

Intra-urban variation in body condition, body size and oxidative status of Rufous-collared sparrow relate to urban green space attributes in a Latin American metropolis

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Abstract

Urbanization impacts public, wildlife and environmental health. Studies comparing rural and urban populations reveal negative effects of urban life on animal health, however, there is a paucity of research on its intra-urban variation. Specifically, whether body condition, body size and oxidative status of Neotropical birds varies with green space attributes in cities remains poorly understood, which limits strategies for healthier urban environments. In the city of Santiago (Chile), we compared body condition, body size and oxidative status between Rufous-collared sparrow (*Zonotrichia capensis*) that inhabit Urban Natural Remnants (UNRs) and urban parks and assessed the relationship of these variables with the vegetation attributes. We computed principal component analysis (PCA) for describing body condition, used tarsus length as body size indicator and measured the levels of lipid peroxidation (T-BARS) and total antioxidant capacity (TAC) for building an index of oxidative status (T-BARS/TAC ratio). We found that birds living in UNRs exhibited better body condition, larger body size and lower levels of oxidative stress than birds living in parks. UNRs providing greater woody plant richness promoted a better body condition and lower levels of oxidative stress in birds. Body size also increased with the percentage of shrub cover of the UNRs. These results evidence that body condition, body size and oxidative status of birds relate to green space type and vegetation attributes. These findings also highlight the importance of conserving UNRs, promoting shrub cover and prioritizing native plants in afforestation projects to build healthier urban environments for native birds.

Keywords Urban Natural Remnants (UNRs) · Urban parks · Zonotrichia capensis · Vegetation · Bird health

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Introduction

Urbanization is a main threat to biodiversity as it is a driver of land use change (DESA 2018). Due to the profound transformations of the environments, urban ecosystems differ from natural or semi-natural ecosystems in a variety of abiotic (Künzli et al. 2006) and biotic factors (Evans et al. 2009), affecting wildlife in the city. Evidence on how urbanization influences animals come mainly from intraspecific comparisons of the body condition and/or body size between birds inhabiting urban and rural environments (Peig and Green 2010; Alberti et al. 2017; Meillère et al. 2017). Body condition represents the energetic reserves of an animal as a result of feeding (Clancey and Byers 2014) and its body size reflects the nutritional quality of food during its developmental stages (Yom-Tov and Geffen 2011). Several studies have found that birds that inhabit urban environments show a poorer body condition and/or smaller body size compared with birds that inhabit natural environments (Meillère et al. 2015; Biard et al. 2017; Jiménez-Peñuela et al. 2019).

Another less manifest marker of animal health is the oxidative status, which represents the functional balance between the presence of oxidative damage (OD) and antioxidant defenses (AD) (Costantini 2008, 2019). Metabolic activity generates reactive oxygen species (ROS) able to produce OD and lead to oxidative stress, adversely affecting the physiology and health of individuals (Costantini et al. 2010, 2014). Therefore, aerobic organisms have developed AD mechanisms (endogenous AD, such as antioxidant enzymes, or exogenous AD like carotenoids and vitamins that are obtained from dietary sources) to counteract the toxicity of ROS (Cohen et al. 2009). Urban life alters the oxidative status of animals, including birds (Costantini et al. 2014; Isaksson 2020). In pro-oxidant conditions, such as in an urban environment, there may be an imbalance between the generation of ROS and AD (Andersson et al. 2015). In such conditions, ROS can generate OD (Costantini and Verhulst 2009).

Although previous research has found that body condition, body size and oxidative status differ between rural and urban populations (Isaksson et al. 2009; Herrera-Dueñas et al. 2017; Caizergues et al. 2021), to our knowledge there is a paucity of research on intra-urban variation, especially for neotropical birds. Different vegetation attributes influence native birds in cities (Chace and Walsh 2006; Villaseñor et al. 2020), but whether these variables affect body condition, body size and oxidative status has not been investigated. For instance, larger urban green spaces (Muñoz et al. 2018), with high plant diversity (Estades 1995; Shwartz et al. 2013), more woody species (Stagoll et al. 2012; Benito et al. 2019) and predominantly native species (Díaz and Armesto 2003; Chace and Walsh 2006) are inhabited by a higher number of native birds. However, the importance of these variables on the body condition, size and oxidative status of birds remains unclear. Moreover, habitat variables (e.g., percentages of native woody species and shrub cover) differ among urban green spaces based on their planning, design and management, which might lead to differences in habitat quality for wildlife (Ikin et al. 2015).

