



## Avian scavengers living in anthropized landscapes have shorter telomeres and higher levels of glucocorticoid hormones



L. Gangoso<sup>a,b,\*</sup>, A. Cortés-Avizanda<sup>c,d</sup>, A. Sergiel<sup>e</sup>, B. Pudifoot<sup>c</sup>, F. Miranda<sup>f</sup>, J. Muñoz<sup>c</sup>, A. Delgado-González<sup>c</sup>, M. Moleón<sup>g</sup>, J.A. Sánchez-Zapata<sup>h</sup>, E. Arrondo<sup>c,h</sup>, J.A. Donazar<sup>c</sup>

<sup>a</sup> Department of Wetland Ecology, Doñana Biological Station-CSIC Avda, Américo Vespucio 26, 41092 Seville, Spain

<sup>b</sup> Department of Biodiversity, Ecology and Evolution, Faculty of Biology, Complutense University of Madrid, C/José Antonio Novais 2, 28040 Madrid, Spain

<sup>c</sup> Department of Conservation Biology, Doñana Biological Station-CSIC Avda, Américo Vespucio 26, 41092 Seville, Spain

<sup>d</sup> Animal Demography and Ecology Unit, IMEDEA CSIC-UIB, C. Miquel Marqués 21, 07190 Esporles, Spain

<sup>e</sup> Department of Wildlife Conservation, Institute of Nature Conservation, Polish Academy of Sciences, Adama Mickiewicza 33, 31120 Krakow, Poland

<sup>f</sup> Ecophysiology Laboratory, Doñana Biological Station-CSIC Avda, Américo Vespucio 26, 41092 Seville, Spain

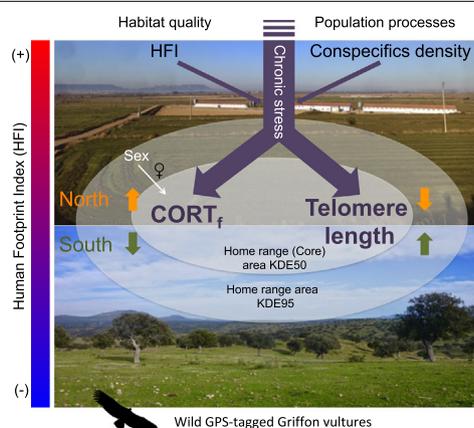
<sup>g</sup> Department of Zoology, University of Granada, Avda. Fuentenueva, s/n, E-18071 Granada, Spain

<sup>h</sup> Department of Applied Biology, Miguel Hernández University, Avda. de la Universidad, s/n, 03202 Elche, Alicante, Spain

### HIGHLIGHTS

- Organisms are exposed to environmental stressors in human-transformed landscapes.
- We measured  $CORT_f$  and telomere length in GPS-tagged griffon vultures.
- Telomere length decreased in parallel to anthropization and density of conspecifics.
- $CORT_f$  levels were higher in females and in more anthropized areas.
- Environment- and population-related stressors affect overall individual quality.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 11 November 2020

Received in revised form 29 March 2021

Accepted 30 March 2021

Available online 6 April 2021

Editor: Damia Barcelo

#### Keywords:

Chronic stress

Feather corticosterone

Eurasian griffon vulture

Conspecific density

### ABSTRACT

Habitat anthropization is a powerful stressor affecting the health and fitness of organisms, ultimately impacting their population dynamics. In vertebrates, stressful living conditions are usually associated with elevated glucocorticoids-based responses (GCs) as well as shorter telomeres, which are in turn associated with decreased overall body condition fitness and life expectancy. However, our understanding of how habitat anthropization per se and population processes synergistically, or independently, may affect GCs and telomere dynamics in natural populations is still very limited. Here, we assessed the physiological footprint of habitat anthropization and conspecific density in 65 GPS-tagged Eurasian griffon vultures (*Gyps fulvus*) from two populations of the Iberian Peninsula. We examined how extrinsic (human footprint values and conspecific density within individual activity areas) and intrinsic (sex and home range size) factors determine GCs deposited in feathers ( $CORT_f$ ) and telomere length as proxies of overall individual condition and quality. We found strong differences in both physiological markers between populations, with higher  $CORT_f$  levels and shorter telomeres in vultures living in the northern, more anthropized area. We also found sex-specific patterns of  $CORT_f$ , with females having higher levels than males. In both sexes, telomere length decreased as the density of conspecifics increased. Previous

\* Corresponding author at: Department of Biodiversity, Ecology and Evolution, Faculty of Biology, Complutense University of Madrid, C/José Antonio Novais 2, 28040 Madrid, Spain.  
E-mail address: [lgangoso@ucm.es](mailto:lgangoso@ucm.es) (L. Gangoso).

Predictable food resources  
Telomere length

studies in these populations have shown lower survival rates in individuals who exploit more anthropized areas, and here we show a potential physiological causal link. We highlight the existence of complex effects of chronic stress associated both with living in anthropized environments and with population-related processes likely associated to the spatial distribution of resources.

© 2021 Elsevier B.V. All rights reserved.

## 1. Introduction

Anthropization of landscapes affects vital rates of organisms, their population dynamics and, ultimately, community structure (Loss et al., 2015; Olden et al., 2004). In addition to altering key ecological processes and interactions such as intra- and inter-specific competition for resources (Vitousek et al., 1997), habitat anthropization may be a powerful environmental stressor affecting the health and fitness of organisms (see reviews in Angelier et al., 2018; Dantzer et al., 2014). In vertebrates, the individual response to environmental stress is driven by the activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased levels of glucocorticoids (GCs). GCs are the main hormonal mediators of allostasis (i.e., maintaining homeostasis through change, McEwen and Wingfield, 2003) and are often used as a proxy for an individual's ability to cope with the environment (Angelier et al., 2010; Wingfield et al., 1998). Short-term elevated GC concentrations may help to face successfully life-threatening situations, but chronic activation of the HPA axis and cumulative exposure to circulating GCs may also have deleterious effects, such as reductions in body mass/condition, immune function, fertility, and survival (e.g., Kitaysky et al., 2010; Sheriff et al., 2011). Although consequences of long-term stress caused by natural factors are not necessarily pathological (Boonstra, 2013; Dantzer et al., 2013) and the relationship between GCs and fitness in free-living animals is not uniformly negative (Bonier et al., 2009; Crespi et al., 2013), stress derived from anthropogenic disturbance is likely to exceed that originating from natural sources (e.g., Dantzer et al., 2014; Zbyryt et al., 2018). Thus, understanding how human-mediated stressors affect wildlife populations is of paramount importance in ecology and biodiversity conservation.

In birds, several studies have found significant relationships between anthropization and high GCs levels, low survival, and low breeding success (e.g., Mülner et al., 2004; Strasser and Heath, 2013). However, there are also examples showing no relationships or even the opposite patterns (Buxton et al., 2018; Crino et al., 2013; Rebolo-Ifrán et al., 2015), suggesting that the links between anthropization and GCs are far from being easily generalized. Rather, they may be highly complex and dependent on species-, population-, and individual-level variation in the perception (e.g., urban exploiters vs. avoiders) and response to chronic stress (see Iglesias-Carrasco et al., 2020 and references therein). An example of within species variation is found in the sex-specific response to anthropogenic disturbance, with stronger male than female responses reported in some studies (e.g., Hayward et al., 2011) while the reverse is observed in others (e.g., Strasser and Heath, 2013).

Corticosterone, the primary GC in birds, is deposited in feathers ( $CORT_f$ ) and reflects the cumulative exposure of individuals during feather growth, so it is increasingly used as reliable proxy for long-term stress (Bortolotti et al., 2008). The integration of  $CORT_f$  during the period of feather growth is weeks long, meaning that the values are less sensitive to short-term stressors (e.g., predator attack). Thus, this measure is more suitable for studying responses to environmental stressors that operate over longer time periods such as climate, food availability and habitat quality (Romero and Fairhurst, 2016). Increases in  $CORT_f$  have been found along an urbanization (and pollution) gradient in birds of different ages (Beaugeard et al., 2019; Meillère et al., 2016). However,  $CORT_f$  has also been reported to be similar between urban and rural habitats, with partial and non-linear effects on survival

(Rebolo-Ifrán et al., 2015). In addition, Ibáñez-Álamo et al. (2020) found consistently higher  $CORT_f$  levels in non-urban as compared with urban habitats when analyzing different populations of an urban exploiter species, yet this effect was only apparent in males, a result likely associated with habitat-associated differences in physiology between sexes. Therefore, both the magnitude and the direction of GCs-mediated response of particular species and sexes to anthropization are overall difficult to predict.

Stress also operates through accelerated cell aging, as indexed by shorter telomeres at the ends of chromosomes (Epel et al., 2004; Kotrschal et al., 2007). Telomeres are evolutionarily conserved caps consisting of short tandem repeated DNA sequences that protect eukaryotic linear chromosome ends (Zakian, 1995). The enzyme telomerase replicates DNA in chromosome ends and thus enlarges telomere length that would be otherwise shortened in each cell division (Greider and Blackburn, 1985). Chronic stress and elevated GCs interfere with antioxidative defenses, therefore increasing cellular and DNA oxidative damage, especially affecting the telomeric regions and the restorative ability of the enzyme telomerase (Epel et al., 2004). Chatelain et al. (2020) concluded that exposure to stressors, regardless of its origin, is unequivocally associated with shorter telomeres or greater telomere shortening across taxa. In birds, telomere length and shortening rate have been associated with stress conditions experienced both early in life (e.g., Young et al., 2017) and in other later stages (e.g., Mizutani et al., 2013). Although studies linking anthropization to telomere length and dynamics are still scarce, it has been shown that growing up in urban habitats and human-induced disturbances accelerate telomere shortening (Blévin et al., 2016; Salmón et al., 2016; Stauffer et al., 2017; but see Biard et al., 2017) and that urban-dwelling birds have shorter telomeres than forest-dwelling ones (Ibáñez-Álamo et al., 2018).

The social environment experienced by individuals may be also altered by anthropization, mainly through its effects on population density and the distribution and availability of resources, likely increasing both social and nutritional stress (reviewed in Bonier, 2012). Experimental studies have found higher GC levels under increased social competition (e.g., Hawley et al., 2006; Mora et al., 1996). In wild bird populations, conspecific density and intra-specific competitive interactions have also been associated with increased GCs secretion (e.g., Landys et al., 2010; Silverin, 1998). However, these relationships are not universal and become even more complicated in hierarchical social animals, being further influenced by ecological and environmental factors (reviewed in Creel et al., 2013; Goymann and Wingfield, 2004).

The influence of social variables on telomere dynamics has received much less attention, and most evidence for their effects come from studies with model organisms, showing a negative effect of density (Beloor et al., 2010) and crowding, also dependent on an individual's sex (Kotrschal et al., 2007). Experimental studies with wild passerines and mammals also provide evidence for conspecific resource competition during development, leading to greater telomere shortening (e.g., Nettle et al., 2015), and shorter telomere lengths (e.g., Cram et al., 2017) that may be carried through to adulthood. In free-ranging adult mammals, Lewin et al. (2015) showed the influence of social and ecological factors on telomere length, which were unrelated to individual chronological age.

Although current evidence from long-lived wild bird populations is scarce and individual relationships have reported mixed results,

mounting evidence supports that telomere length in long-lived birds correlates with life-history traits and fitness (e.g., Angelier et al., 2019; Wilbourn et al., 2018) and that these processes are tightly linked to environmental stress (see review in Angelier et al., 2018). However, our understanding of how the habitat per se and ecological processes, including population variables, synergistically or independently affect GCs and telomere dynamics in natural populations is still limited, and even more so with regards to the effects of habitat anthropization.

