Differences in adrenocortical responses between urban and rural burrowing owls: poorly-known underlying mechanisms and their implications for conservation

Antonio Palma1, *, Julio Blas1,2, José L. Tella1, Sonia Cabezas2, Tracy A. Marchant2 and Martina Carrete1,3

1 Department of Conservation Biology, Estación Biológica de Doñana (EBD-CSIC), 41092 Seville, Spain
2 Department of Biology, University of Saskatchewan, S7N 5E2 Saskatoon, Saskatchewan, Canada
3 Departament of Physical, Chemical and Natural Systems, Universidad Pablo de Olavide (UPO), 41013 Seville, Spain

*Corresponding author: Department of Conservation Biology, Estación Biológica de Doñana (EBD-CSIC), 41092 Seville, Spain. Email: palmag.antonio@gmail.com

The hypothalamus–pituitary–adrenal/interrenal (HPA) axis of vertebrates integrates external information and orchestrates responses to cope with energy-demanding and stressful events through changes in circulating glucocorticoid levels. Urbanization exposes animals to a wide variety of ever-changing stimuli caused by human activities that may affect local wildlife populations. Here, we empirically tested the hypothesis that urban and rural owls (Athene cucularia) show different adrenocortical responses to stress, with urban individuals showing a reduced HPA-axis response compared to rural counterparts to cope with the high levels of human disturbance typical of urban areas. We applied a standard capture-restraint protocol to measure baseline levels and stress-induced corticosterone (CORT) responses. Urban and rural owls showed similar circulating baseline CORT levels. However, maximum CORT levels were attained earlier and were of lower magnitude in urban compared to rural owls, which showed a more pronounced and long-lasting response. Variability in CORT responses was also greater in rural owls and contained the narrower variability displayed by urban ones. These results suggest that only individuals expressing low-HPA-axis responses can thrive in cities, a pattern potentially mediated by three alternative and non-exclusive hypotheses: phenotypic plasticity, natural selection and matching habitat choice. Due to their different conservation implications, we recommend further research to properly understand wildlife responses to humans in an increasingly urbanized world.

Key words: CORT, corticosterone, HPA axis, stress response, urbanization

Editor: Steven Cooke
Received 16 September 2018; Revised 8 August 2019; Editorial Decision 31 May 2020; Accepted 17 June 2020

Cite as: Palma A, Blas J, Tella JL, Cabezas S, Marchant TA, Carrete M (2020) Differences in adrenocortical responses between urban and rural burrowing owls: poorly-known underlying mechanisms and their implications for conservation. Conserv Physiol 8(1): coaa054; doi:10.1093/conphys/coaa054.
Introduction

Urbanization induces a wide range of ecological changes (Vitousek et al., 1997), often acting as a selective in natural populations (Alberti, 2015), changing biotic and abiotic environmental factors, and having important consequences for biodiversity conservation worldwide. However, not all animals respond equally to these ecological changes and, while urbanization has been linked to lower diversity and abundance of some species (McKinney, 2008; Aronson et al., 2014), other species are able to live successfully and even flourish in urban environments (Ellegren and Sheldon, 2008).

A growing amount of evidence shows that behaviour often defines the initial response of individuals toward anthropogenic changes and provides clues about how species cope with urbanization (see reviews by Lowry et al., 2013; Sol et al., 2013). Behavioural adjustments aimed at coping with human disturbance may be important for individuals to become urban dwellers (Lowry et al., 2013) since urbanization is associated with high levels of human presence and other activities that are unusual in rural and/or natural habitats. Indeed, behavioural traits often differ between urban and non-urban conspecifics, potentially as a consequence of behavioural flexibility (i.e. phenotypic plasticity), selection of individuals with particular behaviours (i.e. natural selection) or non-random sorting of individuals among habitats (i.e. matching habitat choice). Carrete and Tella (2010, 2011) have proposed that bird species able to colonize urban habitats are those with higher inter-individual variability in their fear of humans (estimated as the distance at which a given individual flees when approached by a human, hereafter flight initiation distance FID). This suggests that from a pool of behaviourally variable rural individuals, only the less fearful ones can colonize cities (Carrete and Tella, 2011). As FID is a repeatable and heritable trait, this non-random distribution of individuals would likely arise through natural selection or matching habitat choice, although some degree of plasticity cannot be completely ruled out (Carrete and Tella, 2010, 2013; Carrete et al., 2016).

However, the successful use of urban habitats may ultimately depend on how individuals within populations respond to the challenges imposed by urban environments (Romero et al., 2009; Bonier, 2012). The hypothalamic–pituitary–adrenal/interrenal (HPA) axis of vertebrates plays a key role integrating external information and orchestrating hormonal and behavioural responses (Blas, 2015). The HPA axis is typically activated in response to increased energy demands (i.e. allostatic load) from both predictable environmental changes (e.g. causing daily and seasonal variations in baseline glucocorticoids, GC, levels) and unpredictable and potentially noxious stimuli (thus triggering stress-related GC elevations). Most studies on urban endocrine ecology have been performed using plasma samples from birds, and the results regarding baseline and stress-induced corticosterone (CORT) levels have revealed no consistent patterns of variation between urban and rural conspecifics (Bonier, 2012). However, circulating CORT levels are subjected to rapid changes following exposure to stress, and plasma samples typically provide information about the short-term endocrine state of the individual. To obtain a longer-term, integrated assessment of individuals’ endocrine state, Rebolo-Ifrán et al. (2015) tested for differences between urban and rural burrowing owls (Athene cunicularia) using feather CORT levels. Since CORT is deposited from the bloodstream into the feather structure over several weeks (i.e. the period of feather growth), feather CORT levels provide a longer-term, retrospective measure of HPA axis activity integrating both baseline and stress-induced plasma CORT levels (Bortolotti et al., 2008; Blas, 2015). Consistent with the hypothesis that individuals occupy breeding sites with different levels of human disturbance according to their tolerance to humans (Carrete and Tella, 2010), they found no significant differences in feather CORT among urban and rural individuals. Nonetheless, the similar levels of CORT deposited in feathers may result from multiple stimuli—not only human presence—perceived by individuals, and the intensity of these stimuli may also vary between urban and non-urban habitats (Bonier, 2012; Rebolo-Ifrán et al., 2015). Therefore, complementary approaches performed on the same species are necessary to better assess potential differences in the HPA axis activity associated with urban life.

