bias in the between-sides variance if not appropriately corrected for (Merilä & Björklund, 1995; Palmer & Strobeck, 1986; Van Dongen, Molenberghs, & Matthysen, 1999). Therefore, feather FA was analyzed through mixed-regression analysis with Restricted Maximum Likelihood (REML) parameter estimation (Van Dongen et al., 1999). While yielding identical FA estimates as two-way mixed ANOVA models, the REML method allows to test for FA significance, to model heterogeneity in FA and measurement error among populations or treatments, to test for directional asymmetry (DA), and to obtain estimates of individual FA that are unbiased with respect to ME and DA. Fixed intercepts estimate overall trait size, fixed slopes estimate DA, and the random intercepts and slopes (both estimated *within* individuals) estimate the variation in individual trait value and individual FA, respectively (Van Dongen et al., 1999). Based on this analysis, variance due to ME was not heterogeneously distributed between sampling sites (likelihood-ratio tests: all $p > 0.05$), hence a single error component was estimated. Variance in signed FA was highly significant (likelihood-ratio test: $p < 0.001$) 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.

Estimation of body condition

We calculated body condition according to the scaled mass index (SMI, Peig and Green (2009)), 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). We ran a correlation analysis between body mass and tarsus length on a log–log scale ($r = 0.421$, $P < 0.001$) and consequently applied that variable to scale body mass. After excluding 3 outliers (i.e., |standardized residual| > 3), the regression slope was 1.51, whereas average tarsus length was 18.74 mm. We thus calculated the scaled mass index as body mass × $(18.74/\text{tarsus length})^{1.51}$ (Peig & Green, 2009, 2010).

Statistical analyses

In order to reduce the complexity of statistical models, we decided to work only with capture sites embedded in an urban or rural landscape, while excluding sites in suburban landscapes. Therefore, the number of capture sites was reduced to 26 sites (10 Urban and 16 Rural) and 107 females and 143 males were included in the models.

The local urbanization ratio fitted a normal distribution after log transformation, as well as CORT_f and FA measurements. Continuous predictors considered in the models were first z-transformed to a mean of zero and standard deviation of one.

To test whether the response (CORT_f, ptilochronology, FA, SMI, tarsus length, wing length, bill height) was affected by urbanisation at different scales, we used general linear mixed models (lmer function of R packages lme4 and lmerTest; Bates, Maechler, Bolker, and Walker (2015)) in which we included "local urbanisation" as a continuous and "landscape urbanisation" as a categorical predictor, as well as the two-way interaction between both fixed effects. In order to test if both sexes responded in a similar way to urbanization, "sex" was also included as fixed effect, as well as the two-way interactions between sex and local and sex and landscape urbanization. Finally, the three-way interaction between sex*local*landscape was also considered. Population was considered a random effect to account for the non-independence of individuals from the same population.

We checked whether the assumptions of normally distributed and homogeneous residuals were fulfilled by visually inspecting a qqplot and the residuals plotted against fitted values (both indicated no obvious deviations from these assumptions in any of the models). Variance Inflation Factors (VIF, Field (2005)) were derived using the function vif of the R-package car (Fox & Weisberg, 2011) applied to a standard linear model excluding the random effect.

Given that populations differ in distance to each other which could result in spatial autocorrelation, we tested the residuals for our models on spatial autocorrelation by using the Moran's I test (moran.test function in R). The residuals for none of our models resulted significantly spatially auto-correlated.

We adopted a frequentist approach whereby full models (i.e. models containing all explanatory variables considered here) were reduced in a stepwise manner, by dropping non-significant interaction terms before main effects from the full model, until only $p < 0.05$ predictors remained. Denominator degrees of freedom are computed by the default Satterthwaite's method. In each step, the AIC of models with and without the dropped effect were also compared by performing a likelihood ratio test using the anova() function with argument test set to "Chisq". After model reduction, a likelihood ratio test was used to test for the significance of the complete reduced model compared to the null model (including only the random term).

Given that CORT is passively deposited during feather formation (Bortolotti, 2010; Jenni-Eiermann, Helfenstein, Vallat, Glauser, & Jenni, 2015; Romero & Fairhurst, 2016), $CORT_f$ is likely influenced by the length or quality of the feather (Patterson, Kitaysky, Lyons, & Roby, 2015). In our dataset, $CORT_f$ was indeed significantly correlated to feather length, mass, and quality (mass corrected for length), but most strongly so to feather mass (estimate 0.097 ± 0.027, $F_{1,248} = 12.46$, P=0.00049). Therefore, in the model with $CORT_f$ as response, feather mass was included as a covariate (in the null model). All statistical analyses were performed in R statistical computing software (R Core Team, 2016).

Results

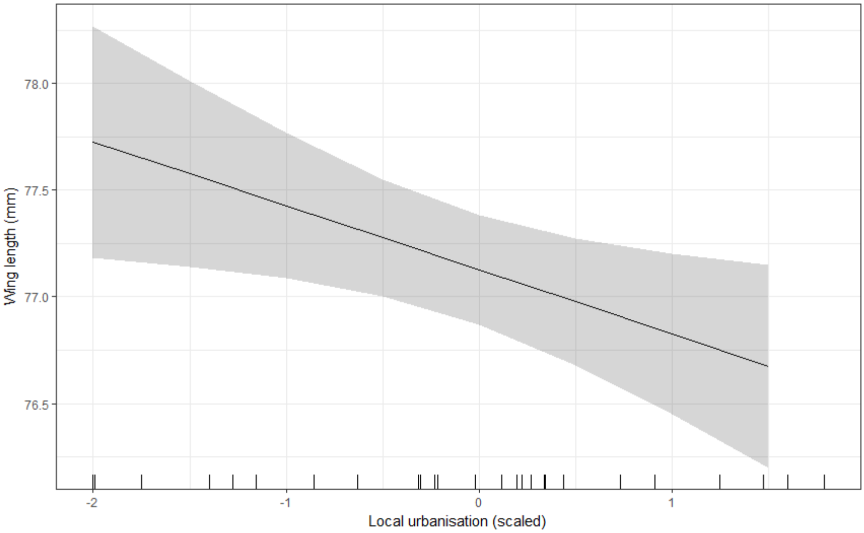
Stress proxies

None of the considered predictors remained in the model to explain significant variation in CORT$_f$ levels across sparrow populations (likelihood ratio tests with the null model, all P>0.64). The same applied to feather ptilochronology (likelihood ratio tests with the null model, all P>0.24) and fluctuating asymmetry (likelihood ratio tests with the null model, all P>0.38).