Here, we evaluated the physiological trace of habitat anthropization in two populations of long-lived Eurasian griffon vultures (*Gyps fulvus*) inhabiting areas with uneven human landscape transformations in the Iberian Peninsula (Arrondo et al., 2020a). We assessed how extrinsic (levels of landscape anthropization and conspecific density within individual activity areas) and intrinsic (sex and home range size) factors relate to  $CORT_f$  and telomere length of adult birds. We used the Human Footprint Index (HFI; Venter et al., 2016) to evaluate the degree of anthropization of the home range exploited by birds determined from GPS tracking data (Arrondo et al., 2020a) and an index of conspecific density as an evaluator of intraspecific competition. The effects of the environmental variables considered on the individual physiological response could differ depending on the degree of exposure, which may in turn depend on the frequency of use of different habitats. Therefore, we assessed the relative effect of these variables at both small (kernel 50%) and large (kernel 95%) spatial scales, indicating the core activity and full range areas exploited by vultures, respectively, and at the population level. We predict that vultures living in more anthropized environments will have shorter telomeres and higher  $CORT_f$ . This would be consistent with the already observed lower individual survival rates in vultures using the more anthropized area, which are mainly associated with human activities and infrastructure (Arrondo et al., 2020a). Predicting the effects of sex and density of conspecifics on these physiological evaluators is not straightforward given the complex relationships and contrasting patterns already reported in different species (see above). However, we expect that a higher density of conspecifics competing for resources, even in this social species, will increase levels of environmental stress for both sexes.

## 2. Material and methods

### 2.1. Focal species, study area, and field procedures

We targeted the Eurasian griffon vulture, a large (up to 12 kg), long-lived avian scavenger that roosts and breeds colonially in cliffs and feeds on wild and domestic ungulate carcasses. Griffons cover hundreds of kilometers when foraging (Cortés-Avizanda et al., 2014), yet most of the foraging activity of breeding birds concentrates within 50 km from the nest site (Arrondo et al., 2020a). Historically, the species occupied dry and open regions of northern Africa, southern Europe, the Middle East, and central Asia. In the Western Palearctic, griffons have disappeared or declined all over their former range except the Iberian Peninsula, which currently holds 95% of the European population with around 34,000 breeding pairs (Del Moral and Molina, 2018).

We captured 30 adult griffons in the southern Iberian Peninsula between December 2014 and January 2015. Another 35 adults were captured in the north between December 2015 and March 2016 (SM1). The southern breeding area corresponds to the Sierras de Cazorla Segura y las Villas Natural Park, a mountainous region with an altitude ranging from 500 to 2107 m. These birds mainly forage in Sierra Morena, an east-to-west ridge mostly covered by Mediterranean woodlands and pasturelands, predominantly dedicated to traditional livestock farming, big game hunting, and forestry (see details in Martín-Díaz et al., 2020). The northern population breeds in the hills and mountains of the upper Ebro Valley, a region with plains largely transformed for intensive agricultural crops and where traditional, extensive sheep farming has also been partially replaced by intensive farming (Martín-Queller et al., 2010). The two study areas hold 12%

and 28% of the total breeding population of the target species in Spain, respectively (Del Moral and Molina, 2018).

Individuals were captured using cannon nets and livestock carcasses as baits. All tagged birds were adults (>6 years old), classified according to morphological traits such as plumage, eye, and bill characteristics (Zuberogoitia et al., 2013). All birds were weighed using a Pesola scale ( $\pm 5$  g). Wing chord length was measured using a 100 cm ruler to the nearest mm and used as a proxy for body size. Birds were equipped with 98 g GPS/GPRS-GSM devices from e-Obs digital telemetry (<https://www.e-obs.de>). The weight of the device and the harness account for less than 1.1% of the mean adult weight, so negative effects on the individual behavior and fitness are not expected (Martín-Díaz et al., 2020). We monitored all individuals from capture until December 2018, except in cases of device failure or individual mortality (Arrondo et al., 2020a). More details on the study model and fieldwork procedures can be found in Martín-Díaz et al. (2020) and Arrondo et al. (2020a).

At the time of capture for each individual, we collected a 5 ml blood sample by venipuncture of the brachial vein. Blood samples were preserved in absolute ethanol for molecular analyses (sex and telomere length determination). In addition, we collected a new and unworn scapular feather from each individual for hormone analyses ( $CORT_f$  determination), and stored the samples in paper envelopes at room temperature until analysis. This particular feather can be easily identified, which facilitates the standardization of sample collection (the same feather is collected from all individuals), and is of sufficient size while not being an essential flight feather. Although we do not know the exact growth rate of scapular feathers of griffon vultures, the growth rate of flight feathers in birds of prey is similar across species (about 4 mm/day, Zuberogoitia et al., 2018). Assuming this growth rate and considering that the mean length of scapulars used here was 129 mm (see below), the sampled feathers would have required about 30 days to achieve full growth. Body feathers are replaced every 2–4 years, but less active molt is observed during the breeding period in adult individuals (Zuberogoitia et al., 2013). Our sampling was done in winter and early spring. Thus, because we chose new, unworn feathers, which are darker and brighter than worn feathers molted in previous years (SM2), they were likely to have grown in the immediately preceding post-breeding autumn and winter months, thus standardizing the environmental conditions experienced by sampled birds.

### 2.2. Determination of $CORT_f$

$CORT_f$  was measured using the acetonitrile/hexane extraction and enzyme immunoassay (EIA) method, following a modified protocol originally proposed by Kouwenberg et al. (2015). Firstly, we silanized the borosilicate tubes using 99% toluene and 1% acetonitrile in an extractor hood. Tubes were rinsed with 1 ml methanol and air-dried before a 30 min incubation. After removing the after-feather, calamus, and any debris, the length ( $\pm 1$  mm) and weight ( $\pm 1$  mg) of the rachis were measured using a ruler and an analytical balance, respectively (mean length = 129 mm, range = 82–192 mm; mean weight = 89.22 mg, range = 23.8–191.5 mg). Feathers were then cut into small pieces with scissors and introduced into silanized tubes with 2 ml of methanol. We included a blank extraction tube with no feather. Subsequently, the tubes were sonicated for 30 min and introduced in a shaking water bath for 16 h at 50 °C and 50 rpm. Then, methanol was filtered using ESF-GF-25-100 (25 mm 1  $\mu$ m) filters in silanized tubes, and two additional milliliters of methanol were added to collect and filter all corticosterone. Tubes were dried for 1 h at 60 °C in a nitrogen evaporator (N-Evap).

For the corticosterone purification, we first added 2 ml of hexane in each tube, vortexed, then added 2 ml of acetonitrile and vortexed again. Samples were centrifuged at 1250  $\times$ g and 10 °C for 15 min to facilitate separation of the Acetonitrile and hexane layers. The upper phase (hexane layer) was then transferred to a clean tube, and 2 ml of acetonitrile

was added. Two milliliters of hexane were added to the tube with the lower phase (acetonitrile layer). Tubes were vortexed and centrifuged again using the same conditions as above. We then eliminated the hexane layer of every tube and merged the two acetonitrile layers from the same sample for evaporation under nitrogen flow in a N-Evap. Samples were kept at  $-80^{\circ}\text{C}$  until the corticosterone analysis. Corticosterone was quantified using a commercial CORT EIA kit (501,320, Cayman Chemical Company, Ann Arbor, MI) previously validated for the measurement of feather corticosterone (Kouwenberg et al., 2015), following manufacturer instructions. Dried extracts were re-suspended in 125  $\mu\text{l}$  of EIA buffer and run in duplicates, with intra- and inter-assay coefficient of variation (CV) of 3.70 and 8.86%, respectively. Cross-reactivity values of this assay for corticosterone, 11-deoxycorticosterone, prednisolone, 11-dehydrocorticosterone, cortisol, and progesterone are 100, 15.8, 3.4, 2.9, 2.5, and 1.4%, respectively, and  $< 0.5\%$  for remaining structurally related molecules (potential cross-reactants). The feather growth rate is rather uniform between and within species (Rohwer et al., 2009). Consequently, the hormone exposure is time-dependent and feather corticosterone should be standardized by length. Therefore, corticosterone values were expressed as pg CORT per mm of feather (pg/mm).

### 2.3. Determination of telomere length

Telomere length was evaluated using DNA extracted from erythrocytes. Although the biological age of each individual was unknown, the fact that all samples came from the same tissue and all birds were adults facilitated the comparison of telomere length among them (Nussey et al., 2014). Telomere length was estimated by a quantitative PCR assay (qPCR) (Cawthon, 2002; Criscuolo et al., 2009). Relative telomere length was measured by determining the ratio (T/S) of telomere repeat copy number (T) to single control gene copy number (S) in target samples when compared with a non-telomeric reference sequence sample. Hence, the qPCR method measures relative telomere lengths and not absolute telomere lengths (i.e., kb), as in other methods such as Telomere Restriction Fragment (TRF) (Cawthon, 2002).

We followed the procedure described in Gangoso et al. (2016), with some modifications; we carried out telomere and control single copy gene (Glyceraldehyde-3-phosphate dehydrogenase, GAPDH) real-time amplifications on different plates using two biological replicates per sample. In addition, amplifications for all samples were run on two different plates (i.e.,  $N = 40$  and  $N = 25$ ,  $N_{\text{total}} = 65$  samples). Each PCR reaction for the telomere (or GAPDH) was performed using 20 ng/ $\mu\text{l}$  of DNA with sets of primers Tel1b/Tel2b (or GAPDH-F/GAPDH-R), each used at a concentration of 500 nM, in a final volume of 20  $\mu\text{l}$  containing 10  $\mu\text{l}$  of Fast Start Universal SYBR Green Brilliant Master (Roche, Diagnostics GmbH, Mannheim, Germany) and 0.5  $\mu\text{l}$  of BSA (Bovine Serum Albumin) at a concentration of 20 ng/ $\mu\text{l}$ .

PCR conditions for the telomere portion of the assay were 10 s of pre-incubation at  $95^{\circ}\text{C}$  followed by 30 cycles of 10 s at  $95^{\circ}\text{C}$ , 10 s at  $56^{\circ}\text{C}$ , and 1 min at  $72^{\circ}\text{C}$ . A final melting assay of 15 s at  $95^{\circ}\text{C}$  followed by a temperature ramp from  $65^{\circ}\text{C}$  to  $95^{\circ}\text{C}$  at a ramp rate of  $2.2^{\circ}\text{C/s}$  was also performed. Conditions for the GAPDH portion of the assay were 10 s of pre-incubation at  $95^{\circ}\text{C}$  followed by 50 cycles of 10 s at  $95^{\circ}\text{C}$ , 10 s at  $56^{\circ}\text{C}$ , and 40 s at  $72^{\circ}\text{C}$ . Identical conditions to the telomere portion for the final melting assay were followed for GAPDH.

All PCRs were performed in a Light Cycler 480 RT-PCR System (Roche). To test the efficiency of each PCR reaction, a standard curve was produced in every plate by serially diluting a mix of DNA obtained from five random samples (40, 10, 2.5 and 0.66 ng/ $\mu\text{l}$  of DNA per well) and by running it in duplicate. The slopes of the standard curves ranged from  $-3.11$  to  $-3.59$ ; efficiencies ranged from 1.95 and 2.09 (mean efficiency<sub>telomere</sub> = 1.95; mean efficiency<sub>GAPDH</sub> = 1.99), thus falling within the acceptable range of efficiencies for qPCR assays (see review in Horn et al., 2010). The CV of the quantification cycle ( $C_t$ ) values for the GAPDH and telomere amplifications were  $< 5\%$  in all samples

following Criscuolo et al. (2009). We then calculated the threshold of quantification cycle values ( $C_t$ ) of this reference sample for each plate. All other samples were run in duplicate on the plates, and mean values per plate were used to calculate relative ratios of target individual relative to the reference individual. Mean intra- and inter-plate CV was 0.71% and 2.32%, respectively, for the  $C_t$  values of GAPDH assays, while intra- and inter-plate CV was 0.41% and 0.97%, respectively, for the  $C_t$  values of telomere assays. To take into account the variation of efficiencies between telomere and GAPDH amplifications, we calculated relative telomere length by transforming  $C_t$  values into normalized relative quantities (NRQs) following Hellemans et al. (2007).

### 2.4. Determination of environmental variables: Human Footprint Index and vulture density

We performed kernel density models in the adehabitatHR package (Calenge and Fortmann-Roe, 2013) run in R version 3.0.3 (R Development Core Team, 2018) to estimate core area (kernel 50%, hereafter KDE50, small spatial scale) and the home range area (kernel 95%, hereafter KDE95, large scale) of individual vultures (Table 1, Fig. 1). We estimated the spatial use of each individual with the ad hoc method as a smoothing parameter and with a resolution of 1 ha (Margalida et al., 2016).