In this study, we compared the adrenocortical response of urban and rural (non-urban) burrowing owls using the standard protocol of capture and restraint with repeated blood sampling (‘stress series’; Wingfield et al., 1982). Based on our previous knowledge of our study model, we predicted that (i) urban and rural birds will show similar baseline CORT levels, as birds occupy sites according to their tolerance to human disturbance (Carrete and Tella, 2010, 2011; Rebolo-Ifrán et al., 2015); (ii) rural birds will show higher variability in the CORT levels in the bloodstream, encompassing the variability shown by urban ones, as expected from previous behavioural studies assessing variability in fear of humans (Carrete and Tella, 2010, 2011); and (iii) urban birds will show lower stress-induced CORT levels, as only individuals with particular physiological responses are able to live close to humans without experiencing chronic stress (Lowry et al., 2013; Rebolo-Ifrán et al., 2013). Our results supported all of these predictions, contributing to a better understanding of how wildlife copes with urbanization. Although our study does not allow us to properly identify the mechanisms involved, we discuss the three alternative and non-mutually exclusive hypotheses that can explain these results, namely phenotypic plasticity, natural selection and matching habitat choice, and discuss the conservation implications derived from each scenario.

Methods

Study system

The burrowing owl is a small-sized Strigiform distributed across American open landscapes, breeding in self-excavated...
burrows or those excavated by fossorial mammals. Breeding pairs are territorial and show diurnal activity, and are easily located in the surroundings of their nest (Carrete et al., 2016). Individuals breed for the first time when they are <1 year of age, although some individuals delay breeding (authors’ unpublished data).

The study site encompasses ca. 5400 km² of urban and rural areas around the city of Bahia Blanca (Argentina). Rural owls breed in natural grasslands, pastures and cereal crops, where human presence is sporadic and largely restricted to a few, mostly unpaved roads. Urban owls nest in private and public gardens in residential areas, unbuilt spaces among houses, street curbs and boulevards, living in continuous contact with humans and intense road traffic. Previous studies show that urban owl populations were founded by individuals from the neighbouring rural areas a few decades ago (ca. 40 years; Mueller et al., 2018). Individuals thriving in urban areas have experienced lower predation pressure, which allowed them to reach higher breeding success and densities compared to their rural counterparts (Rodríguez-Martínez et al., 2014; Rebolo-Ifrán et al., 2017). For more details, see Carrete and Tella (2010, 2011, 2013), Rebolo-Ifrán et al. (2015, 2017) and Mueller et al. (2018, 2020).

Field procedures

During the chick-rearing period (December to January), we performed a standardized-capture and restraint protocol (Wingfield et al., 1982) in 27 breeding adults (urban: 7 females and 7 males; rural: 7 females and 6 males), which were captured using bow nets at their nests during daylight. All captured individuals were attending pre-fledging chicks of similar ages (between 3 and 4 weeks, estimated visually by their size and plumage development), thus controlling for potential effects of breeding phenology on CORT (Blas, 2015). We serially obtained five blood samples (100 μL each) per individual from the brachial vein at pre-determined time intervals following capture (i.e. within the first ca. 3 min post-capture as well as 15, 30, 45 and 60 min post-capture). Birds were housed individually in dark metal cages (20 × 15 × 15 cm) between successive bleedings. Blood samples were preserved in a plastic storage box surrounded by ice coolers, inside an isothermal bag, until centrifuging (10 min at 10577 g) within the same day. Plasma was stored by ice coolers, inside an isothermal bag, until centrifuging allowed them to reach higher breeding success and densities among similar ages (between 3 and 4 weeks, estimated visually) while in random slope models their responses can vary even among the 10 groups’ means (i.e. urban and rural, for ca. 3, 15, 30, 45 and 60 min post-capture).

We then assessed whether the CORT responses among individuals were similar in urban and rural areas or, conversely, whether they were more variable among rural than among urban ones, so that the latter represent a subset of the former. For this purpose, we compared a random intercept and a random slope model for each group of birds using log-likelihood tests. In random intercept models, the effect of the explanatory variable on the response is the same for all individuals, while in random slope models their responses can differ (i.e. CORT responses are different among individuals).