Body size and condition

Males had larger bills than females (likelihood ratio test, $\chi 2$ = 6.01, df=1, P= 0.014, estimate 0.099 ± 0.039), but bill height was not affected by urbanization (likelihood ratio test comparing to null model, all P>0.23). Males also had longer wings than females (estimate 2.23 ± 0.24) and both sexes had shorter wings with increasing local urbanization (estimate -0.30 ± 0.13, Fig. 2). The reduced model with sex and local urbanization as predictors explained significantly more variation in wing length than the null model (likelihood ratio test, $\chi 2$ = 78.74, df=2, P<0.001).

Fig 2. Predicted wing length in relation to local urbanization level (z-transformed).



Both landscape and local urbanization scale were related to tarsus length and SMI, but differently so in males and females (Table 1, Table 2). Tarsus length decreased in males in relation to local urbanization, while no such trend was observed in females (Fig. 3A). On the other hand, SMI decreased in females in relation to local urbanization, while no such trend was observed in males (Fig. 3B). In populations in rural landscapes, males and females did not significantly differ in tarsus length or SMI, but in populations embedded in urban landscapes, females had smaller tarsi, but higher SMI compared to males. In addition, SMI varied differently according to local urbanization, depending on if the population was embedded in an urban or rural landscape (Table 2). Sparrows of populations embedded in an urban landscape tended to decrease SMI according to increasing local urbanization (though the relationship did not seem to reach significance, Fig. 4), while no relationship with local urbanization was observed in populations embedded in a rural landscape.

Table 1. Results from the reduced general linear mixed model that best explained variation in tarsus length. Significant effects are shown in bold. Estimates of fixed factors show differences of male *vs* female and urban *vs* rural landscape. Likelihood ratio test with the null model, df=5, χ^2=11.49, P=0.042.

Explanatory variable	Chisq	P-value	Estimate ± SE
Sex	1.239	0.266	-0.15 ± 0.15
Landscape	0.008	0.927	-0.36 ± 0.23
Local	0.846	0.358	0.15 ± 0.11
Sex*Landscape	**4.962**	**0.026**	**0.67 ± 0.29**
Sex*Local	**8.862**	**0.003**	**-0.42 ± 0.14**

Table 2. Results from the reduced general linear mixed model that best explained variation in SMI. Significant effects are shown in bold. Estimates of fixed factors show differences of male *vs.* female and urban *vs.* rural landscape. Likelihood ratio test with the null model, df=6, χ^2=12.08, P=0.060.

Explanatory variable	Chisq	P-value	Estimate ± SE
Sex	0.061	0.805	0.57 ± 0.35
Landscape	0.337	0.561	1.51 ± 0.60
Local	0.293	0.588	-0.33 ± 0.28
Sex*Landscape	**4.956**	**0.026**	**-1.54 ± 0.69**
Sex*Local	**8.261**	**0.004**	**0.93 ± 0.32**
Landscape*Local	**4.864**	**0.027**	**-0.94 ± 0.43**

Fig 3. Predicted values ± 95CI of A) tarsus length and B) SMI in relation to local urbanization (z transformed) in male and female house sparrows

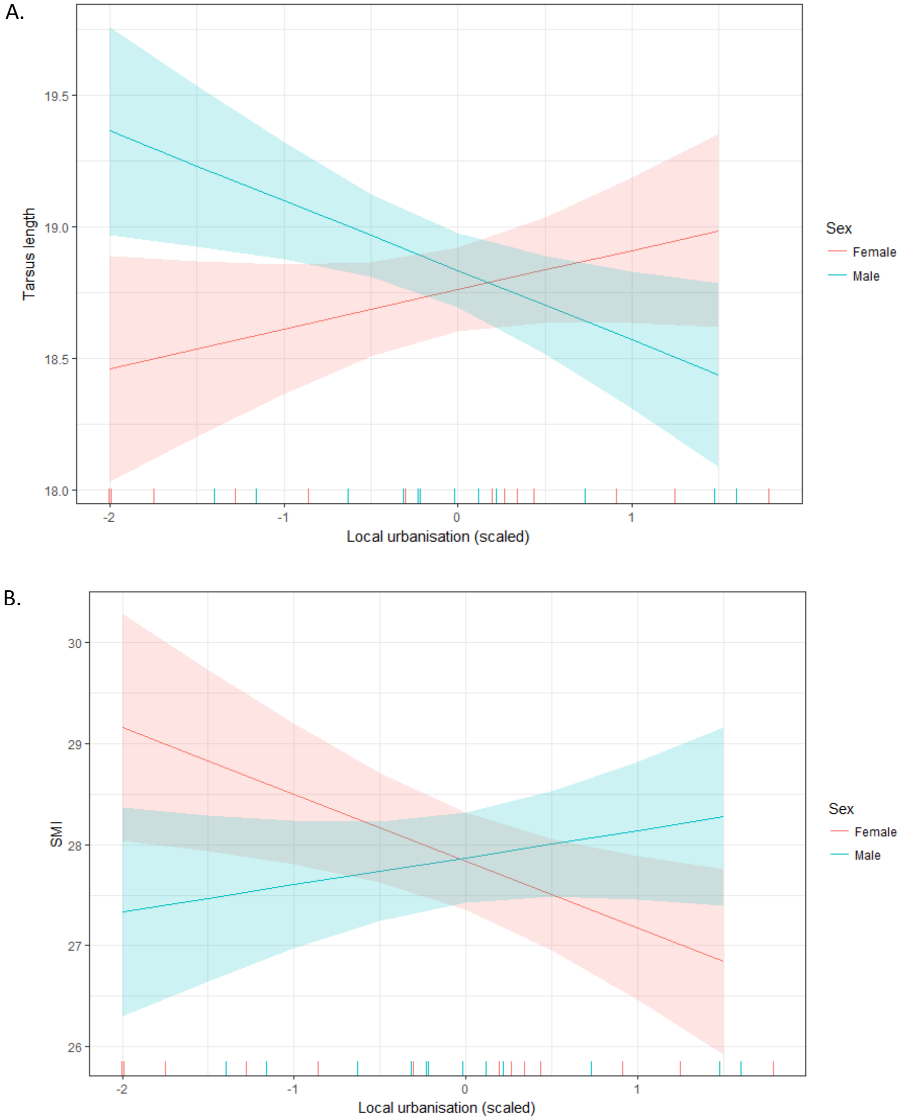


Fig 4. Predicted mean ± SE of **A)** Tarsus length and **B)** SMI of males and females of populations embedded in an urban or rural landscape