As proxy for anthropization we used the Human Footprint Index (HFI, <https://wchumanfootprint.org/>), which expresses, on a scale from 0 to 50, the overall human influence in a particular area (1 km<sup>2</sup> resolution) by integrating the human population density and land use: crop surface, pasturelands surface, nightlights, built environments, railway coverage, major roadway coverage, and navigable waterways (Venter et al., 2016). The variable HFI was not normally distributed in our study area, but the data were skewed and contained clear outliers (see Arrondo et al., 2020a). Therefore, we used the median for further calculations. We quantified the median HFI at KDE50 and KDE95 for each individual bird by intersecting each individual's respective KDE with the European Human Footprint map.

An index of conspecific density was calculated for both KDE50 and KDE95 home ranges as follows. Firstly, and for each  $10 \times 10$  km grid cell encompassing the Iberian Peninsula, we calculated the number of potential foraging vultures as the number of breeding individuals according to the griffon vulture census database of 2008 (Del Moral, 2009) within a radius of 50 km from the grid center. This radius was chosen in the light of the existing data showing that most of the foraging activity of the birds concentrates within 50 km from the breeding sites (Arrondo et al., 2020a). Subsequently, and for both home range estimators, we calculated the mean number of conspecifics/km<sup>2</sup> on the basis of the total number of grid cells within the respective area.

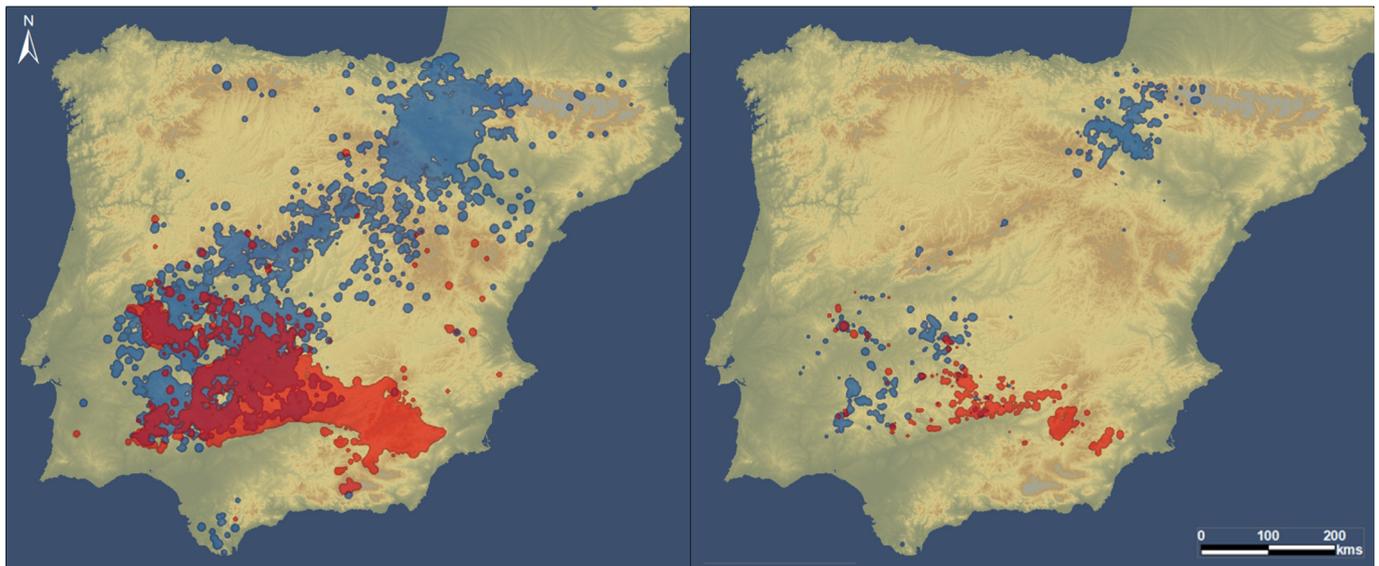
### 2.5. Statistical analysis

We fitted Linear Models (LMs) considering both the concentration of feather corticosterone/mm (CORT<sub>f</sub>) and telomere length (TL) as response variables. These two variables were not correlated (Pearson

**Table 1**

Mean  $\pm$  standard deviation (SD) size (km<sup>2</sup>) of the core area (kernel 50%, KDE50) and the home range area (kernel 95%, KDE95) of 65 GPS-tagged Eurasian griffon vultures from the northern and southern study populations. Sample sizes (N) for each sex and population are indicated.

|       |         | North     |         |    | South     |         |    |
|-------|---------|-----------|---------|----|-----------|---------|----|
|       |         | Mean      | SD      | N  | Mean      | SD      | N  |
| KDE50 | Males   | 451.53    | 551.50  | 19 | 467.45    | 557.36  | 19 |
|       | Females | 830.44    | 570.62  | 16 | 937.62    | 571.51  | 11 |
| KDE95 | Males   | 6709.52   | 8386.43 | 19 | 9888.89   | 6152.36 | 19 |
|       | Females | 10,886.63 | 8803.21 | 16 | 13,600.34 | 5985.50 | 11 |



**Fig. 1.** Sum of the individual Kernels (left panel KDE95, right panel KDE50) of 65 GPS-tagged Eurasian griffon vultures from the northern (blue) and southern (red) study populations in the Iberian Peninsula.

correlation test,  $r = -0.125$ ,  $p = 0.34$ ,  $N = 60$ ). Initial explanatory variables accounting for individual variability in these response variables were: location (North/South), sex (male/female), HFI and conspecific density. The latter two variables were calculated at small and large spatial scales (i.e., KDE50 and KDE95). Although vultures concentrate their activity within relatively small areas (KDE50, core areas), usually during winter and the earlier reproductive season, they may perform much larger foraging movements (KDE95, whole home range) over the year (Table 1, Fig. 1). Factors operating at these two scales may be different because, obviously, the individuals can be constrained by the habitat characteristics and availability of resources near the breeding areas, but they can be more selective when larger areas are available to forage.

In the first instance, we also considered working with explanatory variables describing the size of the home range ( $\text{km}^2$ ) because  $\text{CORT}_f$  levels may be positively associated with locomotor activity, particularly under nutritional stress (e.g., Carrete et al., 2013). In fact, Carrete et al. (2013) found differences in  $\text{CORT}_f$  values in Egyptian vultures (*Neophron percnopterus*) associated with very large differences in home range sizes (i.e., 33 times larger in African wintering than in European breeding areas). In our study birds, females had larger home ranges than males, but the difference was comparatively much smaller both at KDE50 and KDE95 (Table 1). Preliminary analyses showed covariation ( $p < 0.05$ ) between sex and home range at both spatial scales, i.e., KDE50 and KDE95, so these variables should not be included in the same model to avoid multicollinearity (Graham, 2003). Moreover, it is important to note that vultures are specialized in soaring-gliding flight for wide-range movements, which minimizes energy expenditure (about 1.5 x basic metabolic rate; Norberg, 1996) while searching for food (Duriez et al., 2014). Thus, large differences in  $\text{CORT}_f$  values associated with differences in home range size between sexes of such magnitude would not be expected. Even so, and to assess the potential effect of home range size, we fitted preliminary models for both  $\text{CORT}_f$  and TL with home range sizes (both KDE50 and KDE95) as the only predictors. In all cases, we found no relationships with these variables (in all cases estimates overlapped zero). Home range was therefore excluded from the LM analyses, while sex was maintained, as differences in  $\text{CORT}_f$  between sexes are likely due to sex-linked features other than differences in their home range size.

We also considered including individual body condition as an explanatory variable. Thus, a body condition index was calculated

separately for males and females by obtaining the standardized residuals of the regression of body mass against the wing chord. A preliminary examination of the data showed that both male and females of the northern population had higher body masses than their southern counterparts, even though the body size (wing chord) was similar between populations in both sexes (SM3). The regression between body mass and wing chord gave non-significant relationships (males:  $p = 0.112$ ; females:  $p = 0.651$ ), indicating that the body condition index showed similar variation to body mass. Given the aforementioned covariation with “location” and given that the correlation between the body condition index of both sexes with the response variables  $\text{CORT}_f$  and TL was non-significant (SM3), we did not incorporate the body size index in subsequent analyses.

Before model fitting, we made preliminary analyses to assess potential covariations among explanatory variables (Spearman’s correlation coefficient higher than  $|0.5|$ ). We found that the variables “HFI” and “conspecific density” at both spatial scales were not correlated. On the contrary, the variable “location” covaried with both HFI and conspecific density at both spatial scales (Wilcoxon tests for independent samples, in all cases,  $p < 0.05$ ). To help discriminate between these correlated variables that in turn determine the most suitable spatial scale to be used, we first included these variables into a single model and, following Zuur et al. (2009), we performed a marginal test using the “drop1” function in the package *lme4* (Bates et al., 2015). The results (see SM3) showed that, for the response variable “ $\text{CORT}_f$ ”, including “location” resulted in models with lower AICc as compared to including the “HFI” and “conspecific density” at both spatial scales. This means that the AICc explaining mean (population) values is identical to the AICc explaining individual values, so individual variation does not add explanatory power. According with this, we found no within-locality significant correlations among response and explanatory variables (Spearman R correlations,  $p > 0.05$  in all cases). Thus, slopes of intralocality regressions probably do not differ from zero. Consequently, in a further step, we fitted models with only two explanatory variables: “location” and “sex”. For the response variable “TL”, we found similar results (SM3). The exception was the explanatory variable “Consp\_KDE95”. Dropping this variable also resulted in a noticeable increase in AICc. Therefore, this variable was also included in the model assessing variation in TL together with “location” and “sex”. A similar trend was observed for the variable “Consp\_KDE50”, although the significance of the difference was slightly lower (SM3).

Accordingly, we performed two final modelling trials. In relation to the response variable  $CORT_f$ , we considered two explanatory variables: “location” and “sex”. In relation to the response variable TL, we considered these same two variables plus “Consp\_KDE50”. In both trials, and in order to account for sex-specific patterns, we included the interaction between “sex” and the other explanatory variables. Before model fitting, continuous variables were scaled by subtracting the mean and dividing by standard deviation. Model selection was based on the small-sample-size corrected version of the Akaike Information Criterion (AICc, Burnham and Anderson, 2002). Competing models (i.e., models differing  $<2\Delta AICc$ ) were averaged. Analyses were done using the packages *MuMIn* (Barton, 2019) and *AICcmodavg* (Mazerolle, 2020) in R 3.5.1 (R Development Core Team, 2018).

### 3. Results

We used GPS data and physiological measures (levels of  $CORT_f$  and TL) of 65 griffon vultures from two different populations (north: 19 males and 11 females, and south: 19 males and 16 females, see Table 1 and Fig. 1). Data on  $CORT_f$  levels could not be obtained from five birds. Overall, levels of  $CORT_f$  and, especially TL differed between the two study sites, with slightly higher  $CORT_f$  levels and significantly shorter TL found in the northern population. In addition, the Human Footprint Index (HFI) and conspecific density also showed significantly higher values in the northern population at both spatial scales (KDE50 and KDE95) (Table 2, Fig. 2). Within each population, values of these two variables were similar at both scales for vultures living in the northern population. On the contrary, HFI and conspecific density decreased from the core (KDE50) to the large individual home range scale (KDE95) in the southern population (Table 2).

The fitting of  $CORT_f$  gave two equivalent models, which when averaged, showed that levels of  $CORT_f$  were lower for the southern population (Tables 3 and 4). Additionally, males had lower  $CORT_f$  levels than females, especially in the southern population (see also Fig. 2), yet the interaction between these variables did not significantly affect  $CORT_f$  (95% CI overlapped zero). The average of the three equivalent models for the response variable TL (Table 3) showed that vultures from the northern population had shorter telomeres, and that TL was also negatively associated, yet to a lower extent (lower relative importance, see

Table 4), with the density of conspecifics at the large-scale home range (KDE95) (Tables 3 and 4, see also Fig. 3).

### 4. Discussion

Our study shows that living in anthropized landscapes entails important individual physiological costs in the adult fraction of the population of a top, long-lived avian scavenger. Exploitation of anthropized and likely more stressful habitats, together with the density of conspecifics within individual activity areas, was associated with poorer overall individual condition and quality, as evidenced by shorter telomeres (see below). In the same study birds, Arrondo et al. (2020a) found a direct relationship between anthropization and lower survival probabilities. Our study takes this a step further and shows a potential physiological causal link. By combining two physiological markers and individual GPS-based delimitation of exposure areas, this is the first study to our knowledge showing that the interaction of human-mediated landscape transformations and intra-population patterns in wildlife lead to cumulative negative effects on individual stress levels and cell aging, which may ultimately affect fitness.