An example of such varying habitat attributes across green spaces is found in Santiago de Chile. In this city, urban parks are green spaces designed to fulfill a social role; hence, they commonly present infrastructure for recreational, cultural and/or sports activities (GORE RMS 2014; INE 2020), are dominated by exotic species with practically neither native nor shrub vegetation (Mella and Loutit 2007) and more than 90% do not exceed 5 ha in size (Reyes and Figueroa 2010; Picon and Barrera 2019). In contrast, Urban Natural Remnants (UNRs) defined as "natural areas that have been partially or completely isolated by an urban matrix, but that still retain compositional and structural characteristics of the original natural habitat" (Fernández et al. 2019), commonly offer large open areas where native vegetation remains (Forray et al. 2012). In Santiago city, UNRs are larger than parks averaging 226.11 ha (Arriagada 2017). Whether these contrasting habitats and their vegetation variables can lead to differences on body condition, size and oxidative status of birds remains unknown.

Rufous-collared sparrow (Zonotrichia capensis) is wellsuited to assess whether green space type and vegetation variables influence on body condition, body size and oxidative status of a Neotropical bird in Latin American cities because they reside in a variety of environments, including urban, peri-urban and natural areas from Mexico to Cape Horn (Chapman 1940; Laiolo 2011). Rufous-collared sparrow is an omnivorous species that feeds mainly on fruits, seeds and insects (Lopez-Calleja 1995). During the breeding season it defends territories about 0.6 ha, where feeds and feeds the young (Miller and Miller 1968). It appears to occupy the same range areas year-round (Cheviron and Brumfield 2009; Poblete et al. 2018). Variations in behavior and physiology of rufous-collared sparrow along latitudinal (Sabat et al. 2006, 2009; Van Dongen et al. 2010) and altitudinal gradients (Ruiz et al. 1995; Poblete et al. 2018, 2020) show their adaptability to different environmental conditions. Nevertheless, comparisons among urban and peri-urban populations show the negative influence of urbanization on body mass and hematological stress (Ruiz et al. 2002; Egli and Vásquez 2018), but until now no evidence has been presented on intra-urban variation for neotropical birds living in different green spaces in cities.

Here, we compare the body condition, body size and oxidative status between rufous-collared sparrows breeding in UNRs and parks in the city of Santiago (Chile) and assess whether body condition, body size and oxidative status are related with shrub cover, native woody species and woody plant richness in UNRs and parks. We hypothesize that if UNRs offer better habitat quality for rufous-collared sparrows than parks (Mella and Loutit 2007; Muñoz et al. 2018), individuals inhabiting these UNRs will present better body condition, larger body size and lower levels of oxidative stress (ROS/AD ratio) than the individuals inhabiting parks. We also predict that body condition, body size and oxidative status of birds will be directly related with shrub cover, native woody species, as well as woody plant richness, because these vegetation attributes are likely to provide an important feed source and antioxidants to rufous-collared sparrows (Lopez-Calleja 1995). This information will be relevant to inform green space management that is healthier for native birds in cities.