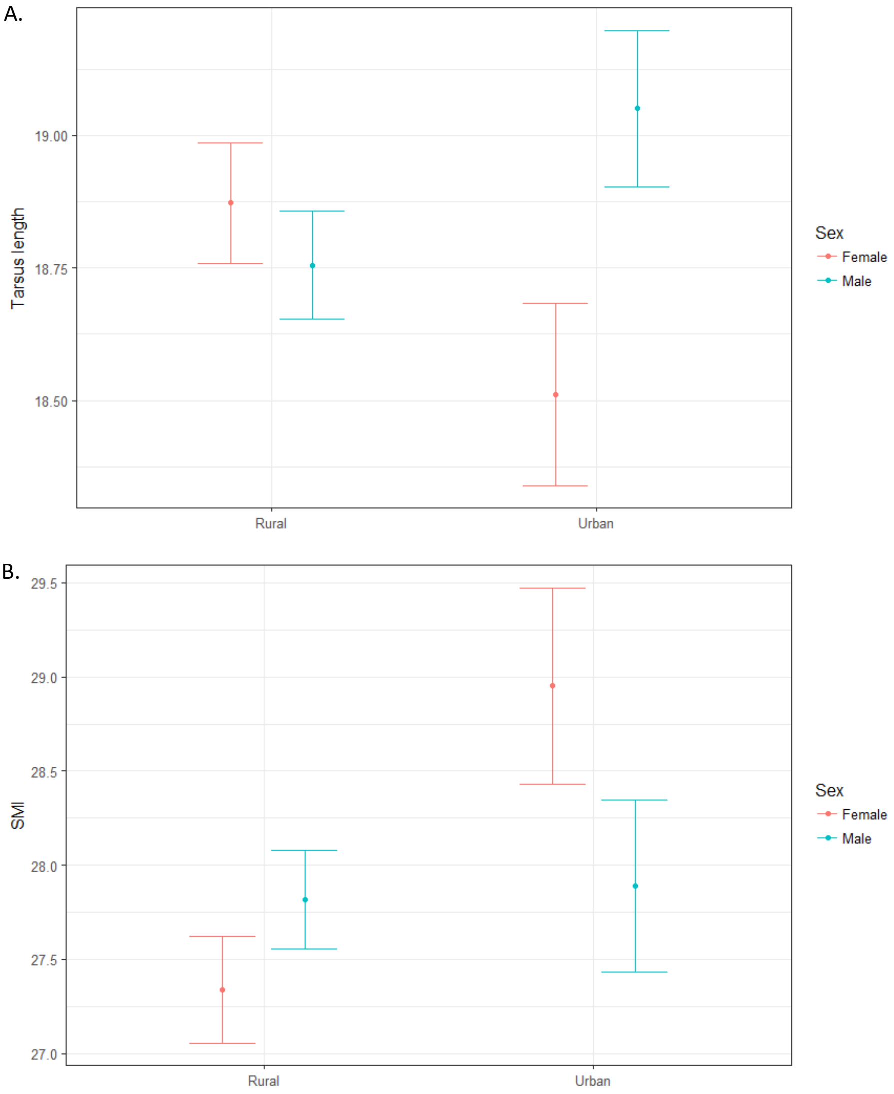
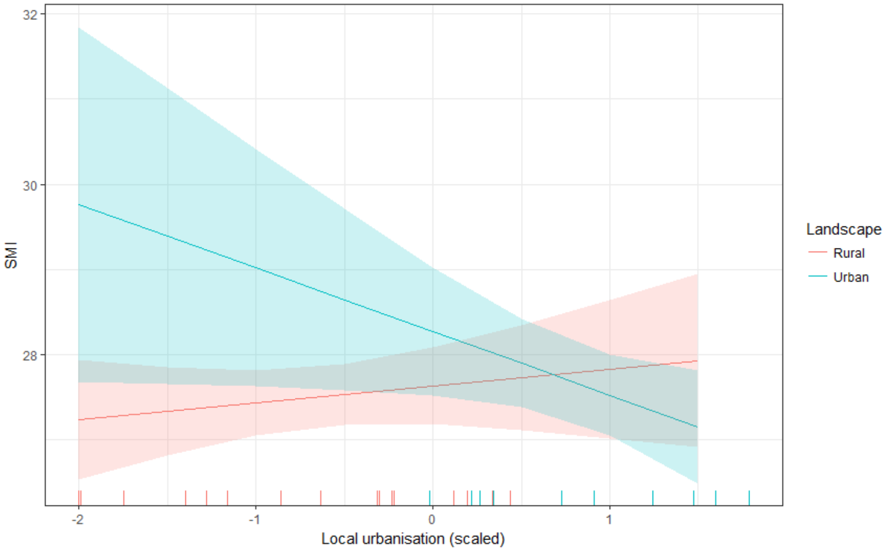


Figure 5. Predicted SMI (±95%CI) in relation to local urbanization ratio in populations embedded in urban and rural landscapes.



Discussion

The results partly support our hypothesis that the urbanisation levels of landscapes that surround the local habitats do interact with the association between phenotypic traits and urbanisation at the local scale. In urban landscapes, birds tended to decrease their SMI as the urbanization intensity increased in local habitats. However, no such effects appeared in rural landscapes. Besides that, we also found that phenotypic traits in relation to local urbanisation vary according to intrinsic factors such as sex. SMI in females and wing length in males was reduced with increasing urbanization level in local habitats.

An interaction effect between local and landscape scales was only found for SMI. A previous study on house sparrows found that SMI plastically responds to food predictability, probably mediated through predation risk (Salleh Hudin et al., 2016). In urban areas, house sparrows are likely exposed to higher predation pressure than in the rural areas following the recent re-colonization of avian predator species (Seress & Liker, 2015). Eurasian sparrowhawks (*Accipiter nisus*), the key predator of house sparrows, reached a higher density in several European cities compared to many natural areas (Seress & Liker, 2015). Moreover, domestic cats (*Felis catus*) increased their abundance in cities so much that it exceeded their natural carrying capacity (Baker, Molony, Stone, Cuthill, & Harris, 2008; Lepczyk, Mertig, & Liu, 2004; Sims, Evans, Newson, Tratalos, & Gaston, 2008). Sparrowhawks and cats have a home range of about 1550 m (Marquiss & Newton, 1982; Selås & Rafoss, 1999) and 79 m in radius, respectively, and thus coincide within the foraging range of house sparrows at the landscape and the local scales. The higher presence of sparrowhawks within urban landscape scales exerts predation pressure on urban house sparrows at the local scale where cats also present. These conditions put house sparrows under high predation pressure especially in highly urbanized local habitats embedded within urban landscapes. To lower the risk of being depredated, house sparrows may reduce their SMI which enables them to escape better from predators (Salleh Hudin et al., 2016) through faster take off (Yom‐Tov, Yom‐Tov, Wright, JR Thorne, & Du Feu, 2006). Birds can maintain low SMI without exposing themselves to the risk of starvation because food supplies are abundant and highly predictable in urban landscapes, and thus decrease the needs to keep high body reserves (Shochat, Warren, Faeth, McIntyre, & Hope, 2006).