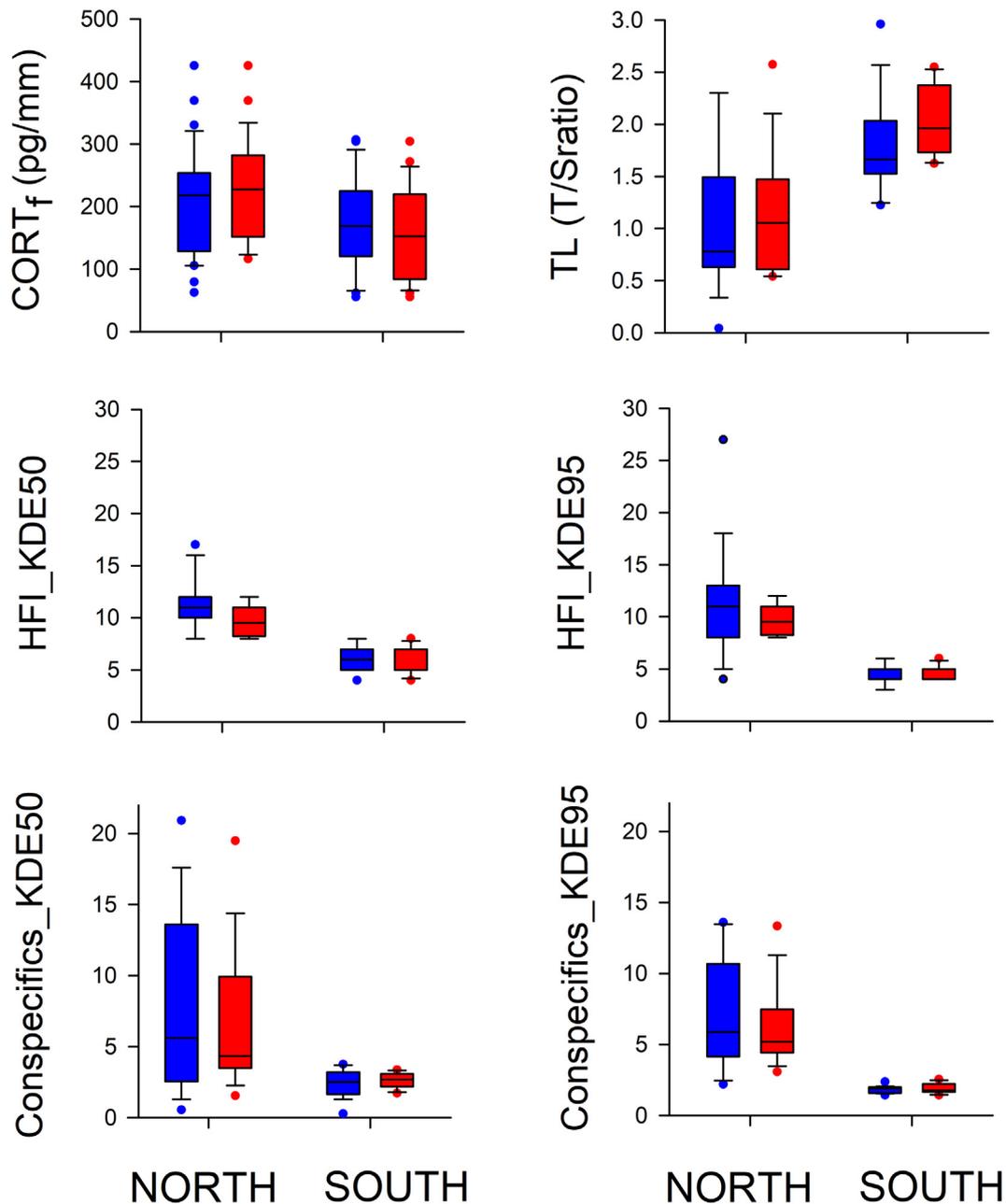
Wildlife populations living in proximity to humans generally experience reductions in population size and connectivity, a process that frequently decreases biodiversity (McKinney, 2002). Nonetheless, human-altered environments may create opportunities for some species by, for example, increasing environmental heterogeneity and resource availability or reducing predation pressure (reviewed in Chace and Walsh, 2006), with some species thriving in moderately altered environments (Luck, 2007). Although negative effects of anthropization on individuals' performance likely outweigh the positive effects, contrasting patterns and even absence of effects have been reported with regard to physiological stress responses (see reviews in Dantzer et al., 2014; Iglesias-Carrasco et al., 2020). Even so, it may be expected that, in general, wild urban-avoiders inhabiting areas with a higher degree of anthropization suffer higher chronic stress.

Vultures have been associated with human activities since ancient times, mainly benefiting in terms of food acquisition (Gangoso et al., 2013; van Overveld et al., 2018). Foraging in more anthropized areas may have several cost-benefits in terms of energy balance and stress. On the one hand, availability and predictability of food is likely higher, which is supported by shorter foraging trips (smaller home ranges, particularly at KDE95, Table 1) and higher body mass in vultures exploiting this area (SM3). However, we found that individuals from the north had overall higher  $CORT_f$  values than those from the south. This pattern could be associated with a lower food quality for vultures in the north, as poor nutritional status contributes to stress and elevated levels of circulating GCs (Kitaysky et al., 2006; Pokharel et al., 2019). In fact, by analyzing GPS locations classified as “foraging” on the basis of accelerometer data, it was shown that the use of landfills and intensive livestock farms was much higher in the northern population (north: mean =  $12.20 \pm 11.54$  SD and  $35.88 \pm 13.31$ , respectively, vs. south:  $0.04 \pm 0.23$  and  $24.13 \pm 24.16$ ; authors, unpublished). This easy to find food may also entail a risk of ingesting veterinary drugs and other pollutants that may in turn affect vultures' immunity-disease interactions (Blanco et al., 2019; Plaza et al., 2019). Glucocorticoids, by orchestrating allocations among physiological systems, may eventually influence life-history trade-offs such as those arising between responses to nutritional and oxidative stress and immune function (see review in Hasselquist and Nilsson, 2012). Body condition of griffon vultures measured at sampling was, however, unrelated to both  $CORT_f$  and TL. This further suggests that the quality rather than the quantity of food obtained at highly anthropized areas may entail physiological consequences. Nonetheless, other more subtle effects should also be considered, such as the risk of ingesting lead from ammunition sources. By analyzing blood samples of vultures from the same study sites, Arrondo et al. (2020b) found higher lead concentrations derived from hunting activities in individuals from the southern population. This

**Table 2**

Descriptive statistics of the response variables: feather corticosterone  $CORT_f$  (pg/mm feather) and relative telomere length TL (T/S ratio) and the explanatory variables: Human Footprint Index (HFI) and conspecific density (mean number of individuals/km<sup>2</sup>) in Eurasian griffon vultures from the two study populations (North/South). In each case, the mean, the standard deviation (in brackets) and the range are shown. We also show the results of comparisons between populations (one-way ANOVA, right column) and within populations (Wilcoxon signed rank tests, below pairs of variables at different spatial scales). Note that difference in degrees of freedom is due to the fact that five individuals have no data on  $CORT_f$ .

|                               | North<br>N = 35            | South<br>N = 30            | Comparison<br>between<br>populations |
|-------------------------------|----------------------------|----------------------------|--------------------------------------|
| $CORT_f$                      | 206.0 (86.5)<br>62.3–425.4 | 164.0 (75.1)<br>55.2–306.7 | $F_{1,58} = 3.89$ ,<br>$P = 0.0531$  |
| TL                            | 1.11 (0.68)<br>0.04–3.14   | 1.89 (0.43)<br>1.22–2.96   | $F_{1,63} = 29.41$ ,<br>$P < 0.001$  |
| HFI_50                        | 10.57 (2.18)<br>8.0–17.0   | 5.90 (1.18)<br>4.0–8.0     | $F_{1,63} = 109.3$ ,<br>$P < 0.001$  |
| HFI_95                        | 10.02 (4.34)<br>4.0–27.0   | 4.47 (0.86)<br>3.0–6.0     | $F_{1,63} = 47.38$ ,<br>$P < 0.001$  |
| Comparison within populations | $P = 0.1037$               | $P < 0.001$                |                                      |
| Consp_KDE50                   | 7.14 (5.65)<br>0.55–20.91  | 2.48 (0.79)<br>0.27–3.75   | $F_{1,63} = 20.08$ ,<br>$P < 0.001$  |
| Consp_KDE95                   | 6.72 (3.41)<br>2.20–13.60  | 1.86 (0.27)<br>1.42–2.54   | $F_{1,63} = 60.65$ ,<br>$P < 0.001$  |
| Comparison within populations | $P = 0.8652$               | $P < 0.001$                |                                      |



**Fig. 2.** Values of the response variables: feather corticosterone CORT<sub>f</sub> (pg/mm feather) and relative telomere length TL (T/S ratio), and the explanatory variables: Human Footprint Index (HFI) and conspecific density (mean number of individuals/km<sup>2</sup>) at KDE50 and KDE95 spatial scales in male (blue) and female (red) Eurasian griffon vultures from the North and South study populations (respectively: 19 males, 16 females; 19 males, 11 females). The lines within the boxes indicate the median, the edges of the boxes the first (Q1) and third (Q3) quartiles, and the whiskers extend to 1.5-times the interquartile range.

finding is inversely related to the anthropization levels between the two study populations we focused on. Indeed, game hunting mostly occurs in the south, where large undisturbed areas maintain large populations of free-ranging ungulates.

Interestingly, we found sex-associated asymmetries in levels of CORT<sub>f</sub>, with females being apparently more responsive than males. It is important to note that the GC-mediated response is a plastic trait, also dependent on several individual features such as sex and reproductive status (e.g., Martínez-Mota et al., 2007). In addition, individual behavioral skills may further modulate the magnitude of the physiological response to environmental stressors (Dantzer et al., 2014). For instance, more experienced, often older individuals may be more efficient while foraging and breeding. In this regard, Angelier et al. (2007)

found that first-time breeding black-browed albatrosses (*Thalassarche melanophris*) had higher CORT levels than experienced ones and that baseline CORT levels were positively correlated to time spent fasting/brooding on the nest. Moreover, male vultures may have different stress coping strategies than females. Sex-related differences in GCs or their metabolite levels in response to anthropogenic disturbance have been widely reported in birds and other taxa (e.g., Ahlering et al., 2013; Hayward et al., 2011; Strasser and Heath, 2013), yet the more responsive sex appears to be species-specific. Although in our study species both sexes use similar food resources (herbivore carcasses and garbage dumps), patterns of resource use may differ slightly between males and females (authors' unpublished data). For example, home range sizes of females surpass that of males (Table 1), which could be associated

**Table 3**

Competing models assessing variation in the response variables feather corticosterone  $CORT_f$  (pg/mm feather) and relative telomere length TL (T/S ratio) in adult GPS-tagged Eurasian griffon vultures. For each response variable we show alternative models with different combinations of explanatory variables: sex, location and conspecific density (Consp). We also considered the null model (only intercept) and interactions between these variables (denoted as “:”). For each model, the degrees of freedom (df), log-likelihood (logLik) and Akaike Information Criterion corrected for small sample sizes (AICc) are shown. Delta AICc ( $\Delta AICc$ ) was calculated as the difference in AICc between each model and the best model in the set. We also computed the Akaike weights ( $\omega$  AICc) to assess the weight of evidence in favor of each candidate model. Higher ranked, equivalent models ( $\Delta AICc < 2$ ), are bolded.