Finally, we compared the maximum CORT titre in blood and the subsequent minimum levels reached between rural

Corticosterone radioimmunoassay

Plasma CORT concentration was determined through radioimmunoassay (RIA) using extraction with diethyl ether (Fisher Chemical, Fair Lawn, New Jersey, USA) following the protocol described in Blas et al. (2005). All plasma samples were extracted in one extraction procedure and the extraction efficiency, estimated in three samples spiked with 5000 CPM of [3H]-CORT, was 95.5%. All reconstituted plasma extracts (300 μL of phosphate-buffered saline, 0.05 M and pH 7.6) were analysed in duplicate in four separate RIAs. Antiserum (C8784; lot 092M4784) and purified CORT (C2505, Lot 22K1439) for standards were obtained from Sigma-Aldrich Chemicals (Saint Louis, MO, USA). We measured assay variability as the coefficient of variation resulting from repeated measurement of six samples with a known CORT amount in each RIA. The intra- and inter-assay coefficients of variation were 5.7 and 11.6%, respectively. The average detection limit (ED 80: SD) was 22.49 ± 1.08 pg per assay tube, and CORT plasma values from all samples were above this limit.

Statistical analyses

Differences in CORT among individuals across time (ca. 3, 15, 30, 45 and 60 min post-capture) were tested using linear mixed models in R 3.1.3, including habitat (urban or rural) and its interaction with time post-capture (linear and quadratic effects) as explanatory variables, and bird identity as a random term. Other factors may affect stress-induced CORT responses in birds, such as time of day (Romero and Remage-Healey, 2000), reproductive effort (Bonier et al., 2011), body condition (Pérez-Rodríguez et al., 2006) or sex (Blas et al., 2011). Thus, we fitted into the model the sex of the individuals, their body condition (estimated as the residuals from a [log]body mass on [log]wing chord regression; female: F1,600 = 28.04, p < 0.001; male: F1,409 = 5.32, p = 0.022), the hour of the day and the brood size (as a proxy of reproductive effort) to control for their potentially confounding effects. The interaction between habitat and time post-capture was tested using Tukey’s post hoc tests for all pairwise comparisons among the 10 groups’ means (i.e. urban and rural, for ca. 3, 15, 30, 45 and 60 min post-capture).
and urban birds to obtain a better visualization of CORT responses. For this purpose, we used linear models, controlling for body condition, brood size, sex and diel effects. Dependent variables in these models were (i) absolute maximum: the maximum stress-induced CORT level of each individual, (ii) relative maximum: the fold change from baseline to reach the maximum CORT level (calculated by dividing maximum by baseline CORT titre), (iii) absolute minimum post-peak: the minimum CORT level of each individual after reaching the maximum and (iv) relative minimum post-peak: the fold change from baseline to reach minimum post-peak CORT level (calculated dividing minimum post-peak by baseline CORT titre). When there was no reduction in CORT level after the maximum within our sampling timeframe, we considered the minimum post-peak values to be equal to the maximum ones.

Results

Urban and rural birds showed statistically similar baseline CORT levels but differed in the time and magnitude of their stress-induced CORT responses. While urban individuals reached maximum CORT titres 15 min post-capture with a smooth decrease afterwards, rural individuals maintained a steady increase in plasma CORT up to 30 min after capture, when they reached a plateau (Fig. 1A, groups representing

### Table 1: Linear-mixed model explaining variability in CORT (baseline and stress-induced) levels in urban and rural breeding burrowing owls *Athene cunicularia* across the 60-min capture and restraint protocol.

| Explanatory variables | Estimate | SE  | F-test   | p-value |
|-----------------------|----------|-----|----------|---------|
| Time                  | 6.20     | 1.08| $F_{1,50} = 31.23$ | <0.0001 |
| Time$^2$              | −4.32    | 0.60| $F_{1,106} = 52.02$ | <0.0001 |
| Habitat (urban)       | −2.71    | 2.21| $F_{1,47} = 1.50$ | 0.2263 |
| Sex (females)         | 2.91     | 1.51| $F_{1,127} = 3.72$ | 0.0559 |
| Body condition        | −1.22    | 0.82| $F_{1,127} = 2.17$ | 0.1429 |
| Hour of the day       | 0.84     | 0.88| $F_{1,127} = 0.90$ | 0.3450 |
| Brood size            | −0.41    | 0.80| $F_{1,127} = 0.26$ | 0.6078 |

The random slope model did not show significant differences in the post hoc Tukey tests. These differences between urban and rural individuals remained statistically significant while controlling for sex, body condition, brood size and hour of the day (Table 1).

Models run separately for urban and rural birds showed that inter-individual CORT response were different for the two groups. While the random slope model did not
Table 2: Linear models explaining variability in CORT levels in urban and rural breeding burrowing owls *Athene cunicularia*.

