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ORIGINAL ARTICLE

Human pressure may pose a threat to Mediterranean Golden Eagle's (*Aquila chrysaetos homeyeri*) nestlings' welfare

Laura Ciot[1] · **Annaïs Carbajal**[1] · **Javier Gamonal Talens**[2] · **Marta Peláez**[3] · **Pablo Ortega Martín**[4] · **Enrique Navarro Hernández**[2] · **Manel Lopez-Bejar**[1]

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Abstract

Mediterranean Golden Eagles (*Aquila chrysaetos homeyeri*) are crucial for maintaining the balance of the ecosystem they live in. Human presence and some human activities are recognized as major disturbance factors affecting their welfare. In the present study, we evaluated through the measurement of feather corticosterone (CORTf), the welfare state of nestlings subjected to different levels of human pressure. Nestlings were sampled in different locations in Spain and Portugal for two consecutive years (2018, 2019). CORTf levels were higher in groups of individuals living in most populated areas and positively correlated with the proximity to airports, suggesting that human presence and noise pollution generated by aircraft may be a source of stress for developing eaglets, affecting their physiological state. CORTf levels were also related to mortality, finding low mean levels in individuals dying in the short-run. Finally, the relation between CORTf and other commonly used stress indicators such as the intensity of the color of the hue of cere and the number of fault bars in the tail of the nestlings was investigated. Considering the hue of cere, a significant negative strong correlation with the corticosterone levels in nestlings was found in samples from 2018 suggesting that nestlings in poorer nutritional conditions may present higher stress levels, whereas no correlation with the number of fault bars was found.

Keywords Feather corticosterone · Animal welfare · Human pressure · Golden Eagle · Wild birds

Zusammenfassung

Stress durch Menschen ist möglicherweise eine Bedrohung für die Gesundheit der Jungen des mediterranen Steinadlers *(Aquila chrysaetos homeyeri)*

Die Steinadler im Mittelmeerraum (*Aquila chrysaetos homeyeri*) sind für die Erhaltung des Gleichgewichts in ihrem Ökosystem von entscheidender Bedeutung. Die Anwesenheit von Menschen und einige ihrer Aktivitäten gelten als wichtige Störfaktoren, die das Wohlergehen der Vögel beeinträchtigen. In dieser Untersuchung haben wir durch Corticosteron-Messungen in Federn (CORTf) den gesundheitlichen Gesamtzustand von Nestlingen ermittelt, die unterschiedlichem Stress durch Menschen ausgesetzt waren. Nestlinge wurden in zwei aufeinanderfolgenden Jahren (2018, 2019) an verschiedenen Orten in Spanien und Portugal getestet. Die CORTf-Werte waren in Tieren in den am stärksten besiedelten Gebieten höher

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✉ Laura Ciot
lauraciot00@gmail.com

1 Departmento de Sanidad y Anatomía Animal, Universitat Autònoma de Barcelona, Barcelona, Spain

2 Fundación TAGONIUS, Nuevo Baztan, Madrid, Spain

3 Departamento de Sistemas y Recursos Naturales, Universidad Politécnica de Madrid, Madrid, Spain

4 Departamento de Ecología, Facultad de Ciencias Biológicas, ACE-Asociación del Corzo Español, Universidad Complutense de Madrid, Madrid, Spain

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und korrelierten positiv mit der Nähe zu Flughäfen, was bedeuten könnte, dass die Anwesenheit von Menschen und der Lärm von Flugzeugen Stressquellen für die Adlerjungen während ihrer Entwicklung sein und ihre physiologische Verfassung beeinträchtigen könnten. Die CORTf-Spiegel wurden auch zusammen mit der Sterblichkeit betrachtet, wobei für diejenigen Tieren, die kurze Zeit später starben, niedrige Durchschnittswerte gemessen wurden. Schließlich wurde auch ein möglicher Zusammenhang zwischen CORTf und anderen üblicherweise verwendeten Stressindikatoren wie Farbton der Wachshaut und Anzahl von Fehlstellen im Schwanzgefieder der Nestlinge untersucht. Bei den Proben aus dem Jahr 2018 wurde eine starke, signifikant negative Korrelation des Farbtons der Wachshaut mit den Corticosteronwerten der Nestlinge festgestellt, was darauf hindeutet, dass Nestlinge unter schlechteren Ernährungsbedingungen möglicherweise höhere Stresslevel zeigen, wohingegen keine Korrelation mit der Anzahl der Fehlstellen im Gefieder gefunden wurde.