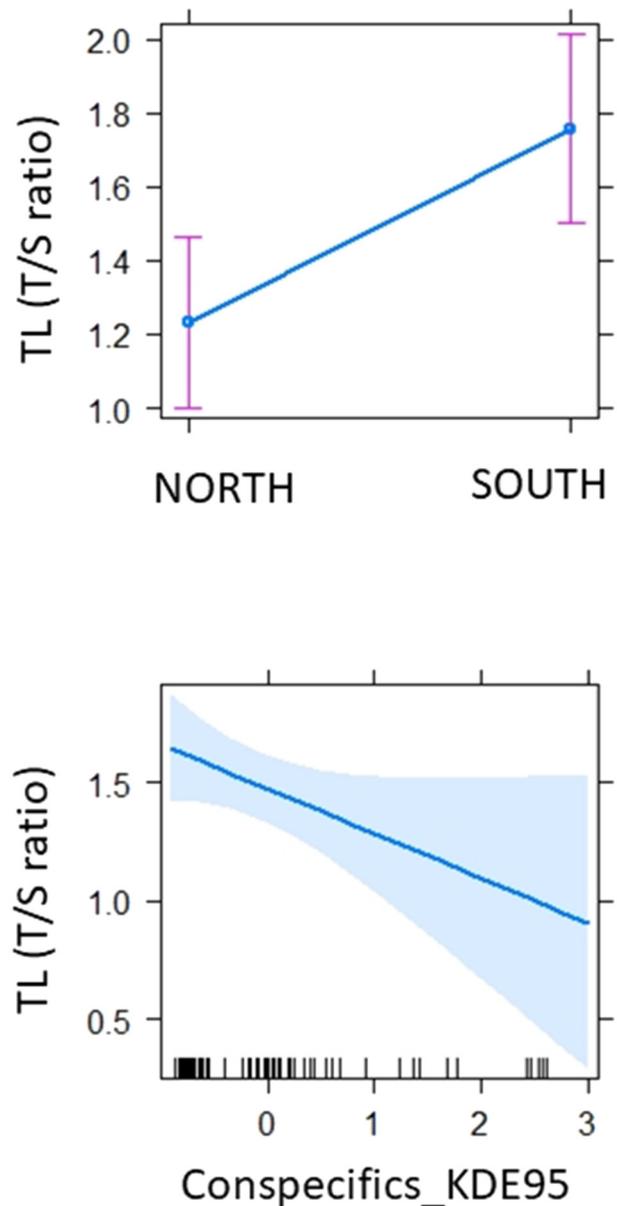
|   | df       | logLik         | AICc         | $\Delta AICc$ | $\omega$ AICc |
|---|----------|----------------|--------------|---------------|---------------|
| <b><math>CORT_f</math></b>                                      |          |                |              |               |               |
| <b>Location + sex + sex: Location</b>                           | <b>5</b> | <b>-345</b>    | <b>701.1</b> | <b>0</b>      | <b>0.40</b>   |
| <b>Location + sex</b>   | <b>4</b> | <b>-347</b>    | <b>701.8</b> | <b>0.73</b>   | <b>0.28</b>   |
| Location  | 3        | -348           | 703.2        | 2.13          | 0.14          |
| Sex   | 3        | -349           | 703.5        | 2.44          | 0.12          |
| Null  | 2        | -350           | 704.9        | 3.81          | 0.06          |
| <b>TL</b>   |          |                |              |               |               |
| <b>Consp_KDE95 + Location</b>                                   | <b>4</b> | <b>-53.633</b> | <b>115.9</b> | <b>0</b>      | <b>0.39</b>   |
| <b>Location</b>   | <b>3</b> | <b>-55.615</b> | <b>117.6</b> | <b>1.69</b>   | <b>0.17</b>   |
| <b>Consp_KDE95 + Location + sex</b>                             | <b>5</b> | <b>-53.406</b> | <b>117.8</b> | <b>1.9</b>    | <b>0.15</b>   |
| Location + sex  | 4        | -55.256        | 119.2        | 3.25          | 0.08          |
| Consp_KDE95 + Location + sex + Location: sex                    | 6        | -53.096        | 119.6        | 3.71          | 0.06          |
| Consp_KDE95 + Location + sex + sex: Consp_KDE95                 | 6        | -53.391        | 120.2        | 4.3           | 0.05          |
| Consp_KDE95   | 3        | -56.937        | 120.3        | 4.34          | 0.04          |
| Consp_KDE95 + Location + sex + sex: Consp_KDE95 + Location: sex | 7        | -52.551        | 121.1        | 5.14          | 0.03          |
| Location + sex + Location: sex                                  | 5        | -55.093        | 121.2        | 5.27          | 0.03          |
| Consp_KDE95 + sex   | 4        | -56.894        | 122.5        | 6.52          | 0.02          |
| Consp_KDE95 + sex + sex: Consp_KDE95                            | 5        | -56.885        | 124.8        | 8.85          | 0.01          |
| Null  | 2        | -68.066        | 140.3        | 24.39         | 0             |
| Sex   | 3        | -68.027        | 142.4        | 26.52         | 0             |

with their observed higher  $CORT_f$  levels. GCs have metabolic functions regulating the uptake, storage, and release of energy, and can also have a stimulatory effect on locomotor activity (Landys et al., 2006), so a direct relationship between  $CORT_f$  and movement/foraging activity could be expected. However,  $CORT_f$  levels were unrelated to the home

**Table 4**

Determinants of feather corticosterone  $CORT_f$  (pg/mm feather) levels and relative telomere length TL (expressed as T/S ratio) in GPS-tagged Eurasian griffon vultures: sex, conspecifics density (Consp; mean number of individuals/km<sup>2</sup>) and location (North/South). Interactions between explanatory variables are denoted as “:”. Estimates, standard errors (SE), and 95% confidence intervals (CI; lower 2.5% and upper 97.5% limits) of the variables retained after model averaging (Table 1) are shown. The application of 90% CI identified the same informative parameters. Variables receiving the strongest support (i.e., higher estimates whose CI do not overlap zero) are bolded. RI and CM show the relative importance and the number of models containing each variable, respectively. The coefficient of determination  $R^2$  and the AICc of each model are shown.

| Model                       | Estimate      | SE           | CI 2.5%        | CI 97.5%      | RI          | CM       |
|-----------------------------|---------------|--------------|----------------|---------------|-------------|----------|
| <b><math>CORT_f</math></b>  |               |              |                |               |             |          |
| <b>(Intercept)</b>          | <b>245.44</b> | <b>20.31</b> | <b>205.63</b>  | <b>285.26</b> |             |          |
| <b>Sex[male]</b>            | <b>-70.51</b> | <b>27.17</b> | <b>-123.77</b> | <b>-17.25</b> | <b>1.00</b> | <b>2</b> |
| <b>Location[south]</b>      | <b>-82.06</b> | <b>31.23</b> | <b>-143.27</b> | <b>-20.84</b> | <b>1.00</b> | <b>2</b> |
| Sex[Male]:Location[South]   | 71.51         | 41.4         | -9.63          | 152.64        | 0.59        | 1        |
| $R^2 = 0.16$                |               |              |                |               |             |          |
| AICc = 701.08               |               |              |                |               |             |          |
| <b>Telomere length (TL)</b> |               |              |                |               |             |          |
| <b>(Intercept)</b>          | <b>1.267</b>  | <b>0.14</b>  | <b>1.014</b>   | <b>1.465</b>  |             |          |
| <b>Location[south]</b>      | <b>0.525</b>  | <b>0.20</b>  | <b>0.121</b>   | <b>0.894</b>  | <b>1</b>    | <b>3</b> |
| <b>Consp_KDE95</b>          | <b>-0.189</b> | <b>0.10</b>  | <b>-0.390</b>  | <b>-0.001</b> | <b>0.76</b> | <b>2</b> |
| Sex (male)                  | -0.094        | 0.14         | -0.325         | 0.589         | 0.21        | 1        |
| $R^2 = 0.38$                |               |              |                |               |             |          |
| AICc = 116.81               |               |              |                |               |             |          |



**Fig. 3.** Model results showing that relative telomere length TL (T/S ratio) was shorter in adult Eurasian griffon vultures from the northern population and was also negatively associated with increasing values of conspecific density at large spatial scale (whole home range-kernel 95%, KDE95). Dashed area encloses the 95% CI.

range size of vultures, which gives little support to this possibility. On the other hand, males spend more time feeding at garbage dumps and are bolder than females when facing risky situations (e.g., they are more likely to be captured in enclosures, authors' unpublished data). Therefore, we cannot rule out a differential response of sexes to environmental risks at small spatial scales, as occurs with other top avian scavengers (e.g., Andean condors *Vultur gryphus*, Gangoso et al., 2016).

Telomere length was significantly shorter in the northern population. This trend could reflect chronological age, with vultures living in the north being older than those from the south. All birds included in our study were adults (see Material and Methods section for details) but griffon vultures have a lifespan of up to 40 years, so the exact age of each sampled individual was unknown. However, we know that birds from the northern population, which forage in highly anthropized areas, have significantly lower survival rates (Arrondo et al., 2020a). This suggests that a higher turnover of individuals may exist in this northern region, with juveniles soon recruiting to replace dead adults,

as occurs in other populations of long-lived species (Fay et al., 2015). Consequently, longer telomeres would be expected in the northern population, but we found the opposite. A possible explanation is that the age of recruitment of griffon vultures, particularly in a dense population with large numbers of floaters, could be delayed to offset potential differences between individuals and populations. It is important to note that in long-lived birds, rates of telomere attrition are faster in early life and a large telomere loss with chronological age seems not to be the rule (see Angelier et al., 2018 and references therein). Several longitudinal studies conducted with long-lived birds that involved individuals of known age found that, although adults have generally shorter telomeres than nestlings, telomere length is unrelated to chronological age (e.g., Mizutani et al., 2013; Pauliny et al., 2012; Rattiste et al., 2015). Furthermore, telomere length may vary widely among individuals of the same age, suggesting that individuals differ in their postnatal exposure or response to telomere-shortening stress factors such as harsh environmental conditions or demanding biological processes (Chatelain et al., 2020).

Alternatively, observed differences in adult telomere length could have arisen at earlier life stages, either associated with environmental conditions during development (e.g., low quality food, see above) or with parental telomere length. Separating environmental from genetic effects on a dynamic trait such as telomere length is difficult, but recent evidence shows high heritability estimates of this trait in wild birds (Vedder et al., 2021). Therefore, we cannot rule out that the two populations are genetically structured, with vultures from the northern population having consistently short telomeres, which are further passed to their offspring. Although empirical data are scarce, observations from ringed individuals indicate that natal dispersal distances of griffon vultures are very low (less than 50 km in most cases,  $N = 150$  recruits, G. Blanco pers. comm.), which suggests that genetic flow (including telomere length) between these populations may also be low, despite the high mobility that characterizes this species. More research would be needed to unravel whether the patterns found are caused by habitat anthropization and associated population effects. In fact, although additive genetic variance has been found to account for a substantial part of the phenotypic variation in telomere length, this is not against the growing evidence for the role of stressful conditions in telomere length and shortening rate (Chatelain et al., 2020; Vedder et al., 2021).

According to our results on  $CORT_f$ , together with the association between GCs-modulated oxidative stress and telomere dynamics (Costantini et al., 2011; Haussmann and Marchetto, 2010), we would expect shorter telomeres in females under the same degree of anthropization. However, we found that telomere length did not differ between sexes in both study populations. These findings suggest that males, despite being less responsive to stress than females in terms of GCs levels, respond differently to anthropization, suffering higher levels of oxidative stress, ultimately translated into accelerated telomere shortening. It has been proposed that telomeres are either directly or indirectly related to individual variation in life-history strategies and behavior, a relationship that may occur in a state-dependent manner (Bateson and Nettle, 2018; Giraudeau et al., 2019; Vernasco et al., 2020; Young, 2018). Interestingly, it has been shown that individuals with shorter telomeres display bolder and more aggressive personalities (e.g., captive brown trouts *Salmo trutta*, Adriaenssens et al., 2016) and that telomere attrition during nestling development is associated with shorter telomeres and boldness during adulthood (e.g., wild European starlings *Sturnus vulgaris*, Bateson et al., 2015; Bateson and Nettle, 2018), likely causing early death (Wilbourn et al., 2017). The study by Arrondo et al. (2020a) found higher mortality rates of vultures in the northern population as compared to the south (41.7% vs. 10.0%), with non-natural causes of mortality (run over by vehicles, poisoning, and accidents with infrastructure) being predominant. Living in a more anthropized area clearly increases risk exposure and levels of chronic stress for vultures. Birds in poorer overall condition (i.e. shorter telomeres) may perform worse when dealing with these challenges,

being in addition more prone to death. It has been suggested that telomeres, by combining the information not only of internal physiological processes, but also of environmental cues, may modulate pace-of-life strategies in response to environmental conditions (Giraudeau et al., 2019). Contrasting environmental conditions in both study populations could then favor different life-history strategies involving reproductive, behavioral and physiological traits (Réale et al., 2010). Following this argument, the environmental pressures characterizing the northern population (i.e., poorer nutritional conditions and higher mortality risk) would favor a faster pace-of-life strategy. Although current data prevent us from testing this possibility, we hypothesize that male griffon vultures that breed and forage in more anthropized and stressful areas are those with shorter telomeres that may in turn show bolder behaviors in a risky human-dominated landscape and, consequently, have lower survival rates.