| Dependent variable                      | Explanatory variables | Estimate | SE  | F-test     | p-value |
|-----------------------------------------|-----------------------|----------|-----|------------|---------|
| **Absolute maximum**                    | Habitat (urban)       | -4.02    | 4.63| *F*₁,₂₁ = 4.54 | 0.0450  |
|                                         | Body condition        | -104.25  | 54.05| *F*₁,₂₁ = 5.12 | 0.0343  |
|                                         | Sex (females)         | 4.35     | 3.71| *F*₁,₂₁ = 1.78 | 0.1958  |
|                                         | Hour of the day       | 0.24     | 0.42| *F*₁,₂₁ = 0.25 | 0.6213  |
|                                         | Brood size            | -1.78    | 1.43| *F*₁,₂₁ = 1.55 | 0.2266  |
| **Relative maximum**                    | Habitat (urban)       | -7.95    | 3.79| *F*₁,₂₁ = 4.72 | 0.0413  |
|                                         | Body condition        | -1.69    | 44.21| *F*₁,₂₁ = 0.14 | 0.7145  |
|                                         | Sex (females)         | 1.47     | 3.03| *F*₁,₂₁ = 0.08 | 0.7832  |
|                                         | Hour of the day       | 0.37     | 0.34| *F*₁,₂₁ = 1.18 | 0.2898  |
|                                         | Brood size            | -0.04    | 1.17| *F*₁,₂₁ = 0.01 | 0.9753  |
| **Absolute minimum post-peak**         | Habitat (urban)       | -5.65    | 4.95| *F*₁,₂₁ = 4.78 | 0.0402  |
|                                         | Body condition        | -94.35   | 57.75| *F*₁,₂₁ = 3.75 | 0.0664  |
|                                         | Sex (females)         | 2.26     | 3.96| *F*₁,₂₁ = 0.40 | 0.5354  |
|                                         | Hour of the day       | 0.23     | 0.44| *F*₁,₂₁ = 0.21 | 0.6498  |
|                                         | Brood size            | -1.15    | 1.53| *F*₁,₂₁ = 0.57 | 0.4584  |
| **Relative minimum post-peak**         | Habitat (urban)       | -7.50    | 3.59| *F*₁,₂₁ = 3.49 | 0.0759  |
|                                         | Body condition        | 15.47    | 41.88| *F*₁,₂₁ = 0.01 | 0.9700  |
|                                         | Sex (females)         | 0.66     | 2.87| *F*₁,₂₁ = 0.01 | 0.9780  |
|                                         | Hour of the day       | 0.42     | 0.32| *F*₁,₂₁ = 1.70 | 0.2069  |
|                                         | Brood size            | -0.04    | 1.11| *F*₁,₂₁ = 0.01 | 0.9678  |

SE: standard error.

fit data well for urban birds, showing that the CORT response was rather similar among individuals (likelihood ratio tests between the random intercept and the random slope models; \( \chi^2 = 0.29, \, df = 2, \, p = 0.8639 \)), the response of rural birds was more variable, as shown by the better fit of the random slope model (likelihood ratio tests between the random intercept and the random slope models; \( \chi^2 = 13.99, \, df = 2, \, p = 0.0009 \)). Despite these differences, the CORT response of both urban and rural birds followed a significant quadratic trend (Table S1). Also important, the more restricted range of CORT responses of urban individuals was encompassed within the wider variability displayed by their rural counterparts (Fig. 1B).

In accordance with the above results, absolute CORT maximum, relative CORT maximum, absolute CORT minimum post-peak and relative CORT minimum post-peak were also significantly higher in rural birds compared to urban owls, even while controlling for potential confounding effects such as sex, body condition, brood size and hour of the day (Table 2). Not only did rural birds show higher absolute CORT (absolute values, Fig. 2A and C), but they also reached more than twice the proportional CORT recorded by their urban counterparts (relative values, Fig. 2B and D). See also Fig. S1 for urban and rural comparison, after controlling for sex and body condition.

**Discussion**

Our results show that, as predicted, urban and rural burrowing owls display similar baseline CORT levels but differ in their stress-induced responses. The adrenocortical response of urban owls reached its maximum 15 min after capture and started the termination sooner than rural birds, which reached a maximum later (30 min), reached higher plasma CORT levels and showed no sign of termination after 60 min. Also in agreement with our predictions, the HPA axis response was more homogeneous among urban birds, while rural owls showed a higher inter-individual variability that encompassed the range of values reported for urban owls. A lower stress-induced CORT response in urban than in non-urban individuals has been found in other bird species (Partecke *et al.*, 2006; Atwell *et al.*, 2012; Grunst *et al.*, 2014) and suggests that the close interaction with humans represents a source of stress, which can be tolerated only by particular phenotypes.

Previous studies performed in our burrowing owl populations have found that only individuals with less fear of
Figure 2: Absolute maximum (A), relative maximum (B), absolute minimum post-peak (C) and relative minimum post-peak (D) CORT levels in urban and rural breeding burrowing owls *Athene cunicularia*. Urban owls showed lower values compared to rural counterparts for all four measures (see Table 2 for results).

Humans (i.e. those with lower FID) can persist in the urban environment (Carrete and Tella, 2010, 2011). This results in an uneven occupation of territories by individuals with different tolerances to humans, which cannot be completely explained through habituation. Indeed, FID is highly repeatable throughout an individual’s adulthood (Carrete and Tella, 2010, 2013), and it is correlated with other behaviours such as exploration, anti-predator behaviour and dispersal within behavioural syndromes that differ between urban and rural birds (Carrete and Tella, 2017; Luna et al., 2019a, 2019b). Recent full-genome sequencing approaches performed in different burrowing owl populations (including those of our study area) reinforce the idea that cities were colonized a few decades ago by a small number of founders from the surrounding natural areas (Mueller et al., 2018), with an enrichment of different genes related to personality, behavioural control, memory and cognitive/learning functions (Mueller et al., 2020). Heritability of FID has also been found to be high (Carrete et al., 2016), although the lower values observed among urban birds can be related to differences in the ability of these individuals to adjust their FID adaptively (Vincze et al., 2016). Thus, although much of our previous work points toward selective processes on behavioural traits during urban invasion by burrowing owls (i.e. through natural selection or matching habitat choice), phenotypic plasticity cannot be completely ruled out. Although less is known, it is likely that these same mechanisms that promote changes in
the behavioural profiles of urban and rural populations also act to produce differences in their physiological profiles (i.e. the functioning of individual’s HPA axis) recorded within the frame of this study.