Introduction

The Mediterranean Golden Eagle *(Aquila chrysaetos homeyeri* Severtzov, 1888) occupies almost the entire Iberian Peninsula (Tapia et al. 2007) with a high percentage of the breeding pairs concentrated in the center (Blanco et al. 2017). The selection of breeding areas depends on the presence of appropriate cliffs for nesting, adequate prey availability, and low human pressure (Watson 2010). The Mediterranean Golden Eagle occupies the top of the food chain and, therefore, plays a crucial role in maintaining the health of the ecosystems where it lives, regulating the densities of populations of mesopredators and prey (Lyly et al. 2015). Differently from many other raptors, the percentage of mammalian predators, such as the Red Fox (*Vulpes vulpes*), in their diet is relatively high, ranging from 2% to 10–20% (Watson 2010). As a consequence, if their population declines, populations of mesopredators will increase, and induce extensive cascading effects on the lower trophic levels, as suggested by the mesopredator release hypothesis (Prugh et al. 2009; Lourenço et al. 2011). Recently, Golden Eagle populations in Spain have been observed foraging on young Roe Deers (*Capreolus capreolus*). This is likely not a new foraging habit but an old behavior that has now been reassumed, after the Roe Deer population recovery in the twentieth century and its vertiginous increase in the last three decades, to get to the occupation today of a good part of the territory of the Iberian peninsula. Therefore, the Mediterranean Golden Eagle collaborates in the regulation of the current excesses of Roe Deer populations in Spain (Martínez-Abraín et al. 2019), hence the importance of its conservation.

Many Golden Eagle populations faced a strong decrease during the early twentieth century, mainly due to persecution by humans (Lourenço et al. 2011). Currently, Golden Eagle populations are stable, but their recovery has been slowed down by habitat transformation and increasing human disturbance which remains the major hazard to their welfare (Watson 2010). Anthropogenic disturbance can affect eagles' behavior such as their foraging activity, parental care, self-maintenance, pair bond maintenance, vigilance against predators, territory establishment, or defense (Hansen et al. 2017). Human activities that can cause an important disturbance to eagles are the noise generated by vehicles (Benítez-López et al. 2010; van der Ree et al. 2011), wind energy facilities (Kolar and Bechard 2016), paragliders (Guglielmi et al. 2022), pollutants of human derivation (Ganz et al. 2016) and especially pedestrians such as eco-tourists, hunters, climbers. González et al. (2006) attributed this finding to pedestrians' unpredictable behaviors, such as remaining near eagle 's nests for relatively long periods, stopping at irregular intervals, walking in no particular direction, and looking up toward the sky or at the surrounding area.

In stressful situations, the hypothalamic–pituitary–adrenal axis (HPA) is activated and leads to the secretion of high levels of glucocorticoids and, in birds, especially corticosterone (Bortolotti et al. 2009). A rapid rise in circulating levels of corticosterone coordinates the physiological and behavioral responses to stressors (Iyasere et al. 2017) and can be advantageous when capable of redirecting energies in essential survival processes (Ouyang et al. 2012). Acute stress response may therefore be beneficial but, when maintained over time for a repeated or constant stressor, it can have deleterious effects in the long term, such as the suppression of the immune system or growth, and a reproduction delay (Fairhurst et al. 2012). Moreover, some animals exhibit reduced glucocorticoid levels in response to chronic stress or when they are in poor physiological condition (Cyr et al. 2007). Thus, low glucocorticoid levels do not necessarily indicate low stress levels (Hayward et al. 2011). Corticosterone levels can be assessed in several invasive ways, such as blood analyses, or non-invasive ways, like analyzing feces and urine (de Matos 2008). These measurements provide information about a short period of time (from few minutes to few hours) (Goymann 2005). On the other hand, feathers accumulate corticosterone and can be a temporal archive by reflecting plasma hormone levels during the period of feather growth (Bortolotti et al. 2008; Jenni-Eiermann et al. 2015; Ganz et al. 2018). Moreover, feather corticosterone (CORTf) is resistant to degradation by heat and stable over time (Bortolotti et al. 2009). Researchers frequently apply behavioral metrics, such as changes in breeding or feeding behavior, to examine human disturbance on wildlife (González et al. 2006; Perona et al. 2019). Nevertheless, there is currently no published research investigating the physiological effects of human disturbance on wild Golden Eagles (Hansen et al. 2017).