Finally, we found that the presence of conspecifics was significantly and negatively associated with telomere length. This effect was apparent at all spatial scales, but became more evident when considering the entire home range areas. The negative effect of conspecific density could again indicate chronic stress caused by higher intraspecific competition for food and nesting sites, a common phenomenon in social species such as vultures (Fernández-Bellón et al., 2016). Hierarchy could also play a key role in our focal birds, as occurs in mammal carnivores. Hence, according to Lewin et al. (2015) the status-specific differences in telomere length of spotted hyenas (*Crocuta crocuta*) result from subordinate exclusion from high quality food resources and aggressive interactions, regardless of chronological age. Griffon vultures are highly social birds (see reviews in Cortés-Avizanda et al., 2014; van Overveld et al., 2020) and often engage in aggressive disputes when foraging, especially when food resources become available in a patchy and predictable way. This pattern may be further mediated by a clumped distribution of carrion, gathering more individuals at certain predictable places (Cortés-Avizanda et al., 2010, 2012). Individuals living and foraging in the northern study area must compete for lower quality resources with a greater number of conspecifics in a more ecologically challenging environment, which may explain the negative effect of conspecific density and the overall shorter telomere length found in this population.

It would be expected that increased competition for resources would increase stress levels and hence, levels of circulating GCs (Creel et al., 2013; Goymann and Wingfield, 2004). However, we did not find such an effect on  $CORT_f$ . The fact that vultures do not show a GC-based response to the density of conspecifics does not imply it has no cost. Vultures chronically stressed by the cascading effects of social factors may also be in poorer body condition (e.g., Mora et al., 1996), likely having impaired immune responses (e.g., Hawley et al., 2006), further associated with feeding at landfills and intensive farms (e.g., Blanco et al., 2019; Plaza et al., 2019), ultimately affecting oxidative damage and telomere length (reviewed in Chatelain et al., 2020). Moreover, although circulating GCs may be closely and functionally linked with telomere dynamics, the connection between oxidative stress and cellular aging is not necessarily mediated by these hormones, and the relationships between GCs and telomere length may differ between species, life history stages, and environmental conditions (reviewed in Angelier et al., 2018). Mounting numerous and repeated GC stress responses is costly and reduced sensitivity to particular stressors may be adaptive (Angelier et al., 2018; Boonstra, 2013). Therefore, our findings could suggest that, as occurs with some prey species that do not suffer chronic stress from predation risk (Boonstra, 2013; Zbyryt et al., 2018), griffon vultures may be able to cope well in terms of GCs with social stressors that they have probably dealt with throughout their evolutionary history.

## 5. Conclusions and perspectives

Telomere length and dynamics are increasingly recognized as integrative biological markers of overall individual condition and quality

that may be further involved in the adjustment of the pace-of-life specific to environmental conditions (Angelier et al., 2019; Giraudeau et al., 2019). Glucocorticoids, on their part, are also known to mediate, to a large extent, the links between environmental conditions and telomere dynamics (Angelier et al., 2018). This is especially relevant under current and future levels of habitat anthropization, which is one of the most important environmental challenges that organisms have to face. Therefore, understanding the effects of landscape anthropization on individual physiology and population performance is of major importance. Our study, focused on highly social griffon vultures, highlights the existence of effects of chronic stress associated with living in anthropized environments on overall individual condition. In addition, population processes such as intraspecific competition for resources and sex-specific responses to stress might also underlie the observed differential survival rates between individuals of the two study populations facing different levels of anthropization. These findings give rise to stimulating questions for future evolutionary and ecological research studies, such as the role of telomere length in individual and likely sex-specific personalities and responses to environmental risk (e.g., perception of risk and probability of accidents with human infrastructure when foraging in anthropized landscapes) and, especially, its impacts on population dynamics. Telomere length and dynamics are highly heritable (Vedder et al., 2021), and it has been suggested that the potential signaling function of telomere length as mediators of environmental cues may even reach over generations, advertising on the environmental characteristics during development, and hence boosting the potential for phenotypic plasticity (Giraudeau et al., 2019). The extent to which the relative importance of these processes may vary between short- and long-lived species remains an open question. Undoubtedly, all these interrelated topics represent a fascinating avenue of research that will help understand the response of organisms to environmental changes.

### Ethical statement

Capture banding and tagging of griffon vultures were conducted under permits of the respective autonomous communities (Andalucía and Navarra) and following the ethical protocols approved by the competent authorities (AC: 30/01/2013/94).

### CRediT authorship contribution statement

**L. Gangoso:** Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **A. Cortés-Avizanda:** Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **A. Sergiel:** Writing – review & editing. **B. Pudifoot:** Investigation, Methodology, Writing – review & editing. **F. Miranda:** Investigation, Writing – review & editing. **J. Muñoz:** Investigation, Methodology, Writing – review & editing. **A. Delgado-González:** Methodology, Writing – review & editing. **M. Moleón:** Writing – review & editing. **J.A. Sánchez-Zapata:** Supervision, Funding acquisition, Project administration, Writing – review & editing. **E. Arrondo:** Methodology, Writing – review & editing. **J.A. Donázar:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

### Declaration of competing interest

The authors declare no competing interests.

### Acknowledgements

We thank Manuel de la Riva and the staff of the Programa Conservación de Aves Necrófagas de Andalucía and the Bardenas Reales Natural Park for their help during the field work. We also thank the staff of the LEM and LEFS Labs at the EBD-CSIC for their help during the

sample analyses. This research was funded by the Projects RNM-1925 and P18-RT-1321 (Junta de Andalucía), the Comunidad de Bardenas Reales de Navarra, the Projects CGL2012-32544, CGL2015-66966-C2-1-2-R and CGL2015-66966-C2-1-R2 (Spanish Ministry of Economy and Competitiveness and EU/FEDER), and i-link 0564 (CSIC). ACA was supported by a Post-Doc contract Programa Viçent Mut of Govern Balear, Spain (PD/039/2017) and MM by the Ramón y Cajal Program (RYC-2015-19231).

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.146920>.

### References

- Adriaenssens, B., Pauliny, A., Blomqvist, D., Johnsson, J.I., 2016. Telomere length covaries with personality in wild brown trout. *Physiol. Behav.* 165, 217–222. <https://doi.org/10.1016/j.physbeh.2016.07.005>.
- Ahlering, M.A., Maldonado, J.E., Eggert, L.S., Fleischer, R.C., Western, D., Brown, J.L., 2013. Conservation outside protected areas and the effect of human-dominated landscapes on stress hormones in savannah elephants. *Conserv. Biol.* 27, 569–575. <https://doi.org/10.1111/cobi.12061>.
- Angelier, F., Weimerskirch, H., Dano, S., Chastel, O., 2007. Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. *Behav. Ecol. Sociobiol.* 61 (4), 611–621. <https://doi.org/10.1007/s00265-006-0290-1>.
- Angelier, F., Wingfield, J.C., Weimerskirch, H., Chastel, O., 2010. Hormonal correlates of individual quality in a long-lived bird: a test of the “corticosterone-fitness hypothesis”. *Biol. Lett.* 6, 846–849. <https://doi.org/10.1098/rsbl.2010.0376>.
- Angelier, F., Costantini, D., Blévin, P., Chastel, O., 2018. Do glucocorticoids mediate the link between environmental conditions and telomere dynamics in wild vertebrates? A review. *Gen. Comp. Endocrinol.* 256, 99–111. <https://doi.org/10.1016/j.ygcen.2017.07.007>.
- Angelier, F., Weimerskirch, H., Barbraud, C., Chastel, O., 2019. Is telomere length a molecular marker of individual quality? Insights from a long-lived bird. *Funct. Ecol.* 33 (6), 1076–1087. <https://doi.org/10.1111/1365-2435.13307>.
- Arrondo, E., Sanz-Aguilar, A., Pérez-García, J.M., Cortés-Avizanda, A., Sánchez-Zapata, J.A., Donázar, J.A., 2020a. Landscape anthropization shapes the survival of a top avian scavenger. *Biodivers. Conserv.* 29, 1411–1425. <https://doi.org/10.1007/s10531-020-01942-6>.
- Arrondo, E., Navarro, J., Perez-García, J.M., Mateo, R., Camarero, P.R., Martín-Doimeadios, R.C.R., Jiménez-Moreno, M., Cortés-Avizanda, A., Navas, I., García-Fernández, A.J., Sánchez-Zapata, J.A., Donázar, J.A., 2020b. Dust and bullets: stable isotopes and GPS tracking disentangle lead sources for a large avian scavenger. *Environ. Pollut.* 266, 115022. <https://doi.org/10.1016/j.envpol.2020.115022>.
- Barton, K., 2019. MuMIn: Multi-Model Inference, Version 1.43.6. 1–75.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67 (1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Bateson, M., Nettle, D., 2018. Why are there associations between telomere length and behaviour? *Philos. Trans. R. Soc. B* 373 (1741), 20160438. <https://doi.org/10.1098/rstb.2016.0438>.
- Bateson, M., Brirot, B.O., Gillespie, R., Monaghan, P., Nettle, D., 2015. Developmental telomere attrition predicts impulsive decision-making in adult starlings. *Proc. R. Soc. B* 282, 20142140. <https://doi.org/10.1098/rspb.2014.2140>.
- Beaugueard, E., Brisichoux, F., Henry, P.Y., Parenteau, C., Trouvé, C., Angelier, F., 2019. Does urbanization cause stress in wild birds during development? Insights from feather corticosterone levels in juvenile house sparrows *Passer domesticus*. *Ecol. Evol.* 9, 640–652. <https://doi.org/10.1002/ece3.4788>.
- Beloor, J., Kang, H.K., Kim, Y.J., Subramani, V.K., Jang, I.S., Sohn, S.H., Moon, Y.S., 2010. The effect of stocking density on stress related genes and telomeric length in broiler chickens. *Asian Australas. J. Anim. Sci.* 23 (4), 437–443. <https://doi.org/10.5713/ajas.2010.90400>.
- Biard, C., Brisichoux, F., Meillère, A., Michaud, B., Nivière, M., Ruault, S., Vaugouyeau, M., Angelier, F., 2017. Growing in cities: an urban penalty for wild birds? A study of phenotypic differences between urban and rural great tit chicks *Parus major*. *Front. Ecol. Evol.* 5, 79–92. <https://doi.org/10.3389/fevo.2017.00079>.
- Blanco, G., Cortés-Avizanda, A., Frías, Ó., Arrondo, E., Donázar, J.A., 2019. Livestock farming practices modulate vulture diet-disease interactions. *Glob. Ecol. Conserv.* 17, e00518. <https://doi.org/10.1016/j.gecco.2018.e00518>.
- Blévin, P., Angelier, F., Tartu, S., Ruault, S., Bustamante, P., Herzke, D., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2016. Exposure to oxychlordan is associated with shorter telomeres in arctic breeding kittiwakes. *Sci. Total Environ.* 563, 125–130. <https://doi.org/10.1016/j.scitotenv.2016.04.096>.
- Bonier, F., 2012. Hormones in the city: endocrine ecology of urban birds. *Horm. Behav.* 61 (5), 763–772. <https://doi.org/10.1016/j.yhbeh.2012.03.016>.
- Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009. Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* 24, 634–642. <https://doi.org/10.1016/j.tree.2009.04.013>.
- Boonstra, R., 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Funct. Ecol.* 27, 11–23. <https://doi.org/10.1111/1365-2435.12008>.