Bonier et al. (2007) suggested that the mechanism underlying the broader environmental tolerance of urban birds compared to rural conspecifics should involve physiological characteristics. Lowry et al. (2013) predicted that if bolder (i.e. less fearful) animals are more able to colonize urban environments, they should have a weaker stress-related CORT response compared to rural conspecifics to avoid the detrimental effects associated with chronic physiological stress typical of highly disturbed environments. Our results seem to support their prediction in the context of stress-related habitat matching. As we previously found when analysing feather CORT levels (Rebolo-Ifrán et al., 2015), our current results show similar baseline CORT levels in urban and rural owls, likely reflecting their equal ability to afford daily and seasonal routines, as well as their similar overall energetic requirements (Romero et al., 2009; Blas, 2015) or allostatic load (McEwen and Wingfield, 2003, 2010). Nevertheless, the significantly lower and less variable stress-induced CORT responses of urban owls in our study, which are embedded within the range of responses shown by rural conspecifics, suggest that the colonization of urban environments could be associated with particular physiological profiles able to cope with human disturbance. However, our results do not allow us to know whether these differences in the physiological responses of urban and rural individuals are plastic responses toward differences in the degree of human perturbation or result from a non-random distribution of individuals showing fixed, unchanging, physiological profiles. Although some studies on this topic have been conducted (Schoenemann and Bonier, 2018; Taft et al., 2018), there is no agreement regarding how much of the stress-induced response of individuals remains constant throughout their lives and how much corresponds to individual responsiveness to environmental variation. This information is paramount to understanding the mechanisms underlying the differences observed between urban and rural individuals, as natural selection and matching habitat choice rely on the assumption that the traits of interest remain constant through an individual’s lifespan and are heritable, while habituation (phenotypic plasticity) assumes that organisms are able to respond to variation in the environment by modifying their phenotype (although there are studies showing that plasticity per se can also be heritable and genetically fixed; Hallsson and Björklund, 2012; Scheiner et al., 2012; Gomez-Mestre and Jovani, 2013). Partecke et al. (2006), for instance, have found lower stress-induced CORT responses in urban than in rural blackbirds (Turdus merula) reared in a common-garden experiment, suggesting a genetically based difference. Similar results were obtained by Atwell et al. (2012) using dark-eyed juncos (Junco hyemalis), without ruling out early developmental effects. CORT responses have also been found to be heritable in some bird species (Evans et al., 2006; Angelier et al., 2011; Rensel and Schoech, 2011; Cox et al., 2016), including another owl species (Tyto alba) where baseline and stress-induced CORT levels are genetically correlated and heritable (Béziers et al., 2019). However, other studies have shown a low individual consistency in adrenocortical responses (Romero and Reed, 2008; Ouyang et al., 2011; Lendvai et al., 2015) and CORT levels as defined by genetic components and environmental effects (Jenkins et al., 2014; Ouyang et al., 2019), thus complicating our understanding of the mechanisms underlying the observed patterns.

An important point that should be addressed to understand the role of phenotypic plasticity, natural selection or matching habitat choice in the observed differences between urban and rural birds is the link between CORT response and fitness. Although it is accepted that high levels of CORT secretion may result in chronic physiological stress with detrimental effects (Raber, 1998; Sapolsky et al., 2000; Romero, 2004), the relationships between CORT levels and fitness components in animals are not fully understood (Bonier et al., 2009; Crespi et al., 2013). Nonetheless, different measurements of CORT levels have been significantly related to subsequent survival in some bird species (Brown et al., 2005; Blas et al., 2007; Gouette et al., 2010; Koren et al., 2012; Harms et al., 2015), including our study species (Rebolo-Ifrán et al., 2015). There, we found a quadratic relationship between feather CORT levels and survival of urban burrowing owls, indicating stabilizing rather than directional selection favouring intermediate CORT levels. The absence of such a relationship in rural owls was attributed to a higher predation pressure in this habitat (Rebolo-Ifrán et al., 2017), which could mask any CORT-survival relationship (Rebolo-Ifrán et al., 2015).

Understanding the relative importance of phenotypic plasticity, natural selection and habitat matching choice to explain differences in the CORT responses of urban and rural birds would undoubtedly fill large gaps, not only in our knowledge of stress responses but also in terms of biodiversity conservation. Urbanization is currently one of the fastest, longer-lasting sources of habitat transformation worldwide (Vitousek et al., 1997) and is already eroding and homogenizing biodiversity (McKinney, 2008; Aronson et al., 2014; Sol et al., 2014), also reducing phylogenetic diversity (Sol et al., 2017). Moreover, impacts on biodiversity conservation are expected to increase in the near future, as by 2050 it is expected that almost 70% of the world human population will be living in cities (United Nations, 2016). However, some species such as our study model can thrive better in cities than in their non-urban surrounding habitats (Rebolo-Ifrán et al., 2017), and some cities even constitute conservation hotspots for threatened species (Ives et al., 2016; Luna et al., 2018), which could serve as population and genetic stocks for conservation programs (Gibson and Yong, 2017). Therefore, there is an increasing motivation to make urban environments friendly for wildlife (Miller and Hobbs, 2002; Dearborn and Kark, 2010) and to predict which species will be able to cope with urbanization.