The present study was performed within the framework of the Aequilibrium + project: a monitoring program of *Aquila chrysaetos homeyeri*, established in 2016 in Spain (aequilibrium-project.org). The project results from the partnership between the Spanish Roe Deer Association (ACE), linked to the hunting world, and Grupo Tagonius, working in the conservation field. The project monitors the population of Mediterranean Golden Eagle and Roe Deer in Spain, and serves as an example of how full collaboration between the world of hunting and conservation is possible. Its main goal is to study this raptor ethology focusing on how it has adapted to recent changes in prey availability.

The main objective of this study was to investigate the welfare conditions (expressed in CORTf content) of nestlings of Mediterranean Golden Eagles undergoing different kind of stressors and different levels of human pressure. Specifically, we first investigated whether the size of human populations living nearby nests may affect the level of CORTf in eaglets. Secondly, we tested whether the proximity to airports could have an impact on the CORTf levels in nestlings. Then, it was evaluated if there was a significant difference in CORTf levels of nestling alive and going to die in the near future. Finally, it was checked whether there was a correlation between CORTf and other stress indicators such as the intensity of the hue of cere and the number of fault bars. To achieve these objectives, the feathers of nestlings of Golden Eagles were analyzed and corticosterone concentrations were measured. The number of fault bars and the intensity of the hue of cere for each individual were assessed. Additionally, the population size, the distance of nests to airports, and the weather conditions of the areas where the nestlings lived were noted.

Materials and methods

Study area and feather collection

The present study was conducted in Spain, in the communities of Madrid, Castilla la Mancha, Asturias, Castilla y Leon, and in Portugal, in the Bragança district (Fig. 1). The nestlings were sampled from late May to mid-June for two consecutive years: in 2018, nine nestlings were sampled from eight nests (one nest was hosting a couple of birds), in 2019, 22 nestlings were sampled from 16 nests (six nests were hosting couples of birds). The nests were usually placed on cliffs or, more rarely, on trees.

To evaluate the anthropogenic pressure on eaglets, data concerning the number of inhabitants of the municipality where the nest was located were taken from INE.es (Instituto Nacional de Estadística) and INE.pt (Instituto Nacional de Estatística—Statistics Portugal, Portal do INE).

Subsequently, the airline distance in km between each approximated nest location and the nearest international airport has been evaluated. The airports considered for this study were the Adolfo Suárez Madrid–Barajas Airport, the Francisco Sá Carneiro Airport, the Zaragoza airport, the Bilbao Airport, and the Seve Ballesteros-Santander Airport.

Field procedures

In both years 2018 and 2019, Golden Eagle territories were first monitored in February, at the beginning of the breeding season, for signs of reproductive activity (courtship displays, nest construction, copulations, territorial defense, etc.). Egg laying takes place normally in March and the hatching of the eggs takes place 41–45 days later (Watson 2010). Active nests were then visited and sampling was conducted shortly after hatching (in late May/early June, between 40 and 45 days post-hatching). The eaglets were carefully lowered from the nests in duffel bags for morphometric measurement and minimally invasive sampling (electronic supplementary material Fig. S1). Second natal down feathers were gently pulled from the axillary region and blood was collected for sex determination using the PCR protocol developed by Griffith et al. (1998). Estimated age, weight, number of tail's fault bars, and approximate position of the nests were recorded and nestlings were banded with metal and alpha-numeric-coded plastic rings designed to facilitate post-fledging identification with the use of spotting scopes. Cere's color intensity was assessed through comparison with standardized PANTONE color. After sampling, the nestlings were immediately returned to their nest. All feathers were stored in individually labeled paper envelopes and kept at room temperature until analysis. The nestlings were monitored in the subsequent weeks and nine individuals were found dead ($n = 3$ by predation, $n = 2$ by cainism, $n = 3$ by electrocution, $n = 1$ by unknown causes).

Hormone extraction and analysis

First, a visual quality examination of all feathers was conducted and feathers with low quality were discarded. To obtain the same range of sample mass between individuals (mean ± SD: 22.52 ± 5.21 mg), five to eleven unwashed down feathers for each individual were pooled and weighted with a precision scale to the nearest 0.1 mg. A mass of 13.5 mg per sample was set as the minimum required (2018: $n = 9$, 2019: $n = 22$) (Fig. 2). The calamus was removed from all the feather. For the samples collected in 2018, the selected feathers of each individual were manually minced together into pieces of $< 3\ mm^2$ and the post-minced weight was measured with a precision scale to the nearest 0.1 mg

Fig. 1 Geographical localization of individuals sampled in 2018 (blue) and 2019 (green)