We also found a decrease in female SMI when local urbanization intensity increased, while such effect was absent in males, suggesting sexual differences in predation pressure. Ensminger and Westneat (2012) conducted an experiment where they tested risk taking behavior in house sparrows and they found that females expressed more wariness than males as shown by longer hesitance to feed again following disturbance by threatening objects. Besides that, female house sparrows also display more cryptic colorations compared to the males (Götmark, Post, Olsson, & Himmelmann, 1997). Since crypsis is one of the strategies to reduce predation risk in birds (Götmark et al., 1997), these differences in behaviour and morphology in house sparrows may reflect that females experience a higher perceived predation risk and thus, it is possible that the reduced SMI in females corresponds to an increased perceived predation pressure when local habitats become more urbanized.

Apart from SMI, tarsus length was also negatively related to urbanization level at local scale although this pattern was only present in males. In house sparrows, tarsus length increases linearly from hatching to about 10 d of age, during which it reaches a length close to that of adults (reviewed by Anderson (2006)). Therefore, variations in tarsus length should reflect the conditions during the developmental phase. Studies that investigated variation in house sparrow tarsus length found that shorter tarsi were caused by poor nutrition during development (Bókony et al., 2012; Heiss, Clark, & McGowan, 2009; Meillère, Brischoux, Parenteau, & Angelier, 2015; Seress, Bókony, Heszberger, & Liker, 2011). It is known that invertebrates, which are the main food resources for nestlings, have lower abundance in urban areas (Tryjanowski et al., 2015). Sexual differences in energetic demands and in sensitivity to food restriction during development have been found in several bird species (Oddie, 2000; Potti, 1999), and so, if male house sparrow nestlings have higher energetic demands and are more sensitive to food restriction during development than females, it is possible that poor nutrition in more urbanised habitats can cause reduced tarsus length in males. The fact that variation in male tarsus length was only observed at the local scale may suggest that house sparrow parents mainly restrict their foraging range to the local scale during the breeding season. This idea is in line the average foraging range of breeding house sparrow which is only 30-50 m in radius from the nests (Heij & Moeliker, 1990; Peach, Sheehan, & Kirby, 2014).

Similar to tarsus length, wing length is also influenced by food supplies during chick rearing (Ibáñez-Álamo & Soler, 2010; Seress et al., 2012). However, in adults, wing feathers molt every year, thus differences in wing length should also reflect the environmental conditions when molting takes place i.e. both the nutritional condition and the predation pressure. Predation pressure is stronger in highly urbanized areas, so the shorter wings of house sparrows may serve as a morphological response to escape from predators. For example, Hedenström and Rosén (2001) found that prey species are likely to escape predation when they perform tight turn during fast horizontal flight. Such strategy is common in passerines where the high maneuverability can be achieved by having short wings whereas fast flight is possible when wing loading is low (Hedenström & Rosén, 2001). Since house sparrows reduced their SMI and wing length as local urbanization level increases, it is likely that they can adopt the mentioned escape strategy when they are being chased by predators.

For a highly sedentary species like house sparrows, it is not surprising that most of the morphological traits responded only to the local scale as they mainly move within the local habitats. Thus, for less mobile species, local scale is expected to be more influential in shaping the individuals' responses to the environments. In this study, urbanisation at local, landscape, or the interaction of both was not related to bill dimensions, even if variation in bill size of granivorous birds is related to the size and hardness of seeds (Badyaev, Young, Oh, & Addison, 2008; Herrel, Podos, Huber, & Hendry, 2005). This implies that seeds eaten by house sparrows along urbanization gradient are probably similar in their structures. Similarly, urbanisation at neither scale nor the interaction explained variation in any of the indices of stress levels in house sparrows, thus confirming the previous findings that urban environments may not cause stress to this urban exploiter species.

In conclusion, our study does confirm that urbanisation in adjacent landscapes might have the potential to influence bird responses to urbanisation at local scales. Whether multiple scales are important to explain patterns in morphological responses mainly depends on whether ecological conditions relevant for the species of interest are at play at spatial scales. Our results suggest that (i) different traits (morphology and physiology) are exposed to different selections pressures; and (ii) it is important to identify and consider relevant environmental conditions for the species of interest and whether and how these are affected by urbanisation at different scales. Our knowledge of whether and how urbanisation at local and landscape scale might interact in shaping animal phenotypic responses to urbanization is still very scarce, but our study suggests that it is important to consider this aspect in the future to increase our understanding of how animals respond in urban environments.

Acknowledgement

We thank Pieter Vantieghem, Hans Matheve, Lies Ghysels and Hadia Mahmood for their invaluable field assistance. The field sampling protocol was approved by the Ethical Committee of Ghent University. We are also indebted to Hans Matheve for the spatial analysis in ArcGIS. Our gratitude to the all private landowners for their permission to conduct the field sampling on their lands.

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Supplementary Materials

Table S1. Coordinates and built-up percentages of sampling sites in Flanders, Belgium. Rural: BU<5%, Suburban: 5–10% BU, Urban: BU>10%.