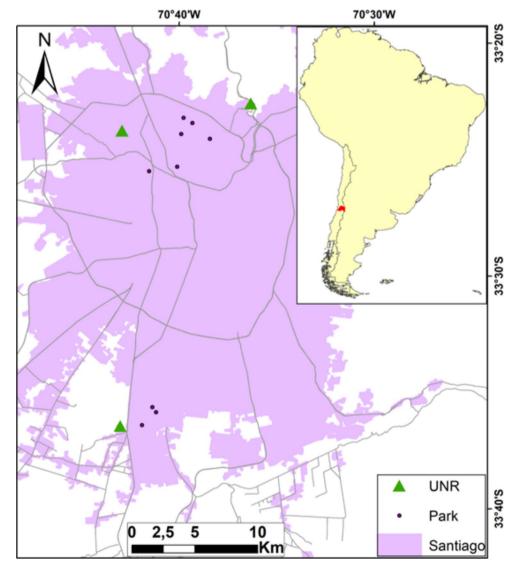
Methods

Study sites

Fieldwork was conducted in Santiago in central Chile (33 $^{\circ}$ 27 ' S, 70 $^{\circ}$ 40 ' W; Fig. 1). Santiago is one of the largest metropolises in Latin America and currently has ~ 7 million inhabitants (MacGregor-Fors and Escobar-Ibáñez 2017). It has a Mediterranean climate, characterized by dry and hot summers and cold and rainy winters according to the Köppen climate classification (updated by Peel et al. 2007). The native vegetation of central Chile is dominated by Mediterranean sclerophyll forests and scrublands (Luebert and Pliscoff 2022), that contain a high proportion of endemic species and is recognized as one of 25 global biodiversity hotspots (Myers et al. 2000). Despite the value of native vegetation for global biodiversity conservation, exotic species dominate the urban landscape, representing approximately 90% of the trees in the city (Hernández and Villaseñor 2018).

Fig. 1 Map showing the study sites in Santiago, Chile (Purple area). The upper-right map shows the location of Santiago in Chile (red area)

We followed a block design to select sampling sites. Each block comprised an UNRs and three surrounding parks (Fig. 1). Given that no study has explored intra-urban variation of body condition, body size and oxidative status of a neotropical bird, we preferred to compare green spaces of high contrast: > 700 ha UNRs vs. < 5 ha managed parks dominated by exotic plants. Thus, we selected the three largest UNRs in the city (>700 ha in size; GORE RMS, 2014) and compared them with parks. We visited those parks mainly covered by plants that were located within 5 km of the UNRs and performed census for detecting rufous-collared sparrows. We found a range 0-3 individuals by park. Thus, we select nine parks with at least 3 adults observed. Based on rufous-collared sparrows movement patterns during breeding season (Miller 1968), we considered 1 km as the minimum distance between sampling sites to promote independence among sites (Cheviron and Brumfield 2009). A description of study sites can be found in supplementary material (Tables S1 and S2).



Bird sampling

During the breeding season, 54 adult individuals of Z. capensis: 10 individuals from each UNRs and 3 individuals from each park were captured using mist nets between 7:00 and 10:00 p.m. from September to December 2019. For each captured bird, bill, tarsus and wings length were measured with digital calipers to the nearest 0.1 cm, and body mass was recorded with a 60 g Pesola scale (± 0.1 g). Before releasing the individuals a small blood sample was collected (c. 50–100 μ L) from the brachial vein using heparinized tubes, which were stored on ice (4 °C) for a maximum of 5 h before reaching the laboratory. Additionally, a blood sample (c. 17 µL) was analyzed with FTA cards (Whatman, Buckinghamshire, UK) for molecular sexing by amplifying the CHD locus using the primers 2550 F (5'-GTTACTGATTC-GTCTACGAGA-3') and 2718R (5'-ATTGAAATGATC-CAGTGCTTG-3') (Fridolfsson and Ellegren 1999) (see supplementary material for details).

Body size and body condition

The tarsus length was used as a body size indicator, given that it remains constant once the bird reaches adulthood (Gosler et al. 1998; Little et al. 2017) and a principal component analysis (PCA) was performed to reduce the dimensionality in our morphological dataset and for computing uncorrelated variables of body condition. The first principal component (eigenvalues \geq 1; c.41.8% of the total variance) was used as a body condition index (BCI; Grant and Grant 2008; see Table S3 for the PCA results; Weeks et al. 2020).

Oxidative status

Blood samples were centrifuged at 8,000 rpm. Plasma was separated and frozen at -80 °C until ROS and TAC could be measured for each sample (Sabat et al. 2017; see below). The total antioxidant capacity (TAC) was used as an indicator of non-enzymatic molecular antioxidants, and the thiobarbituric acid-reactive substances (TBARS) as an indicator of oxidative damage. We estimated the oxidative status of individuals (Costantini et al. 2006), given that both markers have been tested successfully in rufous-collared sparrows (Sabat et al. 2017; Tapia-Monsalve et al. 2018), as well as in other bird species (Gutiérrez et al. 2019).