(mean ± SD: 15.22 ± 6.65 mg). For the samples collected in 2019, the feathers of each individual were pulverized with a milling machine into pieces of < 3 mm^2 and the post-pulverization weight was measured with a precision scale to the nearest 0.1 mg (mean ± SD: 16.55 ± 5.22 mg). To extract CORTf, an optimized protocol for extracting CORT from feathers was followed (Bortolotti et al. 2008; Monclús et al. 2017). All samples were analyzed in the same assay and the intra-assay coefficient of variation was measured using samples from the study (CV intraassay = 8.63%). Methanol (1.5 ml of methanol reagent grade 99.9%) was added to each pulverized sample and they were incubated at 37 °C for 24 h in a shaking water bath (G24Environmental Incubator Shaker; New Brunswick Scientific, Edison, NJ) for steroid extraction. Samples were then centrifuged (Hermle Z300K; Hermle®Labortechnik, Wehingen, Germany) at 9.500 g for 10 min. Then, 1 ml of the resulting supernatant was transferred to a new aliquot that was placed in an oven (Heraeus model T6; Kendro Laboratory Products, Langenselbold, Germany) at 38 °C. Once the methanol was completely evaporated, the dried extracts were reconstituted in 250 µl EIA buffer solution, provided by the EIA assay kit

Fig. 2 Down feathers collected from nestlings

(Corticosterone ELISA kit; Neogen®, Ayr, UK), shaken for 1 min, and stored at − 20 °C until analysis. CORT analysis was performed using a competitive EIA kit (Corticosterone ELISA kits; Neogen®) and carried out following the manufacturer's instructions.

Statistical analysis

Data were analyzed using R software (www.r-project.org, version 4.2.1). All the values are presented as mean ± SD. A p value < 0.05 was established as a criterion for significance. Before analyses, a Shapiro–Wilk test was performed to check normality. Log transformation ($\log_{10}$) was applied to the Corticosterone data and Box–Cox transformation was applied to population data to achieve a normal distribution. The influence of the weight of feathers on CORTf levels was explored using a Pearson correlation test. Pearson correlation tests were performed to evaluate also the correlation between the CORTf concentration and fault bars number, and the CORTf concentration and the intensity of the hue of cere. Independent samples t tests were performed to evaluate the difference in CORTf levels means between 2018 and 2019 samples, between alive animals and close-to-death ones, between the mean number of fault bars in each year, and the intensity of the hue of cere in each year. We calculated nestling body condition using the scaled mass index derived from mass and tarsus length (Peig and Green 2009). The effect of body conditions on CORTf was investigated through simple linear regression. We evaluated multiple linear mixed models (LMMs) to identify the most appropriate model for our data. We tested models including combinations of predictors such as "year", "population size", "distance of the nest to airport", "body condition", "brood size" (the number of birds per nest), and "nestling rank" (first or second of the brood), while keeping "nest" as a random effect to account for inter-nest variability. Each model was fitted using restricted maximum likelihood (REML), and the AIC values were computed. Among the models that incorporate the variables of specific interest for our research (population size, distance to airport), those with the lowest AIC values were considered the most parsimonious and were selected for further analysis. This method ensures that the selected model explains the data well without overfitting.

Results

Differences in CORTf in 2018 and 2019 and relation between CORTf and physiological/morphological variables

The content of corticosterone was determined in feathers from a total of 31 individuals, nine in 2018 and 22 in 2019 ($n=6$ females, $n=11$ males in 2018, $n=16$ females, $n=14$ males in 2019). In general, considering both years, the logarithmic corticosterone content in the feathers varied widely between individuals, ranging from 0.97 pg/mg to 2.06 pg/mg with a mean of 1.56 ± 0.29 pg/mg. The content of corticosterone ($\log_{10}$) in 2018 and 2019 samples differed statistically between years (independent samples t test, $t=2.25$; $p=0.036$) with mean levels in 2018 of 1.74 ± 0.22 pg/mg and 1.49 ± 0.28 pg/mg in 2019. The mass of the samples did not affect the detection of CORTf since no significant correlation between CORTf and the weight of samples was detected (Pearson test, $p>0.05$).