Region	Town	Site	Longitude	Latitude	Built-up % at different radius	
					400m	1600m
Antwerpen	Antwerpen	P10SG	51.2183	43.7529	16.29	7.58
Antwerpen	Antwerpen	P10SR	51.1938	44.1507	30.42	24.64
Antwerpen	Herenthout	P17SG	51.1196	47.1637	0.76	1.91
Antwerpen	Herenthout	P17SR	51.1324	47.3225	6.24	2.82
Antwerpen	Lint	P14SG	51.1360	45.1580	4.10	4.35
Antwerpen	Lint	P14SR	51.1261	44.9620	20.85	11.87
Antwerpen	Mechelen	P12SG	51.0202	44.9838	11.42	16.54
Antwerpen	Mechelen	P12SR	51.0163	44.6885	29.62	22.16
Antwerpen	Pulderbos	P16SG	51.2323	47.0896	1.26	2.57
Antwerpen	Pulderbos	P16SR	51.2229	46.9979	9.00	3.70
Antwerpen	Ruisbroek	P15SG	51.0892	42.9986	0.98	3.10
Antwerpen	Ruisbroek	P15SR	51.0861	43.2991	12.63	6.14
Gent	Beervelde	P05SG	51.0663	38.6527	5.71	4.64
Gent	Beervelde	P05SR	51.0753	38.4093	15.10	7.33
Gent	Gent	P01SG	51.0521	36.9413	10.55	18.29
Gent	Gent	P01SR	51.0560	37.2039	52.51	38.99
Gent	Hillegem	P06SG	50.8779	38.5367	2.35	4.66
Gent	Hillegem	P06SR	50.8971	38.6011	12.62	4.69
Gent	Kalken	P07SG	51.0175	39.3140	1.83	3.76
Gent	Kalken	P07SR	51.0364	39.2207	11.38	4.68
Gent	Melsen	P08SG	50.9340	37.1898	3.22	2.15
Gent	Melsen	P08SR	50.9568	37.0345	9.78	3.85
Gent	Oudenaarde	P03SG	50.8686	35.9354	2.47	9.68
Gent	Oudenaarde	P03SR	50.8609	36.0975	17.23	17.17
Leuven	Houwaart	P25SG	50.9230	48.3591	0.96	1.53
Leuven	Houwaart	P25SR	50.9345	48.6241	5.78	1.72
Leuven	Kerkom	P26SG	50.8394	48.8532	2.07	1.84
Leuven	Kerkom	P26SR	50.8581	48.6813	6.34	2.03
Leuven	Leuven	P19SG	50.8904	46.8944	10.05	16.96
Leuven	Leuven	P19SR	50.8728	47.0604	43.08	27.92
Leuven	Overijse	P24SG	50.7830	45.2451	4.63	8.97

Table S1. (continued)

Region	**Town**	**Site**	**Longitude**	**Latitude**	**Built-up % at different radius**	
					400m	**1600m**
Leuven	Overijse	P24SR	50.7723	45.3562	20.48	8.36
Leuven	Tienen	P21SG	50.8185	49.2943	7.81	10.84
Leuven	Tienen	P21SR	50.8089	49.4066	37.73	17.88
Leuven	Wezemaal	P22SG	50.9622	47.2957	3.39	4.69
Leuven	Wezemaal	P22SR	50.9479	47.5331	14.87	5.41

General Discussion

6

Introduction

Urbanization induced novel selection pressure on animals in which their persistence in these man-made ecosystems is being challenged. Because of that, numbers of studies have attempted to understand how animals respond to the challenges derived from human activities. Animals' phenotype is expected to be the key factor that determines the ability of animals to survive in urban challenges because it acts as the tool that allows animals to interact with the environment. Nevertheless, our knowledge on how animals' phenotype varies with the urban challenges is still limited. This thesis studied the physiological and morphological responses in urban birds when they face urban challenges by focusing on the variations at individual level.

Overview of the main results

Based on the physiological response of house sparrows, urban environments do not seem to impose stress to house sparrows and these results are consistent across geographical areas (Chapter 2, 3, 5). While those results were found in adults, nestlings stress response was also not determined by their origin (Chapter 4). Rather, an increase in the nestling stress level was found when they had access to food supplementation (Chapter 4). In this thesis, I found that variations in stress levels were influenced by the sex and the age of the birds in which stronger stress response was observed in males and juveniles (Chapter 3).

Regarding the morphological response, house sparrows from the more urbanized areas had lower body conditions (Chapter 2, 5). During early in the breeding season, the food supplemented nestlings had larger body condition than those without extra food (Chapter 4). Wing length varies with urbanization level, sex, and age whereby longer wings were shown in birds from urban areas, males, and adults (Chapter 2). However, in Chapter 5 wing length showed a decreasing pattern when urbanization intensifies. Tarsus length reduced as urbanization level increases but such was only observed in males (Chapter 5).

Variations in physiological response to urbanization in different life history stages

Each individual is characterized by different intrinsic factors. Due to that, it is expected that each individual will experience the environments differently even if they are living in the same habitat. In this study, life history stages have been identified as one of the most important factors that influence birds' responses towards urban environments.

Physiological response in nestlings

Nestlings showed different patterns of stress response in relation to urban nutritional conditions. For birds that breed in the urban environments, supplemented food by human serves as an important food source for the nestlings. In many studies, this extra food allows improved reproductive success in birds such as by increasing fledging success (reviewed by Robb, McDonald, Chamberlain, and Bearhop (2008)). In Chapter 4, nestlings that were fed with protein-rich food had an increase in their stress response. The extra food in the birds' territories allows more nestlings to survive which then induced stronger competitions among siblings. As a consequence nestlings had an increase in their stress response as a mechanism to outcompete other siblings. In other study, a similar food provisioning experiment on the American crow nestlings in suburban and rural areas found that stress levels were similar in nestlings with or without food supplementation (Heiss, Clark, & McGowan, 2009). In Heiss et al. (2009), blood samples for CORT quantification were collected at the age of 21-31 days after being hatched, during which the nestlings were almost ready to fledge (average fledging is at the age of 30 days; Chamberlain-Auger, Auger, and Strauss (1990)). If, at this age, brood reduction has already occurred, then we could expect that siblings competitions have relaxed, hence the lower CORT level. Moreover, plasma CORT only reflects short term variations in stress level (Creel, 2001), so it is possible that the CORT secreted earlier within the nesting period, when sibling competition was strongest, was not included. The similar stress response between fed and unfed nestlings shown by Heiss et al. (2009)) seems consistent with the reduction of stress level after brood reduction as expected in Chapter 4.

Physiological response in juveniles

After fledging, juvenile birds are exposed to new environments outside the nests. Those that live in urban environments are likely to face stronger environmental challenges such as rapid habitat change, novel food resources, increased pollution, and higher predation pressure (reviewed by Seress and Liker (2015)). These characteristics of urban environments are likely to cause stress on birds particularly on the juveniles due to their lack of experience compared to the adults. Thus a higher stress response in juveniles when exposed to novel challenges is expected. Increased stress response in juveniles may be adaptive in which it may induce behavioral adjustments that facilitate them in coping with challenges (Anderies, Katti, & Shochat, 2007; Chace & Walsh, 2006; Mennechez & Clergeau, 2006). The effects of life history stage on stress response was evident in juveniles in which they showed higher stress response when challenged with urban stressors than the adults (Chapter 3). In other study, similar trend was also found in juvenile blackbirds (Partecke et al 2006). On the contrary, Meillère, Brischoux, Parenteau, and Angelier (2015) found that stress response was lower in juveniles relative to the adults in house sparrows. The underlying reason for this difference is unknown since they did not relate the observed pattern with any possible influencing parameters. However, they also reported that juveniles had lower body condition and hematocrit level than adults. It is known that low hematocrit level indicates poor health status (Fair, Whitaker, & Pearson, 2007; Hõrak, Ots, & Murumägi, 1998). Less healthy individuals may have less amount of energy, which is indicated by lower body condition, to mount robust stress response (Fokidis, Orchinik, & Deviche, 2009, 2011), so it is possible that their stress response is constrained by the poor health.