Plasma TAC levels were determined using the antioxidant capacity reduction method (Apak et al. 2006; Ribeiro et al. 2011). The assay evaluates the reduction of Copper (II)-neocuproine complex to copper (I)-neocuproine complex by antioxidants present in the plasma. This reaction can be measured by colorimetry at 450 nm. Finally, the sample value was compared with a Trolox standard curve (Ribeiro et al. 2011). TBARS was estimated using the thiobarbituric acid levels (Ohkawa et al. 1979) based on a reaction that evaluates the 1:2 adduct formed by malondialdehyde (MDA; a product of lipid peroxidation) and thiobarbituric acid (TBA). The MDA and TBA adduct was determined to have 532 nm colorimetry (Ohkawa et al. 1979). All analyses were performed in the Ecophysiology Laboratory at the University of Chile in Santiago.

Habitat variables

At each site, habitat variables that were through to be important for *Z. capensis* were estimated in situ, using 11 m radius plots (Hernández and Villaseñor 2018; Benito et al. 2019). All woody species were recorded, to calculate species richness of woody plants and native woody species percentage using the catalogue of vascular plants of Chile (Rodríguez et al. 2018). In addition, the percentage of shrub cover was estimated visually. A shrub was a woody plant smaller than a tree that had several main stems arising near the ground (Rodríguez et al. 2018). Park size was also considered as a habitat variable due to the wide variation observed (0.5 ha -4.9 ha) (see Table S4 for habitat variables at sampling sites).

Statistical tests

We fit linear mixed-effects models with Restricted Maximum Likelihood (REML), using the lme function from the 'nlme' package (Pinheiro et al. 2017) to test whether the birds captured in UNRs exhibited better body condition, larger body size and lower oxidative stress than in parks. Thus, we fitted separate linear models for each response variable: body condition (BCI), body size (tarsus length) and oxidative status (TBARS; TAC and TBARS/TAC). We used urban green space type (UNRs or park) as a predictor variable and included sampling blocks (3 levels) as a random effect. Given that the preliminary analyses did not show significant differences in body condition, body size, or oxidative status between sexes (females: 22; males: 31), sex was not included as a cofactor in the models to limit parameters.

To assess the effect of habitat variables on body condition, body size, and oxidative status of birds in UNRs and parks, we built candidate models for each response variable and selected the best predictive model. To select models, we separately fitted mixed-effects linear models that included body condition, body size and oxidative status as response variables to each habitat type (i.e., URNs and parks). Predictive variables were habitat variables (native woody species, shrub cover, woody species richness and park size), where each model included two habitat variables that were not highly correlated (r < 0.5) to avoid both collinearity and the inclusion of too many parameters due our reduced sampling size ($n_{(UNRs)}=30$; $n_{(parks)}=24$). All models included blocks (3 levels) as a random effect. We used Akaike Information Criterion corrected for small samples (AICc) to select the most parsimonious models (lower AICc; Burnham and Anderson 1998).

We checked model assumptions for all analyses. When model residuals did not meet the assumption of the normal distribution, data were log-transformed, and normality was tested again. Results were considered significant for P < 0.05. All analyses were carried out in R 3.5.3 (R Core Team 2019).

Results

Birds in UNRs exhibited a better body condition, larger body size and lower oxidative stress levels than in parks, the latter as result of significant higher TAC levels (Table 1; Fig. 2).

In UNRs, woody plant richness was positively related with body condition and oxidative status (Table 2; Fig. 3). We also found that the percentage of shrub cover was positively related to body size (Table 2; Fig. 3). The native woody species did not show a significant effect on the body condition, body size, or oxidative status of birds (Table 2; See Table S5 for all models). In parks, habitat variables exhibited no significant effect body condition, body size

Table 1 Results from linear mixed models showing the effect of urban green space type on body condition, body size and oxidative status of rufous-collared sparrows (n=54). Parameter estimates and SE (standard errors) for interaction terms were estimated relative to "park" level in variable "Urban green space". Bold numbers indicate significant P-values (<0.05)

Effect on body	Predictors	Estimate	SE	P-value
condition				
BCI	Intercept	0.31	0.30	0.31
	Urban green	-0.71	0.34	0.04
	space			
Effect on body size	Predictors	Estimate	SE	P-value
Tarsus length	Intercept	20.59	0.12	< 0.001
	Urban green	-0.64	0.18	< 0.001
	space			
Effect on oxidative	Predictors	Estimate	SE	P-value
status				
TAC	Intercept	2.57	0.08	< 0.001
	Urban green	-0.39	0.12	< 0.001
	space			
TBARS	Intercept	-1.49	0.07	< 0.001
	Urban green	0.02	0.10	0.81
	space			
TBARS/TAC	Intercept	-4.06	0.09	< 0.001
	Urban green	0.33	0.13	0.02
	space			

and oxidative status of birds (Table 3; See Table S6 for all models).