Considering the total amount of samples analysed (2018 and 2019), the mean of levels of corticosterone ($\log_{10}$) showed no statistical difference between females and males (independent samples *t* test, $t = 0.32$; $p > 0.05$) with mean levels in females of 1.57 ± 0.32 pg/mg and 1.53 ± 0.25 pg/mg in males. Considering just 2018's samples, we detected a statistical difference between females and males (independent samples *t* test, $t = 3.72$; $p = 0.008$) with mean levels in females of 1.97 pg/mg and 1.64 pg/mg in males. No significant effect of body conditions on CORTf levels was found (simple linear regression, $p > 0.05$) and no significant differences between body conditions in 2018 and 2019 was found (*t* test, $p > 0.05$).

Relation between CORTf and survival/mortality

Considering the samples collected in 2018, a statistical difference in CORTf was detected between nestlings that survived in the following weeks and those who were found dead (independent samples *t* test, $t = 4.91$; $p = 0.004$) with mean levels in alive nestlings of 1.92 pg/mg and 1.53 pg/mg in nestlings soon found dead.

Relation between CORTf and number of inhabitants of the municipality

The population size of the municipality where the nest was located varied from 10 to 195.649 inhabitants. The brood size varied from one to two individuals for each nest. We conducted a LMM analysis to investigate the effects of Box–Cox transformed population size and brood size on log-transformed corticosterone levels in juveniles. The model included "nest" as a random effect. The REML criterion at convergence was 8.5, and the AIC was 20.9, indicating a good fit to the data compared to the other models analyzed that include the variables of interest (electronic supplementary material Table S1). The results of the model showed that the intercept was highly significant (estimate = 1.826, SE = 0.173, $t(22.198) = 10.572$, $p < 0.001$). The effect of population size, as measured by the population variable, was significant (estimate = 0.042, SE = 0.019, $t(14.838) = 2.200$, $p = 0.044$), indicating that an increase in population size is associated with an increase in corticosterone levels. Additionally, brood size had a significant negative effect on cortisol levels (estimate = − 0.326, SE = 0.086, $t(22.656) = -3.775$, $p = 0.001$), suggesting that larger broods are associated with lower corticosterone levels. The random effects in the model revealed a standard deviation of the intercept for NestId of 0.055 and a residual standard deviation of 0.227, indicating variability between nests (Fig. 3, electronic supplementary material Table S2).

Relation between CORTf and distance to airports

The airline distance between the nests and the nearest airports varied from 16 to 148 km. We conducted a LMM analysis to investigate the effects of brood size and distance from the airport on log-transformed corticosterone levels in Golden Eagle nestlings. The model included Nest as a random effect. The REML criterion at convergence was 15, and the AIC was 27.4, indicating a better fit to the data compared to the other models analyzed that include the variables of interest (electronic supplementary material Table S1). The results of the model showed that the intercept was highly significant (estimate = 2.214, SE = 0.175, $t(25.126) = 12.669$, $p < 0.001$). The effect of distance from the airport was marginally significant (estimate = − 0.0024, SE = 0.0014, $t(21.734) = -1.799$, $p = 0.086$), suggesting a trend where increasing distance from the airport is associated with a decrease in corticosterone levels. Additionally, brood size had a significant negative effect on cortisol levels (estimate = − 0.291, SE = 0.090, $t(23.301) = -3.242$, $p = 0.004$), indicating that larger broods are associated with lower corticosterone levels. The random effects in the model revealed a standard deviation of the intercept for Nest of 0.0957 and a residual standard deviation of 0.2198, indicating variability between nests (Fig. 4, electronic supplementary material Table S2).

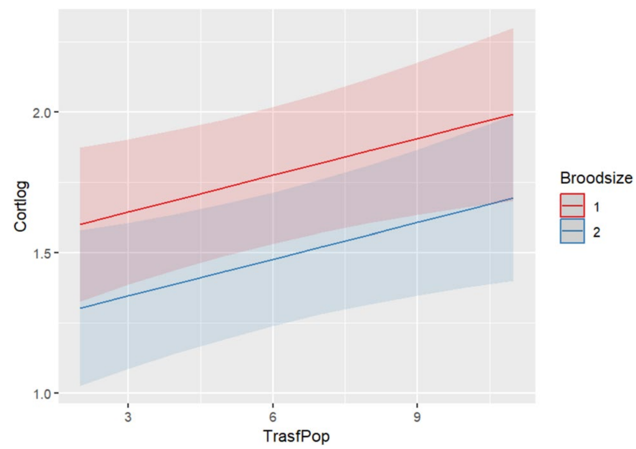

Fig. 3 Relationship between the $\log_{10}$CORTf concentration of Golden Eagle nestlings expressed in pg/mg, box-cox transformed Population (number of inhabitants), and Brood size (1 or 2 eaglets per nest)