The ability of species to thrive (or not) in urban environments has been associated with behavioural (Carrete and
Understanding the relative role played by each of these mechanisms in a wide range of species would allow us to predict the consequences. Future work should be mainly focused on disentangling the mechanisms causing the physiological differences in stress responses observed between individuals living in urban and non-urban environments. The identification of the mechanisms causing the physiological differences in stress responses observed between individuals living in urban and non-urban environments may be paramount to a better understanding of these processes and to forecast further changes in biodiversity and species population dynamics. For example, if between-individual differences in the stress-response of some species mainly arise from phenotypic plasticity, we may expect that any individual of these highly plastic species would be able to physiologically cope with urbanization. These species could easily colonize urban habitats and increase their local and even global population sizes by exploiting these new habitats, provided they offer adequate food (Geis and Pomeroy, 1993; Robb et al., 2008) and resources for reproduction and survival (Chamberlain et al., 2009; Deviche and Davies, 2014), and relief from predation pressure typical in urban environments (Díaz et al., 2013; Rebolo-Ifrán et al., 2017; Luna et al., 2018). However, if differences between urban and rural individuals are related to differences in the colonization of these habitats by individuals showing different, consistent adrenocortical responses, only a proportion of the population (i.e. those individuals pre-adapted to tolerate high human disturbance) could colonize the cities. In terms of invasion biology (Kolar and Lodge, 2001), smaller propagule sizes would slow down a colonization process, highlighting the need to preserve rural populations even among those taxa defined as urban exploiters. Moreover, the effects on population size would greatly differ depending on whether the colonization occurs through natural selection or habitat matching choice. In the first case, urban colonization would be a random process where only pre-adapted individuals would survive, thus reducing the population size of the species. In the second case, however, pre-adapted individuals could actively colonize the city and thrive well there, without detrimental fitness consequences.

Although considerable advances have been made in the field of physiological ecology, further research is still needed. Hypothesis-driven research is particularly important, and future work should be mainly focused on disentangling the mechanisms allowing wildlife adaptation to urban areas. Understanding the relative role played by each of these mechanisms in a wide range of species would allow us to properly design conservation strategies in an increasingly urbanized world.

Authors’ contributions
A.P., M.C., J.B. and J.L.T. conceived the study. A.P. conducted the fieldwork. S.C. and T.A.M. performed the laboratory analyses. A.P., M.C. and J.B. analysed the data. A.P., J.L.T., J.B. and M.C. wrote the paper, and S.C. improved it with suggestions. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Supplementary material
Supplementary material is available at Conservation Physiology online.

Funding
This work was supported by Fundación Repsol, projects CGL2012-31888 and CGL2015-71378 (MEC, Spain) and COOPA20049 (CSIC, Spain). A.P. was supported by International Fellowships Programme Caixa-Severo Ochoa.

Acknowledgements
We acknowledge S. Briones, N. Rebolo-Ifrán, S. Rodríguez-Martínez and N. Tella-Carrete for helping during fieldwork. S. Zalba provided logistic support. Fieldwork was conducted under permits from Argentinean wildlife agencies (22500-4102/09), the guidelines of the Ethic Committee of CSIC (CEBA-EBD-11-28) and the owners of private properties. We acknowledge support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI). We also thank Steven J. Cooke, Amy Newman and two anonymous reviewers for their thoughtful comments that helped us to improve a previous version of the manuscript.

References
Alberti M (2015) Eco-evolutionary dynamics in an urbanizing planet. Trends Ecol Evol 30:114–126.

Angelier F, Ballentine B, Holberton RL, Marra PP, Greenberg R (2011) What drives variation in the corticosterone stress response between subspecies? A common garden experiment of swamp sparrows (Melospiza georgiana). J Evol Biol 24: 1274–1283.

Aronson MF et al. (2014) A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. Proc R Soc B Biol Sci 281: 20133330.

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

Béziers P, San-Jose LM, Almasi B, Jenni L, Roulin A (2019) Baseline and stress-induced corticosterone levels are heritable and genetically correlated in a barn owl population. Heredity 123: 337–348.
Blas J (2015) Stress in birds. In CG Scanes, ed. Sturkie’s Avian Physiology, Ed 2. Elsevier Inc., San Diego, pp 769–810.

Blas J, Baos R, Bortolotti GR, Marchant TA, Hiraldo F (2005) A multi-tier approach to identifying environmental stress in altricial nesting birds. Funct Ecol 19: 315–322.

Blas J, Bortolotti GR, Tell J, Baos R, Marchant TA (2007) Stress response during development predicts fitness in a wild, long lived vertebrate. Proc Natl Acad Sci USA 104: 8880–8884.

Blas J, Sergio F, Wingfield JC, Hiraldo F (2011) Experimental tests of endocrine function in breeding and nonbreeding raptors. Physiol Biochem Zool PBZ 84: 406–416.

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

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

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

Bonier F, Moore IT, Robertson RJ (2011) The stress of parenthood? Increased glucocorticoids in birds with experimentally enlarged broods. Biol Lett 7: 944–946.

Bortolotti GR, Marchant TA, Blas J, German T (2008) Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct Ecol 22: 494–500.

Brown CR, Brown MB, Raouf SA, Smith LC, Wingfield JC (2005) Effects of endogenous steroid hormone levels on annual survival in cliff swallows. Ecology 86: 1034–1046.