- Bortolotti, M.T.A., Blas, J., German, T., 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Funct. Ecol.* 22, 494–500. <https://doi.org/10.1111/j.1365-2435.2008.01387.x>.
- Burnham, K.P., Anderson, D.R., 2002. A Practical Information-theoretic Approach. Model Selection and Multimodel Inference. 2nd ed. Springer, New York <https://doi.org/10.1007/b97636>.
- Buxton, V.L., Santymire, R.M., Benson, T.J., 2018. Mixed effects of urbanization on density, nest survival, and nestling corticosterone of a generalist passerine. *Ecosphere* 9 (12), e02517. <https://doi.org/10.1002/ecs2.2517>.
- Calenge, C., Fortmann-Roe, S., 2013. adehabitatHR: Home Range Estimation. R package version 0.4, 7.
- Carrete, M., Bortolotti, G.R., Sánchez-Zapata, J.A., Delgado, A., Cortés-Avizanda, A., Grande, J.M., Donazar, J.A., 2013. Stressful conditions experienced by endangered Egyptian vultures on African wintering areas. *Anim. Conserv.* 16 (3), 353–358. <https://doi.org/10.1111/acv.12001>.
- Cawthon, R.M., 2002. Telomere measurement by quantitative PCR. *Nucleic Acids Res.* 30 (10), e47. <https://doi.org/10.1093/nar/30.10.e47>.
- Chace, J.F., Walsh, J.J., 2006. Urban effects on native avifauna: a review. *Landsc. Urban Plan.* 74 (1), 46–69. <https://doi.org/10.1016/j.landurbplan.2004.08.007>.
- Chatelain, M., Drobniak, S.M., Szulkin, M., 2020. The association between stressors and telomeres in non-human vertebrates: a meta-analysis. *Ecol. Lett.* 23, 381–398. <https://doi.org/10.1111/ele.13426>.
- Cortés-Avizanda, A., Carrete, M., Donazar, J.A., 2010. Managing supplementary feeding for avian scavengers: guidelines for optimal design using ecological criteria. *Biol. Conserv.* 143, 1707–1715. <https://doi.org/10.1016/j.biocon.2010.04.016>.
- Cortés-Avizanda, A., Jovani, R., Carrete, M., Donazar, J.A., 2012. Resource unpredictability promotes species diversity and coexistence in an avian scavenger guild: a field experiment. *Ecology* 93, 2570–2579. <https://doi.org/10.1890/12-0221.1>.
- Cortés-Avizanda, A., Jovani, R., Donazar, J.A., Grimm, V., 2014. Bird sky networks: how do avian scavengers use social information to find carrion? *Ecology* 95, 1799–1808. <https://doi.org/10.1890/12-0221.1>.
- Costantini, D., Marasco, V., Møller, A.P., 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J. Comp. Physiol. B.* 181 (4), 447–456. <https://doi.org/10.1007/s00360-011-0566-2>.
- Cram, D.L., Monaghan, P., Gillespie, R., Clutton-Brock, T., 2017. Effects of early-life competition and maternal nutrition on telomere lengths in wild meerkats. *Proc. Royal Soc. B* 284 (1861), 20171383. <https://doi.org/10.1098/rspb.2017.1383>.
- Creel, S., Dantzer, B., Goymann, W., Rubenstein, D.R., 2013. The ecology of stress: effects of the social environment. *Funct. Ecol.* 27 (1), 66–80. <https://doi.org/10.1111/j.1365-2435.2012.02029.x>.
- Crespi, E.J., Williams, T.D., Jessop, T.S., 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. <https://doi.org/10.1111/1365-2435.12009>.
- Crino, O.L., Johnson, E.E., Bickley, J.L., Patricelli, G.L., Breuner, C.W., 2013. Effects of experimentally elevated traffic noise on nestling white-crowned sparrow stress physiology, immune function and life history. *J. Exp. Biol.* 216, 2055–2062. <https://doi.org/10.1242/jeb.081109>.
- Crisuolo, F., Bize, P., Nasir, L., Metcalfe, N.B., Foote, C.G., Griffiths, K., Gault, E.A., Monaghan, P., 2009. Real-time quantitative PCR assay for measurement of avian telomeres. *J. Avian Biol.* 40, 342–347. <https://doi.org/10.1111/j.1600-048X.2008.04623.x>.
- Dantzer, B., Newman, A.E., Boonstra, R., Palme, R., Boutin, S., Humphries, M.M., McAdam, A.G., 2013. Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. *Science* 340, 1215–1217. <https://doi.org/10.1126/science.1235765>.
- Dantzer, B., Fletcher, Q.E., Boonstra, R., Sheriff, M.J., 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conserv. Physiol.* 2, cou023. <https://doi.org/10.1093/conphys/cou023>.
- Del Moral, J.C., 2009. El buitre leonado en España. Población reproductora en 2008 y métodos de censo. Spain, SEO/BirdLife, Madrid.
- Del Moral, J.C., Molina, B., 2018. El buitre leonado en España. Población reproductora en 2018 y método de censo. Spain, SEO/BirdLife, Madrid.
- Duriez, O., Kato, A., Tromp, C., Dell’Omo, G., Vyssotski, A.L., Sarrazin, F., Ropert-Coudert, Y., 2014. How cheap is soaring flight in raptors? A preliminary investigation in freely-flying vultures. *PLoS One* 9 (1), e84887. <https://doi.org/10.1371/journal.pone.0084887>.
- Epel, E.S., Blackburn, E.H., Lin, J., Dhabhar, F.S., Adler, N.E., Morrow, J.D., Cawthon, R.M., 2004. Accelerated telomere shortening in response to life stress. *PNAS* 101, 17312–17315. <https://doi.org/10.1073/pnas.0407162101>.
- Fay, R., Weimerskirch, H., Delord, K., Barbraud, C., 2015. Population density and climate shape early-life survival and recruitment in a long-lived pelagic seabird. *J. Anim. Ecol.* 84, 1423–1433. <https://doi.org/10.1111/1365-2656.12390>.
- Fernández-Bellón, D., Cortés-Avizanda, A., Arenas, R., Donazar, J.A., 2016. Density-dependent productivity in a colonial vulture at two spatial scales. *Ecology* 97, 406–416. <https://doi.org/10.1890/15-0357.1>.
- Gangoso, L., Agudo, R., Anadón, J.D., de la Riva, M., Suleyman, A.S., Porter, R., Donazar, J.A., 2013. Reinventing mutualism between humans and wild fauna: insights from vultures as ecosystem services providers. *Conserv. Lett.* 63, 172–179. <https://doi.org/10.1111/j.1755-263X.2012.00289.x>.
- Gangoso, L., Lambertucci, S.A., Cabezas, S., Alarcón, P.A., Wiemeyer, G.M., Sanchez-Zapata, J.A., Blanco, G., Hiraldo, F., Donazar, J.A., 2016. Sex-dependent spatial structure of telomere length in a wild long-lived scavenger. *Ecosphere* 7, e01544. <https://doi.org/10.1002/ecs2.1544>.
- Giraudeau, M., Angelier, F., Sepp, T., 2019. Do telomeres influence pace-of-life-strategies in response to environmental conditions over a lifetime and between generations? *BioEssays* 41 (3), 1800162. <https://doi.org/10.1002/bies.201800162>.
- Goymann, W., Wingfield, J.C., 2004. Allostatic load, social status and stress hormones: the costs of social status matter. *Anim. Behav.* 67 (3), 591–602. <https://doi.org/10.1016/j.anbehav.2003.08.007>.
- Graham, M.H., 2003. Confronting multicollinearity in ecological multiple regression. *Ecology* 84 (11), 2809–2815. <https://doi.org/10.1890/02-3114>.
- Greider, C.W., Blackburn, E., 1985. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* 43, 405–413. [https://doi.org/10.1016/0092-8674\(85\)90170-9](https://doi.org/10.1016/0092-8674(85)90170-9).
- Hasselquist, D., Nilsson, J.A., 2012. Physiological mechanisms mediating costs of immune responses: what can we learn from studies of birds? *Anim. Behav.* 83 (6), 1303–1312. <https://doi.org/10.1016/j.anbehav.2012.03.025>.
- Hausmann, M.F., Marchetto, N.M., 2010. Telomeres: linking stress and survival, ecology and evolution. *Curr. Zool.* 56 (6), 714–727. <https://doi.org/10.1093/czoolo/56.6.714>.
- Hawley, D.M., Lindström, K., Wikelski, M., 2006. Experimentally increased social competition compromises humoral immune responses in house finches. *Horm. Behav.* 49 (4), 417–424. <https://doi.org/10.1016/j.yhbeh.2005.09.003>.
- Hayward, L.S., Bowles, A.E., Ha, J.C., Wasser, S.K., 2011. Impacts of acute and long-term vehicle exposure on physiology and reproductive success of the northern spotted owl. *Ecosphere* 2 (6), 1–20. <https://doi.org/10.1890/ES10-00199.1>.
- Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., Vandesoepel, J., 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol.* 8, r19. <https://doi.org/10.1186/gb-2007-8-2-r19>.
- Horn, T., Robertson, B.C., Gemmill, N.J., 2010. The use of telomere length in ecology and evolutionary biology. *Heredity* 105, 497–506. <https://doi.org/10.1038/hdy.2010.113>.
- Ibáñez-Álamo, J.D., Pineda-Pampliega, J., Thomson, R.L., Aguirre, J.L., Díez-Fernández, A., Faivre, B., Figuerola, J., Verhulst, S., 2018. Urban blackbirds have shorter telomeres. *Biol. Lett.* 14 (3), 20180083. <https://doi.org/10.1098/rsbl.2018.0083>.
- Ibáñez-Álamo, J.D., Jimeno, B., Gil, D., Thomson, R.L., Aguirre, J.L., Díez-Fernández, A., Faivre, B., Tieleman, B.I., Figuerola, J., 2020. Physiological stress does not increase with urbanization in European blackbirds: evidence from hormonal, immunological and cellular indicators. *Sci. Total Env.* 721, 137332. <https://doi.org/10.1016/j.scitotenv.2020.137332>.
- Iglesias-Carrasco, M., Aich, U., Jennions, M.D., Head, M.L., 2020. Stress in the city: meta-analysis indicates no overall evidence for stress in urban vertebrates. *Proc. R. Soc. B* 287 (1936), 20201754. <https://doi.org/10.1098/rspb.2020.1754>.
- Kitaysky, A.S., Kitaiskaia, E.V., Piatt, J.F., Wingfield, J.C., 2006. A mechanistic link between chick diet and decline in seabirds? *Proc. R. Soc. B* 273 (1585), 445–450. <https://doi.org/10.1098/rspb.2005.3351>.
- Kitaysky, A.S., Piatt, J.F., Hatch, S.A., Kitaiskaia, E.V., Benowitz-Fredericks, Z.M., Shultz, M.T., Wingfield, J.C., 2010. Food availability and population processes: severity of nutritional stress during reproduction predicts survival of long-lived seabirds. *Funct. Ecol.* 24, 625–637. <https://doi.org/10.1111/j.1365-2435.2009.01679.x>.
- Kotrschal, A., Ilmonen, P., Penn, D.J., 2007. Stress impacts telomere dynamics. *Biol. Lett.* 3, 128–130. <https://doi.org/10.1098/rsbl.2006.0594>.
- Kouwenberg, A.L., McKay, D.W., Fitzsimmons, M.G., Storey, A.E., 2015. Measuring corticosterone in feathers using an acetonitrile/hexane extraction and enzyme immunoassay: feather corticosterone levels of food-supplemented Atlantic Puffin chicks. *J. Field Ornithol.* 86, 73–83. <https://doi.org/10.1111/jof.12090>.
- Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Endocrinol.* 1482, 132–149. <https://doi.org/10.1016/j.ygcen.2006.02.013>.
- Landys, M.M., Goymann, W., Schwabl, I., Trapschuh, M., Slagsvold, T., 2010. Impact of season and social challenge on testosterone and corticosterone levels in a year-round territorial bird. *Horm. Behav.* 58 (2), 317–325. <https://doi.org/10.1016/j.yhbeh.2010.02.013>.
- Lewin, N., Treidel, L.A., Holekamp, K.E., Place, N.J., Hausmann, M.F., 2015. Socioecological variables predict telomere length in wild spotted hyenas. *Biol. Lett.* 11 (2), 20140991. <https://doi.org/10.1098/rsbl.2014.0991>.
- Loss, S.R., Will, T., Marra, P., 2015. Direct mortality of birds from anthropogenic causes. *Ann. Rev. Ecol. Evol. Systemat.* 46, 99–120. <https://doi.org/10.1146/annurev-ecolsys-112414-054133>.
- Luck, G.W., 2007. A review of the relationship between human population density and biodiversity. *Biol. Rev.* 82, 607–645. <https://doi.org/10.1111/j.1469-185X.2007.00028.x>.
- Margalida, A., Pérez-García, J.M., Afonso, I., Moreno-Opo, R., 2016. Spatial and temporal movements in Pyrenean bearded vultures *Gypaetus barbatus*: integrating movement ecology into conservation practice. *Sci. Rep.* 6, 35746. <https://doi.org/10.1038/srep35746>.
- Martin-Díaz, P., Cortés-Avizanda, A., Serrano, D., Arrondo, E., Sánchez-Zapata, J.A., Donazar, J.A., 2020. Rewilding processes shape the use of Mediterranean landscapes by an avian top scavenger. *Sci. Rep.* 10, 1–12. <https://doi.org/10.1038/s41598-020-59591-2>.
- Martínez-Mota, R., Valdespino, C., Sánchez-Ramos, M.A., Serio-Silva, J.C., 2007. Effects of forest fragmentation on the physiological stress response of black howler monkeys. *Anim. Conserv.* 103, 374–379. <https://doi.org/10.1111/j.1469-1795.2007.00122.x>.
- Martín-Queller, E., Moreno-Mateos, D., Pedrocchi, C., Cervantes, J., Martínez, G., 2010. Impacts of intensive agricultural irrigation and livestock farming on a semi-arid Mediterranean catchment. *Environ. Monit. Assess.* 1671, 423–435. <https://doi.org/10.1007/s10661-009-1061-z>.
- Mazerolle, M.J., 2020. AICcmodavg: Model Selection and Multimodel Inference Based on AICc. R package version 2.3-1, <https://cran.r-project.org/package=AICcmodavg>.
- McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43, 2–15. [https://doi.org/10.1016/S0018-506X\(02\)00024-7](https://doi.org/10.1016/S0018-506X(02)00024-7).