Relation between CORTf and other stress indicators (fault bars and hue of cere)

No significant correlation between CORTf and the number of fault bars in nestlings was found (Pearson test, $p > 0.05$). A statistically significant difference was identified between the mean number of fault bars of individuals in 2018 (12.66 ± 8.68) and in 2019 (5.25 ± 3.64) (independent samples *t* test, $p = 0.037$). Considering only results obtained on 2018, a significant negative correlation between CORTf and the intensity of the hue of cere in nestlings was detected

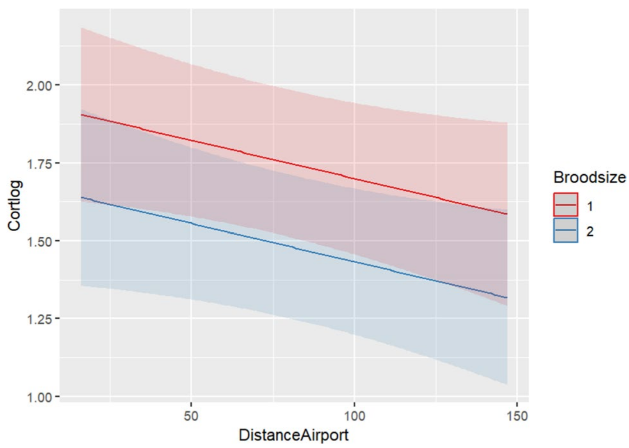

Fig. 4 Relationship between the $\log_{10}$CORTf of Golden Eagle nestlings expressed in pg/mg, the air-distance to an airport expressed in km, and the Brood size (1 or 2 nestlings)

(Pearson test, $p = 0.012$, slope of the line = − 0.786). No significant difference was detected in the intensity of the hue of cere between samples collected on 2018 and samples collected on 2019 (independent samples *t* test, $p > 0.05$).

Discussion

Differences in CORTf in 2018 and 2019

We observed a significant difference between CORTf content in samples from 2018 and 2019, with a higher level of corticosterone in feathers collected in 2018. It is worth mentioning that two different protocols were used for the sample preparation. Samples from 2018 were manually minced, and those from 2019 were pulverized with a milling machine. This difference in protocols could represent a potential source of variation between results. Nevertheless, it is to be noted that the CORTf extraction from manually minced samples usually leads to a lower revelation of corticosterone when compared to pulverized samples (Ataallahi et al. 2020) because of the lower contact surface between feathers and alcohol and consequently the lower extraction capacity. On the contrary, in our study, the levels of corticosterone detected were significative higher for the manually minced samples (2018) compared to the automatically minced ones (2019). We can therefore conclude that the difference between the 2018 and 2019 samples may not be due to the difference in the adopted sample preparation methods.

Relation between CORTf and survival/mortality

Splitting individuals into two groups (alive and going to die in the short-run), we observed a significant difference between mean CORTf levels in samples analyzed from nestlings born in 2018. Individuals going to die in the following weeks showed a lower CORTf concentration compared to those alive.

In nestlings, corticosterone during stress may increase or be downregulated. According to the "stress-response hypothesis", higher CORTf levels reflect energetically demanding or stressful conditions during feather growth. However, in many bird species, particularly altricial ones, the HPA axis reactivity is lowest in younger nestlings as an adaptation to protect developing neurons from high CORT levels and only reaches adult-like stress reactivity just before fledging. Moreover, some species are able to down-regulate their CORT release during food scarcity ("hypo-responsive hypothesis") (Jenni-Eiermann et al. 2022). Thus, low stress hormone levels do not necessarily indicate low stress levels and a meaningful interpretation of nestling CORTf should only be made in the context of species-specific traits.

Although higher short-term glucocorticoids levels have been often associated with a greater survival rate in adults and fledglings (Blas et al. 2007; Cabezas et al. 2007; Rivers et al. 2012) it is still unclear how variations in corticosterone levels influence the survival of individuals. As published findings suggest, high acute levels of corticosterone may promote locomotor activity, allowing a more effective escape from predators, and increment foraging behavior (Rivers et al. 2012; Béziers et al. 2020). Despite the adaptiveness of short-term elevations of CORT, chronic elevations could lead to deleterious effects in the long term such as the suppression of the immune system and growth delay (Fairhurst et al. 2012).