Discussion

The present study found that body condition, body size and oxidative status of a Neotropical bird can vary with green space type across one city (intra-urban approach; Bork-Hueffer et al. 2014). In Santiago, we found supporting evidence that UNRs provide better habitat conditions for the rufous-collared sparrow than urban parks. Birds captured in UNRs had better body condition, larger body size and lower levels of oxidative stress (TBARS/TAC) than individuals in parks, as result of higher TAC levels. UNRs exhibited greater shrub cover, woody plant species richness and greater native species of woody plants than parks. These factors would favor the feeding ecology and habitat quality for this bird, positively influencing their body condition, body size and oxidative status (Cohen et al. 2009; Costantini, 2014).

Wildlife diet in urban lands is characterized by the presence of anthropogenic food sources, such as food waste (Townsend et al. 2019) or seeds and fruits that people intentionally provide for birds (Jones and Reynolds 2008). Although the availability of this food in cities is constant might contribute to some bird species to persist in these environments (Gomes et al. 2014; Andersson et al. 2015), it is important to highlight that this diet may lack essential nutrients and hence alters both the oxidative status (Costantini, 2014; Isaksson, 2020) and body condition of birds (Caizergues et al. 2021). This is consistent with previous studies in Passer domesticus showing that bird body mass (Bókony et al. 2012) and body size (Meillère et al. 2015) decreases with urbanization and increases with habitat quality in urban gradients (Liker et al. 2008). Similarly, a decrease in antioxidants (Herrera-Dueñas et al. 2017) and an altered composition of fatty acids in the urban bird's diet (Andersson et al. 2015; Isaksson et al. 2017) may also affect their oxidative status (Isaksson 2020). In Santiago, parks possibly show higher anthropogenic food sources, artificial light, and noise levels than URNs. Diverse studies have shown negative effects of these factors on behavioral, physiology and morphology of birds (reviewed by Patankar et al. 2021). Thus, the differences observed in body condition, body size and oxidative status of rufous-collared sparrows between UNRs and parks may be strongly linked to different human intervention levels in these urban green spaces (Bókony et al. 2012; Biard et al. 2017; Patankar et al. 2021).

In UNRS, woody plant richness was positively related to body condition and body size of birds. Also, our results show that the levels of oxidative stress (TBARS / TAC) of

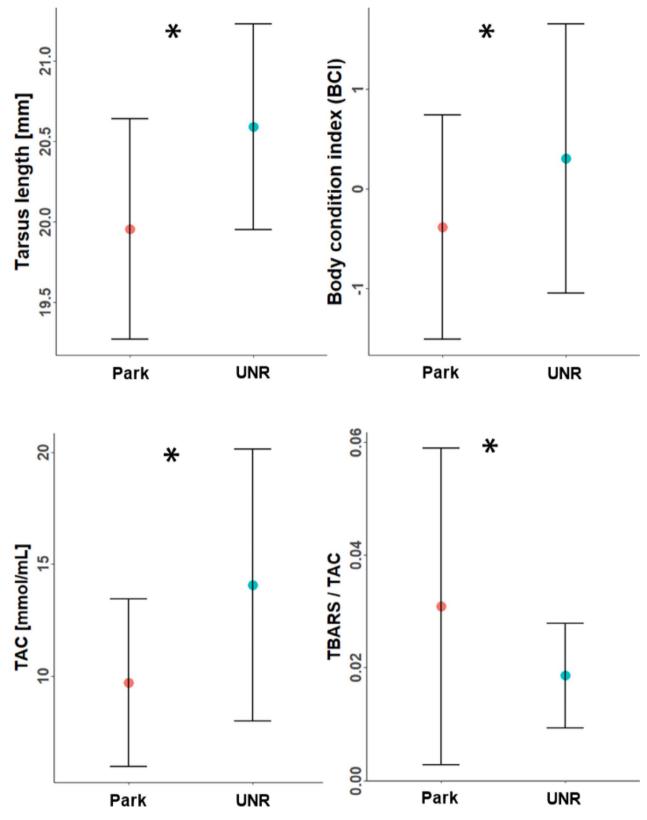


Fig. 2 Differences in body size, body condition and oxidative state in rufous-collared sparrows (n=54) between UNRs (n=30) and parks (n=24). Figure shows that **(a)** tarsus length, **(b)** body condition index