Carrete M, Martínez-Padilla J, Rodríguez-Martínez S, Rebolo-Íñan N, Palma A, Tella JL (2016) Heritability of fear of humans in urban and rural populations of a bird species. Sci Rep 6: 31060.

Carrete M, Tella JL (2010) Individual consistency in flight initiation distances in burrowing owls: a new hypothesis on disturbance-induced habitat selection. Biol Lett 6: 167–170.

Carrete M, Tella JL (2011) Inter-individual variability in fear of humans and relative brain size of the species are related to contemporary urban invasion in birds. PLoS One 6: e18859.

Carrete M, Tella JL (2013) High individual consistency in fear of humans throughout the adult lifespan of rural and urban burrowing owls. Sci Rep 3: 1–7.

Carrete M, Tella JL (2017) Behavioral correlations associated with fear of humans differ between rural and urban burrowing owls. Front Ecol Evol 5: 54.

Chamberlain DE, Cannon AR, Toms MP, Leech DJ, Hatchwell BJ, Gaston KJ (2009) Avian productivity in urban landscapes: a review and meta-analysis. Ibis 151: 1–18.

Cox RM, McGlothlin JW, Bonier F (2016) Hormones as mediators of phenotypic and genetic integration: an evolutionary genetics approach. Integr Comp Biol 56: 126–137.

Crespi EJ, Williams TD, Jessop TS, Delehanty B (2013) Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? Funct Ecol 27: 93–106.

Dearborn DC, Kark S (2010) Motivations for conserving urban biodiversity. Conserv Biol 24: 432–440.

Deviche P, Davies S (2014) Reproductive phenotype of urban birds: environmental cues and mechanisms. In D Gil, H Brumm, eds. Avian Urban Ecology: Behavioural and Physiological Adaptations. Oxford University Press, Oxford, pp 98–115.

Díaz M, Müller AP, Flensted-Jensen E, Grim T, Ibáñez-Álamo JD, Jokimäki J, Markó G, Tryjanowski P (2013) The geography of fear: a latitudinal gradient in anti-predator escape distances of birds across Europe. PLoS One 8: e64634.

Ellegren H, Sheldon BC (2008) Genetic basis of fitness differences in natural populations. Nature 452: 169–175.

Evans MR, Roberts ML, Buchanan KL, Goldsmith AR (2006) Heritability of corticosterone response and changes in life history traits during selection in the zebra finch. J Evol Biol 19: 343–352.

Geis AD, Pomeroy LN (1993) Reaction of wild bird populations to a supplemental food source. In Transactions of the North American Wildlife and Natural Resources Conference, pp 44–61.

Gibson L, Yong DL (2017) Saving two birds with one stone: solving the quandary of introduced, threatened species. Front Ecol Environ 15: 35–41.

Gomez-Mestre I, Jovani R (2013) A heuristic model on the role of plasticity in adaptive evolution: plasticity increases adaptation, population viability and genetic variation. Proc R Soc B Biol Sci 280: 20131869.

González-Oreja JA (2011) Birds of different biogeographic origins respond in contrasting ways to urbanization. Biol Conserv 144: 234–242.

Goutte A, Angelier F, Welcker J, Moe B, Clément-Chastel C, Gabrielsen GW, Bech C, Chastel O (2010) Long-term survival effect of corticosterone manipulation in black-legged kittiwakes. Gen Comp Endocrinol 167: 246–251.

Grunt M, Rotenberry JT, Grunt S (2014) Variation in adrenocortical stress physiology and condition metrics within a heterogeneous urban environment in the song sparrow Melospiza melodia. J Avian Biol 45: 574–583.

Hallsson LR, Björklund M (2012) Selection in a fluctuating environment leads to decreased genetic variation and facilitates the evolution of phenotypic plasticity. J Evol Biol 25: 1275–1290.

Harms NJ, Legagneux P, Gilchrist HG, Béty J, Love OP, Forbes MR, Bortolotti GR, Soos C (2015) Feather corticosterone reveals effect of moulting conditions in the autumn on subsequent reproductive output and survival in an Arctic migratory bird. Proc R Soc B Biol Sci 282: 20142085.

Ives CD et al. (2016) Cities are hotspots for threatened species. Glob Ecol Biogeogr 25: 117–126.
Jenkins BR, Vitousek MN, Hubbard JK, Safran RJ (2014) An experimental analysis of the heritability of variation in glucocorticoid concentrations in a wild avian population. *Proc R Soc B Biol Sci* 281: 20141302.

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends Ecol Evol* 16: 199–204.

Koren L, Nakagawa S, Burke T, Soma KK, Wynne-Edwards KE, Geffen E (2012) Non-breeding feather concentrations of testosterone, corticosterone and cortisol are associated with subsequent survival in wild house sparrows. *Proc R Soc B Biol Sci* 279: 1560–1566.

Lendvai Á, Giraudneau M, Bókony V, Angelier F, Chastel O (2015) Within-individual plasticity explains age-related decrease in stress response in a short-lived bird. *Biol Lett* 11: 20150272.

Lowry H, Lill A, Wong BB (2013) Behavioural responses of wildlife to urban environments. *Biol Rev* 88: 537–549.

Luna Á, Palma A, Sanz-Aguilar A, Tell JL, Carrette M (2019a) Sex, personality and conspecific density influence natal dispersal with lifetime fitness consequences in urban and rural burrowing owls. *Proc R Soc B Biol Sci* 286: 20191215.