A possible interpretation of our results is that higher CORTf levels may be the consequence of high baseline corticosterone levels or repeated short-term incrementations. In both cases, elevated CORT levels could be necessary for the proper regulation of physiological processes essential to increase the response in life-threatening situations, and for a modification of behavior focusing on self-maintenance increasing the chance of survival (Sapolsky et al. 2000). Besides, the lower survival rate of individuals presenting low CORTf concentration could be consequence of chronic stress or expression of the "hypo-responsive hypothesis", which has resulted in the down-regulation of the HPA axis activity. This may have ultimately led to the inability to respond physiologically to stressors and so to deleterious behavioral effects that have impacted their survival (Ritchie and Pilny 2008; Fernando Torres Medina 2019).

For two out of the seven individuals that died, death was the result of obligate siblicide, in three cases of predation, and in two cases of accidental electrocution. It is worth noting that in both the cainism cases, the surviving chick showed a higher weight and higher level of corticosterone. As a matter of fact, it has been noted how higher corticosterone levels could lead to more aggressive behavior in nestlings (Kitaysky et al. 2003; López-Jiménez et al. 2016). A frequently addressed key factor in sibling antagonism in the nest is food availability, that has been linked to effects on stress level (Catitti et al. 2022). Further research with an increased sample size is required to improve the understanding of the relationship between CORTf and mortality in nestlings of Golden Eagles.

Relation between CORTf and human pressure

CORTf and number of inhabitants of the municipality

Results of the present study reveal that the size of human population could have an impact on CORT production, metabolism, and deposition in feathers in nestlings of Golden Eagles. Golden eagles living nearby most populated human settlements presented significantly higher levels of CORTf than nestlings inhabiting less populated areas. A similar result was detected by Beaugeard et al. (2019) when they identified a positive relationship between the degree of urbanization and the CORTf levels in juveniles of House Sparrows (*Passer domesticus*). Human presence can cause direct and indirect disturbances to wildlife in many ways (Mathisen 1968; Steidl and Anthony 2000; Gill et al. 2001; Carrete et al. 2007; Ciuti et al. 2012; Rebolo-Ifran et al. 2015; Remacha et al. 2016; Torres et al. 2016). Known factors that can create a direct disturbance are pedestrian activities (Hansen et al. 2017), ludic activities such as cliff wall climbing or paragliding (Guglielmi et al. 2022), presence of vehicles (van der Ree et al. 2011; Psaralexi et al. 2017; Martínez-Abraín et al. 2019) or noise production (Ortiz-Urbina et al. 2020). Among indirect disturbance factors, we can identify air and noise pollution produced by human facilities (Monclús et al. 2019; Ortiz-Urbina et al. 2020; Randulff et al. 2022) and physical alteration to landscapes or decreasing habitat quality (van der Ree et al. 2011; Ciuti et al. 2012; Perona et al. 2019; Demerdzhiev et al. 2022). The more the size of the human population increase, the higher the probability of occurrence of one of the cited situations. The stressful effect of human disturbance on Golden Eagles has been studied using different behavioral and physiological criteria (Gill et al. 2001) but, to date, to the authors' knowledge, there are no studies on the CORTf alterations attributable to human settlements' proximity. Human presence can create a direct effect on nestlings (Remacha et al. 2016) or indirect impacts by affecting parental care. Anthropogenic activities shape the distribution of raptors' nesting sites (Mathisen 1968; González et al. 1992; Morán-López et al. 2006; Ortiz-Urbina et al. 2020), the laying and hatching success (Perona et al. 2019) and the amount and quality of cares parents supply to nestlings (Guglielmi et al. 2005; González et al. 2006). Moreover, Golden Eagles are territorial raptors with limited nest availability and foraging niches and therefore reluctant to find alternative suitable habitats even when disturbance factors are present (Gill et al. 2001; Perona et al. 2019). The amount of stressors evoked by human disturbance can increase CORTf levels in nestlings allocated closer to highly populated areas. Attenuating threats deriving from human activities is essential for the welfare of wild animals. One of the most suggested initiatives is the creation of spatial and temporal buffers: areas and periods where human activities cease or are severely restricted (Birnie-Gauvin et al. 2016; Hansen et al. 2017).