(BCI) and (c) TAC levels are significantly greater in URNs than parks, while (d) levels of oxidative stress are lower URNs than parks (mean \pm SE). Asterisks represents significant differences (p < 0.05)

Table 2 Results from linear mixed models to assess the effect of the habitat variables on the body condition, body size and oxidative status of rufous-collared sparrows (n=30) in UNRs. The table shows the best models based on AICc values (See Table S5 for all models). Bold numbers indicate significant P-values (<0.05)

Effect on body	Predictors	Estimate	SE	P-value
condition				
BCI	Intercept	-0.83	0.59	0.17
	Woody plant richness	0.23	0.11	0.04
Effect on body size	Predictors	Estimate	SE	P-value
Tarsus length	Intercept	20.38	0.14	< 0.001
	Shrub cover [%]	0.02	0.01	0.01
Effect on oxidative	Predictors	Estimate	SE	P-value
status				
TAC	Intercept	2.41	0.17	< 0.001
	Woody plant richness	0.03	0.03	0.34
TBARS	Intercept	-1.33	0.16	< 0.001
	Woody plant richness	-0.03	0.03	0.27
TBARS/TAC	Intercept	-3.74	0.14	< 0.001
	Woody plant richness	-0.06	0.03	0.02

birds decrease as native woody plant richness increases. Plant richness is important because it increases the diversity of resources (Pellissier et al. 2018) and favors coexistence between species (Söderström et al. 2001). Higher woody plant richness is associated with better quality habitats, which provide greater availability and diversity of foods, while favoring the richness and abundance of insects (Paker et al. 2014; Seress et al. 2020). This is relevant to bird health, given that less diverse diets are related to higher stress levels and susceptibility to disease (Birnie-gauvin et al. 2017). Moreover, birds in habitats with greater availability and diversity of foods could spend less time searching for food (Johnson 2007), which would imply less metabolic activity and consequently less oxidative stress compared with birds that occupy lower-quality habitats (van de Crommenacker et al. 2011).

We also found that birds in UNRs have greater body size as the shrub cover increases. This result was expected, because fruits or seeds from shrub vegetation are an important component of the rufous-collared sparrow diet (Lopez-Calleja 1995). Shrub cover also provides sites for nesting (Egli and Vásquez, 2018). This finding agrees with other studies where the abundance of rufous-collared sparrows increases with shrub vegetation (Benito et al. 2019).

In parks, we failed to detect conclusive statistical evidence for a relationship between body condition, body size and oxidative status of birds with habitat variables. Opposite to UNRs, where vegetation is extensive and mainly of native origin, in parks the vegetation is limited and mainly exotic (Hernández and Villaseñor 2018). Studies performed in the Northern Hemisphere, show that the body condition of birds improves in urban areas where native vegetation dominates (Labbé and King 2020). Thus, although birds may show opportunistic feeding behavior when consuming exotic plants, especially where they are more abundant (Aslan and Rejmanek 2012), native vegetation would be of better quality for native animals (Smith et al. 2013; Applegate 2015). The chemical composition, physiological and phenological characteristics of native vegetation have coevolved with animal consumers (Bascompte and Jordano 2007). In fact, exotic vegetation presents novel characteristics that may not be sufficient to supply the physiological and nutritional requirements of birds (Narango et al. 2018). Additionally, the change of native to exotic vegetation can reduce the diversity of insects (Johnson 2007; Burghardt and Tallamy 2013; Narango et al. 2017), directly affecting the body condition of omnivorous birds, such as the rufous-collared sparrow, which feeds on seeds and insects during the breeding season (López-Calleja, 1995). Thus, parks heavily dominated by exotic plants might not provide healthy food to rufous-collared sparrow, where we found no relationships between habitat attributes and body condition, body size and oxidative status of birds in urban parks. Future research could consider parks with varying degrees of native woody plants to investigate the role of native vegetation on rufous-collared sparrow.