McEwen BS, Wingfield JC (2003) The concept of allostatics in biology and biomedicine. *Horm Behav* 43: 2–15.

McEwen BS, Wingfield JC (2010) What is in a name? Integrating homeostasis, allostatics and stress. *Horm Behav* 57: 105–111.

Mckinney ML (2008) Effects of urbanization on species richness: a review of plants and animals. *Urban Ecosyst* 11: 161–176.

Miller JR, Hobbs RJ (2002) Conservation where people live and work. *Conserv Biol* 16: 330–337.

Mueller JC, Carrete M, Boerno S, Kuhl H, Tell JL, Kempenaers B (2020) Genes acting in synapses and neuron projections are early targets of selection during urban colonization. *Mol Ecol*. doi: 10.1111/mec.15451.

Mueller JC, Kuhl H, Boerno S, Tell JL, Carrete M, Kempenaers B (2018) Evolution of genomic variation in the burrowing owl in response to recent colonization of urban areas. *Proc R Soc B Biol Sci* 285: 20180206

Ouyang JQ, Baldwin D, Munguia C, Davies S (2019) Genetic inheritance and environment determine endocrine plasticity to urban living. *Proc R Soc B Biol Sci* 286: 20191215.

Ouyang JQ, Hau M, Bonier F (2011) Within seasons and among years: when are corticosterone levels repeatable? *Horm Behav* 60: 559–564.

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

Pérez-Rodríguez L, Blas J, Viñuela J, Marchant TA, Bortolotti GR (2006) Condition and androgen levels: are condition-dependent and testosterone-mediated traits two sides of the same coin? *Anim Behav* 72: 97–103.

Raber J (1998) Detrimental effects of chronic hypothalamic-pituitary-adrenal axis activation. *Mol Neurobiol* 18: 1–22.

Rebolo-Ifrán N, Carrete M, Sanz-Aguilar A, Rodríguez-Martínez S, Cabezas S, Marchant TA, Bortolotti GR, Tell JL (2015) Links between fear of humans, stress and survival support a non-random distribution of birds among urban and rural habitats. *Sci Rep* 5: 13723.

Rebolo-Ifrán N, Tell L, Carrete M (2017) Urban conservation hotspots: predation release allows the grassland-specialist burrowing owl to perform better in the city. *Sci Rep* 7: 3527.

Rensel MA, Schoeck SJ (2011) Repeatability of baseline and stress-induced corticosterone levels across early life stages in the Florida scrub-jay (*Aphelocoma coerulescens*). *Horm Behav* 59: 497–502.

Robb GN, McDonald RA, Chamberlain DE, Bearhop S (2008) Food for thought: supplementary feeding as a driver of ecological change in avian populations. *Front Ecol Environ* 6: 476–484.

Rodriguez-Martínez S, Carrete M, Roques S, Rebolo-Ifrán N, Tell JL (2014) High urban breeding densities do not disrupt genetic monogamy in a bird species. *Proc R Soc B* 1: e91314.

Romero LM (2004) Physiological stress in ecology: lessons from biomedical research. *Trends Ecol Evol* 19: 249–255.

Romero LM, Dickens MJ, Cyr NE (2009) The reative scope model - a new model integrating homeostasis, allostatics, and stress. *Horm Behav* 55: 375–389.

Romero LM, Reed JM (2005) Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp Biochem Physiol A Mol Integr Physiol* 140: 73–79.

Romero LM, Reed JM (2008) Repeatability of baseline corticosterone concentrations. *Gen Comp Endocrinol* 156: 27–33.

Romero LM, Remage-Healey L (2000) Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): Corticosterone. *Gen Comp Endocrinol* 119: 52–59.

Rapowsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 21: 55–89.

Scheiner SM, Barfield M, Holt RD (2012) The genetics of phenotypic plasticity. XI. Joint evolution of plasticity and dispersal rate. *Ecol Evol* 2: 2027–2039.

Schoenemann KL, Bonier F (2018) Repeatability of glucocorticoid hormones in vertebrates: a meta-analysis. *PeerJ* 6: e4398.

Silva CP, Sepúlveda RD, Barbosa O (2016) Nonrandom filtering effect on birds: species and guilds response to urbanization. *Ecol Evol* 6: 3711–3720.
Sol D, Bartomeus I, González-Lagos C, Pavoine S (2017) Urbanisation and the loss of phylogenetic diversity in birds. *Ecol Lett* 20: 721–729.

Sol D, González-Lagos C, Moreira D, Maspons J, Lapiedra O (2014) Urbanisation tolerance and the loss of avian diversity. *Ecol Lett* 17: 942–950.

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

Taff CC, Schoenle LA, Vitousek MN (2018) The repeatability of glucocorticoids: a review and meta-analysis. *Gen Comp Endocrinol* 260: 136–145.

United Nations HSP (2016) *Urbanization and Development: Emerging Futures*. World Cities Report, Nairobi.

Vincze E, Papp S, Preiszner B, Seress G, Bókony V, Liker A (2016) Habituation to human disturbance is faster in urban than rural house sparrows. *Behav Ecol.* 27: 1304–1313.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of Earth’s ecosystems. *Science* 277: 494–499.

Wingfield JC, Smith JP, Farner DS (1982) Endocrine responses of white-crowned sparrows to environmental stress. *Condor* 84: 399–409.