During the diet experiment, juveniles that were subjected to urban diet expressed stronger stress response than those that fed on rural diet (Chapter 3). These juveniles were captured in early autumn during which natural food resources such as insects and plant materials were still available in their habitats, either in urban or rural areas. After being transferred into aviaries, the juveniles that fed on urban diet faced larger transition in the nutritional contents i.e. from natural diet to the low-quality urban diet. Meanwhile, the difference should be less in juveniles that were provided with rural diet. This probably had caused the higher stress response in juveniles that were subjected to urban diet. Such effect was also observed in adults although the magnitude was as strong as in the juveniles (Fig. 5C, Chapter 3). In another study, stress responses were similar between juvenile blackbirds from urban and forest areas (Partecke et al 2006). No other studies on passerines that I know of have tested the effects of urbanization on stress response in juveniles exist, therefore, whether the difference in stress response between house sparrows and blackbirds was species specific cannot be confirmed.

Physiological response in adults

Among the adults, variations in stress responses between urban and rural house sparrows were no longer present (Chapter 3). Several studies on house sparrows also found similar pattern (eg. Bókony, Seress, Nagy, Lendvai, and Liker (2012); Chávez-Zichinelli et al. (2010); Fokidis et al. (2009); Meillère et al. (2015)). Stress levels did not vary between urban and rural house sparrows probably because some urban features, such as stable food resources, compensate the stress caused by urban environments. Other urban dweller species such as blackbirds showed lower stress response in urban than the non-urban areas

(Partecke, Schwabl, & Gwinner, 2006), whereas curve-billed thrasher (*Toxostoma curvirostre*), Abert's towhee (*Pipilo aberti*), northern mockingbird (*Mimus polyglottos*) showed the opposite pattern (Fokidis et al., 2009). With the continuous exposure to disturbances in urban areas, it is expected that urban dwellers will ultimately reduce their stress response (Cyr & Romero, 2009) because prolonged elevation in stress level often leads to detrimental consequences (Breuner, Patterson, & Hahn, 2008; Kitaysky, Kitaiskaia, Piatt, & Wingfield, 2003; Koren et al., 2012; Lendvai, Giraudeau, & Chastel, 2007; McEwen, 1998; Wingfield & Romero, 2001). However, some urban dwellers retain a higher stress responsiveness compared to those in non-urban areas probably because their high energetic state allows them to do so (Fokidis et al., 2009, 2011). Therefore, urban dweller species may adopt different physiological responses depending on the balance between environmental pressure and their state of energy.

Current literature on the stress physiology of urban birds showed no clear general patterns (reviewed by Bonier (2012)). Some studies reported both positive and negative relationship between urbanization and birds' stress response while in others no effects were found (Bonier (2012) and references therein). These mixed results suggest that the relationships between urbanization and stress physiology are complex and inconsistent. In addition to that, these differences are not only found in different species, rather, variations may also occur within species. In general, house sparrows showed variations in physiological response to urban environments depending on their life history stages. Such age-related differences have also been observed in the stress response of other species, for example, blackbirds (Partecke et al., 2006) and curve-billed thrashers (Fokidis et al., 2009, 2011). These differences indicate that different selection pressure and ecological processes are acting at different stages of life. Therefore, age effect is an important factor that confound physiological response and future studies on avian physiology should consider this factor in their analysis. Another possible reason for these variations is that urbanization affects the life history of birds. As an example, scrub jays breed earlier when supplementary food is available in their suburban habitats (Schoech & Bowman, 2001). If the physiological response of individuals from this population is compared with those from other habitats that do not contain supplementary food in the same period of time, it is likely that the differences in physiological response is confounded by the differences in their life history stages, rather than the urbanization levels. This emphasize the need to account for the effects of life history stage. In particular, the effects of life history stage should be controlled for when comparing samples from individuals with different life history stages that are collected within the same time window to avoid misinterpretations of results.

Variations of avian stress levels in natural environments

In this study I challenged the house sparrows by manipulating food predictability and diet quality to simulate the urban environmetal stress. However, no evidence that shows that urban environments constitute stressful environments to house sparrows was found (Chapter 2, 3, 5). This finding is consistent with other comparative studies on the stress level of house sparrows from urban and non-urban populations (eg. Bókony et al. (2012), Chávez-Zichinelli et al. (2010); Meillère et al. (2015); Fokidis et al. (2009)). Moreover, a similar trend was also discovered in different urban adaptor species such as blackbirds (Partecke et al., 2006), northern mockingbirds, curve-billed trashers, abert's towhees, canyon towhees (Fokidis et al., 2009), inca doves (Chávez-Zichinelli et al., 2013), and tree sparrows (Zhang et al., 2011). Although urban environments are often characterized by stressful conditions such as higher pollution rate (reviewed by Seress & Liker 2015) and increased predation pressure (reviewed by Robb et al 2008), other factors, for example, stable food supplies (reviewed by Shochat et al 2006) and warmer climate (reviewed by Collins et al 2000) may mitigate the negative impacts such that the final stress levels are similar between the urban and non-urban populations.

Nevertheless, there were few studies that found different trends: urban inca doves had lower stress level in urban areas than in croplands (Chávez-Zichinelli et al., 2013) while male white-crowned sparrows from urban areas had higher stress level than those in rural areas (Bonier et al., 2006). However, these studies suggested that the observed patterns were specific to sex (Bonier et al., 2006) and site (Chávez-Zichinelli et al. 2013), and not related to different physiological response of urban birds. Specifically, Bonier et al. (2006) found that the higher stress level in white-crowed sparrows was only observed in males while females showed similar stress levels between urban and rural habitats. In the case of inca doves, Chávez-Zichinelli et al. (2013) suggested that the higher stress level of birds from croplands was because their field sampling was conducted during the dry season, during which no crops were available, thus food scarcity may raised birds' stress level. Moreover, comparison between stress level between birds from urban areas and forests yielded no significant difference.