In summary, our results support the idea that UNRs provide habitat that promotes better health conditions for rufous-collared sparrows than parks in Santiago and show the importance of vegetation composition and structure on the body condition, body size and oxidative status of urban birds. Within this context, our results provide further evidence for the need to devise urban planning and management strategies to improving habitat conditions for bird species. This can be achieved by protecting remnants of natural vegetation, restoring native vegetation in urban green spaces, such as in UNRs, increasing shrub cover, the diversity of woody plants and prioritizing native plants to support local food webs. Future research efforts could focus on the fitness of birds that inhabit different types of urban green spaces, including measures of breeding success and survival rates. In addition, other measures of habitat quality, such as air, noise, and light pollution levels, will help to assess whether these factors have a differential effect on the body condition, physiology, and health of birds.

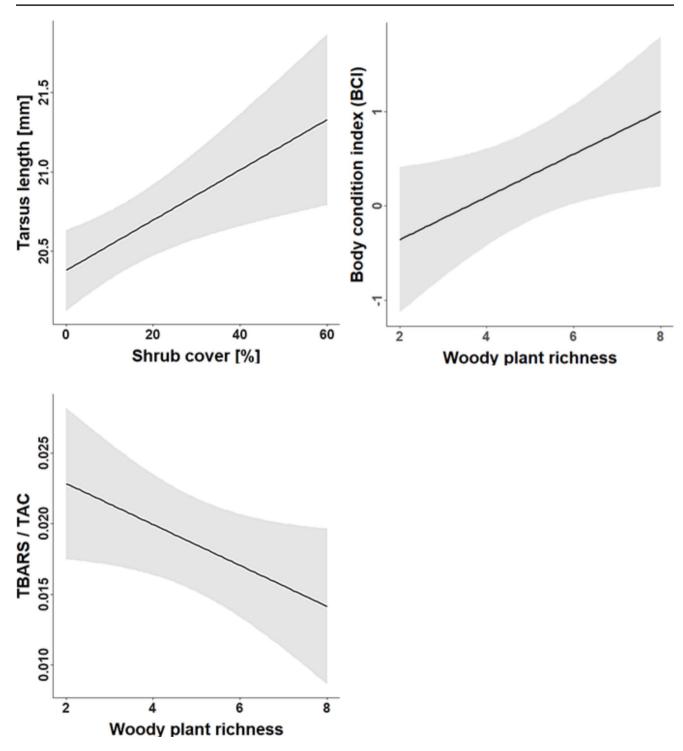


Fig. 3 Significant relationships between body size, body condition and oxidative status with habitat variables in URNs in rufous-collared sparrows. Figure shows that the percentage (a) shrub cover is related

to body size, while woody plant richness is significantly related to (b) body condition (BCI) and (c) oxidative status. Shaded areas represent 95% confidence intervals

Table 3 Results from linear mixed models to assess the effect of the habitat variables on the body condition, body size and oxidative status of rufous-collared sparrows (n = 24) in parks. The table shows the best models based on AICc values (See Table S6 for all models). Bold numbers indicate significant P-values (<0.05)

Effect on body	Predictors	Estimate	SE	P-value
condition				
BCI	Intercept	-1.39	0.62	0.04
	Park size	0.31	0.17	0.08
	(ha)			
Effect on body size	Predictors	Estimate	SE	P-value
Tarsus length	Intercept	20.61	0.45	< 0.001
	Woody plant	-0.15	0.09	0.15
	richness			
Effect on oxidative	Predictors	Estimate	SE	P-value
status				
TAC	Intercept	12.64	2.57	< 0.001
	Park size	-0.76	0.73	0.31
	(ha)			
TBARS	Intercept	-1.36	0.20	< 0.001
	Park size	-0.03	0.06	0.62
	(ha)			
TBARS/TAC	Intercept	-4.45	0.40	< 0.001
	Park size	0.21	0.10	0.06
	(ha)			

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Author Contribution C.F., N.V. and Y.P. Conceived the idea, designed methodology and analyzed the data. C.F. collected the data and wrote the manuscript with input from Y.P and N.V. C.C. Conduced the oxidative state analysis and edited the manuscript. M.A. Conduced DNA extraction and molecular sexing. P.S. Provided assistance with the laboratory analysis and edited the manuscript.

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Data Availability The datasets generated and analyzed during the current study will be archived in the Figshare database (https://doi. org/10.6084/m9.figshare.22320970).

Declarations

Competing interests The authors declare no competing interests.

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