- McKinney, M.L., 2002. Urbanization, biodiversity, and conservation: the impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *Bioscience* 52 (10), 883–890. [https://doi.org/10.1641/0006-3568\(2002\)052\[0883:UBAC\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2).
- Meillère, A., Brischoux, F., Bustamante, P., Michaud, B., Parenteau, C., Marciau, C., Angelier, F., 2016. Corticosterone levels in relation to trace element contamination along an urbanization gradient in the common blackbird *Turdus merula*. *Sci. Total Environ.* 566, 93–101. <https://doi.org/10.1016/j.scitotenv.2016.05.014>.
- Mizutani, Y., Tomita, N., Niizuma, Y., Yoda, K., 2013. Environmental perturbations influence telomere dynamics in long-lived birds in their natural habitat. *Biol. Lett.* 9, 20130511. <https://doi.org/10.1098/rsbl.2013.0511>.
- Mora, A.N.D.L., Drummond, H., Wingfield, J.C., 1996. Hormonal correlates of dominance and starvation-induced aggression in chicks of the blue-footed booby. *Ethology* 102 (5), 748–761. <https://doi.org/10.1111/j.1439-0310.1996.tb01164.x>.
- Mülner, A., Linsenmair, K.E., Wikelski, M., 2004. Exposure to ecotourism reduces survival and affects stress response in hoatzin chicks (*Opisthocomus hoazin*). *Biol. Conserv.* 118, 549–558. <https://doi.org/10.1016/j.biocon.2003.10.003>.
- Nettle, D., Monaghan, P., Gillespie, R., Brilot, B., Bedford, T., Bateson, M., 2015. An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. *Proc. R. Soc. B* 282 (1798), 20141610. <https://doi.org/10.1098/rspb.2014.1610>.
- Norberg, U.M., 1996. Energetics of flight. In: Carey, C. (Ed.), *Avian Energetics and Nutritional Ecology*. Springer, Boston, MA, pp. 199–249.
- Nussey, D.H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmill, N., Hartmann, N., Horn, T., Haussmann, M., Olsson, M., Turbill, C., 2014. Measuring telomere length and telomere dynamics in evolutionary biology and ecology. *Methods Ecol. Evol.* 5, 299–310. <https://doi.org/10.1111/2041-210X.12161>.
- Olden, J.D., Poff, N.L., Douglas, M.R., Douglas, M.E., Fausch, K.D., 2004. Ecological and evolutionary consequences of biotic homogenization. *Trends Ecol. Evol.* 19, 18–24. <https://doi.org/10.1016/j.tree.2003.09.010>.
- Pauliny, A., Larsson, K., Blomqvist, D., 2012. Telomere dynamics in a long-lived bird, the barnacle goose. *BMC Evol. Biol.* 12 (1), 1–8. <https://doi.org/10.1186/1471-2148-12-257>.
- Plaza, P.I., Blanco, G., Madariaga, M.J., Boeri, E., Teijeiro, M.L., Bianco, G., Lambertucci, S.A., 2019. Scavenger birds exploiting rubbish dumps: pathogens at the gates. *Transbound. Emerg. Dis.* 66 (2), 873–881. <https://doi.org/10.1111/tbed.13097>.
- Pokharel, S.S., Singh, B., Seshagiri, P.B., Sukumar, R., 2019. Lower levels of glucocorticoids in crop-raiders: diet quality as a potential ‘pacifier’ against stress in free-ranging Asian elephants in a human-production habitat. *Anim. Conserv.* 22 (2), 177–188. <https://doi.org/10.1111/acv.12450>.
- R Development Core Team, 2018. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rattiste, K., Klandorf, H., Urvik, J., Sepp, T., Asghar, M., Hasselquist, D., Cooley, C., Horak, P., 2015. Skin pentosidine and telomere length do not covary with age in a long-lived seabird. *BioGerontology* 16 (4), 435–441. <https://doi.org/10.1007/s10522-015-9564-1>.
- Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V., Montiglio, P.O., 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos. Trans. R. Soc. B* 365 (1560), 4051–4063. <https://doi.org/10.1098/rstb.2010.0208>.
- Rebollo-Ifrán, N., Carrete, M., Sanz-Aguilar, A., Rodríguez-Martínez, S., Cabezas, S., Marchant, T.A., Bortolotti, G.R., Tella, J.L., 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. <https://doi.org/10.1038/srep13723>.
- Rohwer, S., Ricklefs, R.E., Rohwer, V.G., Copple, M.M., 2009. Allometry of the duration of flight feather molt in birds. *PLoS Biol.* 7 (6), e1000132. <https://doi.org/10.1371/journal.pbio.1000132>.
- Romero, L.M., Fairhurst, G.D., 2016. Measuring corticosterone in feathers: strengths, limitations, and suggestions for the future. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 202, 112–122. <https://doi.org/10.1016/j.cbpa.2016.05.002>.
- Salmón, P., Nilsson, J.F., Nord, A., Bensch, S., Isaksson, C., 2016. Urban environment shortens telomere length in nestling great tits *Parus major*. *Biol. Lett.* 12, 20160155. <https://doi.org/10.1098/rsbl.2016.0155>.
- Sheriff, M.J., Krebs, C.J., Boonstra, R., 2011. From process to pattern: how fluctuating predation risk impacts the stress axis of snowshoe hares during the 10-year cycle. *Oecologia* 166, 593–605. <https://doi.org/10.1007/s00442-011-1907-2>.
- Silverin, B., 1998. Behavioural and hormonal responses of the pied flycatcher to environmental stressors. *Anim. Behav.* 55 (6), 1411–1420. <https://doi.org/10.1006/anbe.1997.0717>.
- Stauffer, J., Panda, B., Eva, T., Rainio, M., Ilmonen, P., 2017. Telomere damage and redox status alterations in free-living passerines exposed to metals. *Sci. Total Environ.* 575, 841–848. <https://doi.org/10.1016/j.scitotenv.2016.09.131>.
- Strasser, E.H., Heath, J.A., 2013. Reproductive failure of a human-tolerant species, the American kestrel, is associated with stress and human disturbance. *J. Appl. Ecol.* 50, 912–919. <https://doi.org/10.1111/1365-2664.12103>.
- van Overveld, T., García-Alfonso, M., Dingemans, N.J., Bouten, W., Gangoso, L., de la Riva, M., Serrano, D., Donazar, J.A., 2018. Food predictability and social status drive individual resource specializations in a territorial vulture. *Sci. Rep.* 8, 1–13. <https://doi.org/10.1038/s41598-018-33564-y>.
- van Overveld, T., Blanco, G., Moleón, M., Margalida, A., Sánchez-Zapata, J.A., de la Riva, M., Donazar, J.A., 2020. Integrating vulture social behavior into conservation practice. *The Condor* 122, duaa035. <https://doi.org/10.1093/condor/duaa035>.
- Vedder, O., Moiron, M., Bichet, C., Bauch, C., Verhulst, S., Becker, P.H., Bouwhuis, S., 2021. Telomere length is heritable and genetically correlated with lifespan in a wild bird. *Mol. Ecol.* <https://doi.org/10.1111/mec.15807>.
- Venter, O., Sanderson, E.W., Magrath, A., Allan, J.R., Beher, J., Jones, K.R., Possingham, H.P., Laurance, W.F., Wood, P., Fekete, B.M., Levy, M.A., 2016. Global terrestrial human footprint maps for 1993 and 2009. *Sci Data* 3, 160067. <https://doi.org/10.1038/sdata.2016.67>.
- Vernasco, B.J., Dakin, R., Majer, A.D., Haussmann, M.F., Brandt Ryder, T., Moore, I.T., 2020. Longitudinal dynamics and behavioural correlates of telomeres in male wire-tailed manakins. *Funct. Ecol.* <https://doi.org/10.1111/1365-2435.13715>.
- Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human domination of Earth’s ecosystems. *Science* 277, 494–499. <https://doi.org/10.1126/science.277.5325.494>.
- Wilbourn, R.V., Froy, H., McManus, M.C., Cheynel, L., Gaillard, J.M., Gilot-Fromont, E., Regis, C., Rey, B., Pellerin, M., Lemaître, J.F., Nussey, D.H., 2017. Age-dependent associations between telomere length and environmental conditions in roe deer. *Biol. Lett.* 13, 0434. <https://doi.org/10.1098/rsbl.2017.0434>.
- Wilbourn, R.V., Moatt, J.P., Froy, H., Walling, C.A., Nussey, D.H., Boonekamp, J.J., 2018. The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philos. Trans. R. Soc. B* 373 (1741), 20160447. <https://doi.org/10.1098/rstb.2016.0447>.
- Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am. Zool.* 38, 191–206. <https://doi.org/10.1093/icb/38.1.191>.
- Young, A.J., 2018. The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. *Philos. Trans. R. Soc. B* 373 (1741), 20160452. <https://doi.org/10.1098/rstb.2016.0452>.
- Young, R.C., Welcker, J., Barger, C.P., Hatch, S.A., Merckling, T., Kitaiskaia, E.V., Haussmann, M.F., Kitaysky, A.S., 2017. Effects of developmental conditions on growth, stress and telomeres in black-legged kittiwake chicks. *Mol. Ecol.* 26, 3572–3584. <https://doi.org/10.1111/mec.14121>.
- Zakian, V.A., 1995. Telomeres: beginning to understand the end. *Science* 270, 1601–1607. <https://doi.org/10.1126/science.270.5242.1601>.
- Zbyryt, A., Bubnicki, J.W., Kuijper, D.P., Dehnhard, M., Churski, M., Schmidt, K., 2018. Do wild ungulates experience higher stress with humans than with large carnivores? *Behav. Ecol.* 29, 19–30. <https://doi.org/10.1093/beheco/axx142>.
- Zuberogoitia, I., De La Puente, J., Elorriaga, J., Alonso, R., Palomares, L.E., Martínez, J.E., 2013. The flight feather molt of griffon vultures *Gyps fulvus* and associated biological consequences. *J. Raptor Res.* 47, 292–303. <https://doi.org/10.3356/JRR-12-09.1>.
- Zuberogoitia, I., Zabala, J., Martínez, J.E., 2018. Molt in birds of prey: a review of current knowledge and future challenges for research. *Ardeola* 65(2), 183–207. doi: 10.13157/arla.65.2.2018.rp1.
- Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media. <https://doi.org/10.1007/978-0-387-87458-6>.