Avian adjustments urbanization through phenotypic plasticity

There is growing evidence that rural and urban individuals differ in physiological and morphological traits (reviewed by Bonier (2012); Partecke et al. (2006)). Factors underlying the differences between urban and rural individuals are phenotypic plasticity and microevolutionary that occurs in the urban environments

(Miranda, 2017). Because birds are highly mobile animals, thus genetic differentiation at local scale is not likely due to gene flow (Partecke, Gil, & Brumm, 2013). However, microevolution still can occur in birds. As an example, Partecke et al. (2006) collected nestlings from city and forest in Germany and later hand-reared them under identical conditions in an experiment. Through the experiment, they found that urban birds had an intrinsic attenuation of stress response compared to the forest birds and they suggested that the variations in stress response of blackbirds could be caused by genetic change as an adaptation to the urban environments (Partecke et al., 2006).

Phenotypic plasticity is defined as the ability of individual that possess a particular genotype to modify its phenotype when experiencing certain environmental conditions. Phenotypic plasticity aimed at increasing individual fitness and it is achieved by altering either the individuals' morphology, physiology, behavior, or phenology so that they suit better with the changes in the environmental conditions (Thibert-Plante & Hendry, 2011). Based on the dual approach of this thesis, I did not find evidence of microevolution in house sparrows, rather, the variations in SMI was caused by phenotypic plasticity (Chapter 3). It is apparent that some traits of house sparrows are more plastic than others and traits can be affected differently by intrinsic factors such as sex and variable environmental conditions, or the interaction between both.

Individual-based approach

Urbanization causes habitat alterations on bird populations. For non-migrating species like house sparrows, changes in the environmental conditions force them to adapt to the novel conditions in their habitats to avoid extinction. An important mechanism for adaptation to environmental changes is through adjustments in phenotypes (Thibert-Plante & Hendry, 2011). By understanding how individuals respond to environmental stressors, we are able to predict the fate of population/species in the changing environments.

One way to predict species persistence is through correlative approach where environmental variables are correlated with the presence and absence of species in an area (Chevin, Lande, & Mace, 2010). What is measured in this approach is the abiotic relationships of a species with its environment which describes the species' realized niche (Chevin et al., 2010). By using this approach, one can identify spatial locations that fulfil the species life requirements (Chevin et al., 2010). Unlike the correlative approach, individual-based approach takes into account the biological processes that influence species adaptation to its environments (Chevin et al., 2010). This approach focuses on individual phenotypic responses which consequently affect population growth (Chevin et al., 2010). The advantage of the individual-based approach is that it allows the identification of biotic factors that may limit population persistence when environmental conditions change (Chevin et al., 2010).

In this study, several important biotic factors that have the potential in affecting birds' responses were identified. By studying morphological response in house sparrows, food predictability and predation showed the potential of limiting house sparrows' survival in urban areas. Diet can also be important but only in nestlings. Moreover, the importance of life history stage is highlighted through studies on house sparrows' physiological response. As urbanization is often progressing in the environments, we could expect that effects stressors will also change in its magnitude. Here, the individual-based approach has the advantage over the correlative approach because it can predict species persistence in changing environmental conditions. In contrast, the correlative approach reflects the relationship between species and the abiotic factors of a habitat during the time when the environmental data were taken. Nevertheless, the drawback of the individual-based approach in predicting species persistence is that it needs sound knowledge on the ecology of the species. Moreover, predictions can only be made if environmental variables are incorporated in the prediction analysis (Chevin et al., 2010). Up to now, individual-based approach is rarely been used in predicting environmental changes induced by urbanization. Further investigates should try to validate the reliability of the use of this approach in predicting species persistence in urbanizing environments.

The effects of spatial scale on avian phenotypic response

The effects of local-landscape scale interaction were not found on the physiological response of house sparrows. While there are no previous studies that I know of have investigated such aspect, several studies have reported that stress levels of urban house sparrows were similar with those from rural areas (Chapter 3; Bókony et al. (2012); Meillère et al. (2015)). Most importantly, these results are consistent despite the variations in the scales used (eg. 100m, Chapter 3; 560m, Bókony et al. (2012); Meillère et al. (2015); 400m and 1600m; Chapter 5). It seems that the physiological response of house sparrow is neither affected by spatial scales nor their interaction. A possible explanation for this is that urban environments do not constitute stressful environments for house sparrows (Chapter 3). So regardless of which scale is used, the pattern will be the same. This proposed reason may also elucidate the similar bill dimensions between the urban and rural house sparrows (Chapter 2, 5).

Wings are longer in house sparrows in urban areas when 100 m scale was used (Chapter 2). However, after taking into account the effects of surrounding landscape (1600 m scale) on morphological response at local habitats (400 m scale), I found that wings are shorter as urbanization level increases in house sparrows that live in populations embedded within urban landscape (Chapter 5). The length of wing bones is influenced by nutritional condition during developmental phase (Ibáñez-Álamo & Soler, 2010; Seress et al., 2012), but the length of feathers on wings should be determined by the nutritional condition and predation pressure during moulting. Here, the contrasting patterns were produced probably because each scale reflects information of different ecological processes i.e. 100 m scale may only include the effects of predation by domestic cats, but the interaction between 400 m and 1600 m scales include the effects of both cats and sparrowhawks. Besides that, SMI had a negative relationship with urbanization level when measured at 100 m scale (Chapter 2), but by combining the 400 m and 1600 m scales I found that such pattern is only present in the house sparrow populations that are embedded within urban landscapes (Chapter 5). This finding improves our understanding of how SMI, a widely used parameter in urban ecological research, responds to the urban environments at local and landscape scales. It is clear that variations in urban birds' morphology would differ depending on the spatial scale used, thus future studies should use scales that correspond to the study species. The incorporation of multiple scales in the analysis should also be considered as surrounding landscapes could potentially affect avian morphology at local scale.

Conservation of house sparrows

Although house sparrows are not negatively affected by urbanization, their urban populations have been undergoing dramatic decline across the European continent during the last decades (De Laet & Summers-Smith, 2007). The fact that they feed on poor diet in urban environments still does not cause negative effects on them either only increased stress response in juveniles but the effects disappear in adults; Chapter 2, 3), thus unable to explain the population decline. One alternative explanation is that urban populations act as demographic sinks and that the decline of urban populations is a result of the decline of populations in rural areas. This hypothesis has been tested by Vangestel et al. (2012) in which they compared genetic diversity and population structure of house sparrows along an urbanization gradient in Flanders, Belgium. Their study discovered unidirectional gene flow of house sparrows from suburban and rural populations into the urban populations resulting in the urban populations which fully consist of individuals from suburban and rural populations, whereas no genetic signatures of urban individuals were found in the suburban and rural populations (Vangestel et al., 2012). If local extinction occurs in the source populations (suburban and rural populations), it is likely that the urban populations will as well extinct.