CORTf and distance to airports

The marginally significant negative effect of distance from the nearest airport may indicate that nests located further from airports experience less stress or disturbance, potentially leading to lower corticosterone levels. Airline activity results in three major ecological impacts: wildlife–aircraft collisions, atmospheric pollution, and, especially, acoustic pollution (Davenport and Davenport 2006). Airports can reach extremely high noise pollution levels (around 140 dB at take-off) (Kurtulus Ozcan and Nemlioglu 2006). In human populations living nearby airports, the effects of elevated noise exposure have been linked to various health problems (Jarup et al. 2008) and higher saliva cortisol levels (Selander et al. 2009; Baudin et al. 2019). The negative impact of airport noise on wildlife has also been reported with panic responses caused by sudden peaks of noise, an increased alert behavior, changes in vocal behavior, and jeopardized reproductive success (Alquezar and Macedo 2019). The proximity of human activity appears to be among the primary factors determining whether and how animals respond to it. Grubb and King (1991) detected an inverse relationship between Bald Eagles' distances from human activities and the severity of their responses to them (i.e., none, alert, flush, depart from the immediate area). Similar to what happens in humans, elevated levels of noise pollution may have an impact on the physiology of Golden Eagles, raising the CORTf levels, and the proximity to the source of noise seems to have a great influence on the intensity of the effect. It should be highlighted that, in general, airports are located near highly populated cities and this could be another possible factor of disturbance for eagles. Undoubtedly, gaining a deeper understanding of human disturbance factors on Golden Eagles is vital to improving decision-making for wildlife conservation.

Relation between CORTf and other stress indicators (fault bars and hue of cere)

Along with corticosterone, other stress indicators are used to assess raptors' welfare. Among the most widely used, there are the number of fault bars, used to estimate stress events occurred during feather growth (Erritzoe 2007; Pape Møller et al. 2009; Jovani and Rohwer 2017), and the intensity of the hue of cere, indicative of nutritional welfare (Martínez-Padilla et al. 2013). Fault bars are bands on the surface of the feather, perpendicular to the rachis, and consist of abnormally formed or missing barbules (Bortolotti et al. 2009; Jovani and Blas 2004). To date, no correlation between the number of fault bars and corticosterone levels has been found in birds (Carbajal et al. 2014; Jovani and Rohwer 2017) and the results of this study support these findings. It is, however, true that, in previous studies, when analyzing the segments of feathers showing fault bars and comparing them with those without, a higher level of CORT was detected (Jovani and Rohwer 2017). Due to the small dimension of our samples, we could not assess this factor in the same manner.

Considering the hue of cere, a significant negative strong correlation with the corticosterone levels in nestlings was found in samples from nestlings born in 2018. Nestlings with a lower hue of cere, and therefore, in poorer nutritional conditions, presented a higher CORTf content. The intensity of coloration of cere has been previously correlated with CORTf levels by Martinez-Padilla et al. (2013). Results, therefore, suggest that nestlings subjected to stressful events may be more carotenoid-limited or less able to allocate carotenoids to cere.

Conclusions

The results of this study show a positive correlation between size of human population and CORTf levels. We conclude that adrenocortical activity and CORTf levels might be affected by human disturbance. Moreover, a negative correlation between CORTf content and the air-line distance of the nest to an airport was detected. The noise pollution generated by aircraft may potentially have an impact on the well-being of Golden Eagles. CORTf was lower in nestlings going to die in the short-run. Higher levels of CORTf could possibly be beneficial for increasing the response in life-threatening situations and therefore affect the chance of survival. No correlation between CORTf and the number of fault bars per individual was found suggesting the independence between the two variables. A negative correlation was found between CORTf levels and the intensity of the hue of cere for 2018 samples. Nestlings subjected to stressful events may possibly be more carotenoid-limited or less able to allocate carotenoids to cere.

Given its importance as a top predator, the future of the Mediterranean Golden Eagle is critical for the maintenance of the entire ecosystem they live in. Biologists and land managers have identified human disturbance as a primary threat to raptor populations (Sumasgutner et al. 2021). This study gives further evidence of the human pressure on Mediterranean Golden Eagles. More studies are needed to identify animal welfare threats and to postulate conservation measures. Conservation is most effective when public awareness and respect for nature are promoted (Fernando Torres Medina 2019). However, activities like ecotourism and bird-watching can conflict with biodiversity conservation, requiring collaboration between managers and scientists in key measures including educating the public on respecting wildlife, restricting access to breeding areas of threatened species, defining itineraries, and using buffer zones and ecological corridors (Hansen et al. 2017), and mitigating airport noise.

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Author contributions LC originally formulated the idea, performed the laboratorial analyses, analyzed the data, and wrote the manuscript. JGT, MP, POM, and ENH conducted the fieldwork and samples collection. ACB developed methodology, provided editorial advice and supervision. MLB supervised the project.

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval.

Ethics approval was not required for this study according to local legislation 32/2007. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Consent to participate Not applicable.

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