The reduction in the abundance of house sparrows in suburban and rural areas has begun since ~50 years ago (Robinson et al 2005; De Laet & Summer-Smith 2007). However, the rural populations seem to have stabilized although at a lower level (De Laet & Summer-Smith 2007). Therefore, the decline of urban populations could be caused by a higher rate of local extinction in urban areas relative to immigration from the suburban and rural population. Conservation plans should, thus, focus on increasing house sparrows' survival in urban areas. Chamberlain, Toms, Cleary-McHarg, and Banks (2007) investigated the habitat used of house sparrows in urban areas and they found that house sparrows' occurrence is highly associated with residential areas, allotments, and farm buildings. Furthermore, the presence of private garden within residential areas will triple the house sparrows' density compared to the areas without gardens (Chamberlain et al., 2007). Similarly, De Coster et al (2015) also found that the number of house sparrows is positively related to the proportion of green elements. A conservation plan to overcome the population decline of urban house sparrows should encourage developers to preserve green areas in the cities. The importance of having green elements is that they may probably provide food resources and hiding places from predators. This is in line with the suggestions from the individual-based approach where food predictability and predation are the limiting factors of house sparrows survival. Due to the continuous urbanization processes, green elements such as private gardens are under high pressure to be replaced by buildings due to housing demands (Chamberlain et al., 2007). Therefore some alternative solutions are to create shared gardens within residential areas and green walls which is effective in increasing bird abundance (Chiquet, Dover, & Mitchell, 2013). However, it is also important to consider the distance between one green element to another because house sparrows are highly sedentary. This aspect is important to allow movements of house sparrows between the green patches to avoid transformations of continuous populations into patchy ones.

Recommendations and suggestions for future studies

Past studies on stress response in birds have mainly used blood samples to quantify the concentration of

CORT. However, plasma CORT only provides a snapshot information of individuals' stress level, thus limit the ability to understand whether the changes in CORT level indicate an adaptation or an effect of environmental changes (Bartolotti et al 2008 and the references therein). An alternative to this is quantifications of CORT concentration from feather samples. A major advantage of CORT_f is that it integrates information of CORT hormone secreted over the duration of feather growth (Bartolotti et al 2008). However, interpretation of CORT_f is complex because high plasma CORT and poor nutrition can reduce feather density (DesRochers et al., 2009; Lattin, Reed, DesRochers, & Romero, 2011). Therefore, an increase in plasma CORT can only be detected up to the level where CORT concentration does not affect feather growth. Beyond this level, increment in plasma CORT may fail to be detected due to restricted feather materials (reviewed by Romero and Fairhurst (2016)). Therefore, the usage of CORT_f as an index to environmental stress needs further investigation to validate its reliability.

This study applied a dual approach where field sampling and experiment are combined. Field sampling provides information about the variations of physiological and morphological traits in nature but the patterns are shaped by multiple factors that present in the environments. Aviary experiment allows us to remove the confounding factors but to mimic natural conditions in the aviary settings is not an easy task. For example, during the aviary experiment (Chapter 2, 3), producing diets that represent the natural food of urban and rural house sparrows in their natural habitats was challenging. Because of these, I would recommend the implementations of field experiment as the best compromise between the field sampling and the aviary experiment. A cross-fostering experiment, such as swapping broods between urban and rural areas, would shed light on genetic vs. non-genetic adaptation. However, such method is only feasible for species that breed in nest boxes.

The usage of multiple components i.e. combinations of physiological and morphological responses, as well as the different indices of stress such as fluctuating asymmetry (FA) and ptilochronology in this study facilitate the interpretations of results. Such integrations of multiple components are especially important for traits that can be interpret in different ways. For instance, high CORT concentration may indicate that individual is experiencing chronic stress. Otherwise, it may also mean that the individual is attempting to increase its fitness by adjusting its physiological response. In this study, the importance of using multiple components is shown in Chapter 2. Lower scaled body mass (SMI) of urban house sparrows might have been interpreted as inferior body condition compared to their heavier rural conspecifics. However, results from FA and ptilochronology suggested that it is not necessarily so. Apart from that, the observed patterns in birds' response may be the results from different environmental stressors. For non-specific response such as physiology, combination with other stressor-specific index such as ptilochronology may help to explain the results. I suggest that future studies should apply the multi-component approach as it may provide views from different aspects of birds' life.

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Acknowledgement

My deepest gratitude goes to my supervisor Prof. Dr. Luc Lens for giving me the opportunity to work on this interesting project, for his unlimited enthusiasm, and his motivation throughout my study. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on the research is priceless. I could not have imagined having a better supervisor for my PhD study.

I would also like to express my sincere thanks to Dr. Liesbeth De Neve and Dr. Diederik Strubbe for their guidance in statistical analysis, results interpretation, and academic writing. The stimulating discussions we had are the cornerstones of this thesis work. It has been a real pleasure to work with you.

I thank the rest of my thesis committee members: Prof. Dr. An Martel, Assoc. Prof. Dr. Joël White, Dr. Liliana D'Alba Altamirano, and Prof. Dr. Dries Bonte for their insightful comments and suggestions which helped me widen my research perspectives and improve this thesis.

I am indebted to the Ministry of Higher Education Malaysia who had granted me with the scholarship for the four years of my PhD study. I would also like to extend my gratitude to the dean of Faculty of Science & Mathematics and the head of the Department of Biology at my home university, Universiti Pendidikan Sultan Idris, for encouraging me to pursue my study this far towards achieving my career goals.

Thanks also to all the members in TEREC for the enjoyable working atmosphere in this group. To Pieter Vantieghem, Hans Matheve, Aimeric Teyssier, and Lieze Rouffaer who helped with me during the field sampling, I personally thank you for the months of hard work in collecting samples. The valuable experience out in the field will never be forgotten.

Special thanks to my family and friends who have provided me with moral and emotional support in my life. Your prayer for me was what sustained me this far. To my beloved husband, Anuar, thank you for being here with me throughout this PhD journey. Words cannot express how grateful I am for all of the sacrifices that you have made on my behalf. Also, thanks to my dearest child, Aamily, for her patience and understanding whilst doing my work at home.

Finally, to everyone who directly or indirectly has lent their hands in this venture, this accomplishment would not have been possible without you. Thank you!