

# Stress associated with group living in a long-lived bird

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**Many long-lived avian species adopt life strategies that involve a gregarious way of life at juvenile and sub-adult stages and territoriality during adulthood. However, the potential associated costs of these life styles, such as stress, are poorly understood. We examined the effects of group living, sex and parasite load on the baseline concentration of faecal stress hormone (corticosterone) metabolites in a wild population of common ravens (*Corvus corax*). Corticosterone concentrations were significantly higher in non-breeding gregarious ravens than in territorial adults. Among territorial birds, males showed higher stress levels than their mates. Parasite burdens did not affect hormone levels. Our results suggest a key role of the social context in the stress profiles of the two population fractions, and that group living may be more energetically demanding than maintaining a territory. These findings have implications for understanding hormonal mechanisms under different life styles and may inspire further research on the link between hormone levels and selective pressures modulating gregarious and territorial strategies in long-lived birds.**

**Keywords:** Białowieża forest; common raven; *Corvus corax*; glucocorticoids; parasites; sociality

## 1. INTRODUCTION

Social interactions and changes in the social environment, such as dispersal, territory acquisition or flocking are among the most potent stressors in vertebrates [1–4]. Short-term ‘acute’ activation of the stress response (a hormonal cascade that results in an increase in glucocorticoid secretion, e.g. corticosterone) is adaptive and aids survival; however, when the stressors persist, the response may become chronic and can induce detrimental symptoms. The social context can modulate individuals’ hormonal stress profile,

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which may affect immunocompetence and individual fitness [1,5–8].

Studies on social stress have mainly focused on the stress levels and response of dominant versus subordinate individuals within the same group, and their relation to group stability or to different social structures (e.g. [1,2,4,6,8]). In birds, cooperative and colonial breeding species have received particular attention (e.g. [2,7,9,10]). However, although many species of birds adopt a different social system at different life stages—gregarious at juvenile and sub-adult stages and territorial at adulthood—there is a shortage of studies integrating stress levels of those fractions in the same population. This information is essential to understanding the role of social stressors in modulating the trade-off between delayed territoriality and fitness enhancement [11]. Whether stress will fall more heavily on dominant adults or on subordinate non-breeders depends on the physiological costs associated with each social rank [1,6]. In group-living animals, social support and cooperation, like food sharing, may decrease stress levels, whereas resource competition, aggressive interactions and parasite transmission may increase them [1,3,6,8,12]. On the other hand, individuals maintaining a territory need to make greater investments in territory defence.

Here, we examined faecal corticosterone concentrations in a wild population of a long-lived passerine, the common raven (*Corvus corax*). It is a good model species, as non-breeding birds form large unstable flocks which roost communally, share information and together exploit food resources, typically large carcasses. Access to this food generally involves active recruitment to overcome the defences of the territorial pair (see [13] and references therein). Adult ravens maintain life-lasting territories throughout the year, which are strongly defended from conspecifics. We asked whether social status affected baseline corticosterone concentrations and whether this effect was mediated by sex and parasite load. We expected higher levels of glucocorticoid metabolites in territorial individuals.

## 2. MATERIAL AND METHODS

### (a) Field procedures

We studied the raven population of the Białowieża forest (northeast Poland), the best preserved lowland temperate forest of Europe (see [14] and references therein). The density of raven breeding pairs was about five pairs per 100 km<sup>2</sup>. A communal roost with around 60 non-breeding ravens was located at the edge of the forest. We collected 258 fresh faecal samples from 15 January to 4 February 2008, outside the breeding season. Droppings from non-breeding ravens ( $n = 113$ ) were found during field inspections at the roost and at three ungulate carcasses attended by flocks of 20–30 ravens. Droppings from raven pairs ( $n = 145$ ) were obtained by exposing carrion baits at 14 known territories. Only very fresh droppings were collected and immediately frozen until analysis. Below zero temperatures during the study period guaranteed the proper preservation of faecal hormones in the field.

### (b) Analysis of faecal hormone metabolites and parasites

Faecal glucocorticoid metabolites (ng g<sup>-1</sup> dry weight), indicative of corticosterone levels, were quantified following Wasser *et al.* [15]. They were extracted from faeces and then assayed using a corticosterone radioimmunoassay kit (ICN Biomedicals), following methods previously validated for glucocorticoid metabolite determinations in raven faeces ([3]; see electronic supplementary material). Assay sensitivity was 2.5 ng ml<sup>-1</sup>. Intra-assay coefficients of variation were less than 9 per cent and inter-assay coefficients of variation were 8.6 and 7.9 per cent. The faecal steroid extraction method of Wasser

*et al.* [16] was used with minor modifications to quantify the concentrations ( $\text{ng g}^{-1}$  dry weight) of testosterone and oestradiol in order to sex the birds by means of a discriminant function that classified all males and females correctly. The faecal concentration of testosterone and oestradiol also allowed us to distinguish adults from non-breeders in the samples collected at carcasses, where one or more territorial pairs could mix with the flock (electronic supplementary material). Assay sensitivity was  $0.25 \text{ ng ml}^{-1}$  for both testosterone and oestradiol. Intra-assay coefficients of variation were less than 10 per cent for testosterone and 8.4 for oestradiol. Inter-assay coefficients of variation were 8.6 and 8.9 per cent for testosterone and 9.1 and 7.4 per cent for oestradiol.

We determined the presence of multiple parasites and pathogens in faecal samples, including protozoans, helminths, bacteria and viruses (hereafter termed parasites; see the electronic supplementary material for a detailed list of the parasites identified, their frequencies and methodological issues). We assessed parasite presence and richness as the total number of parasite species per sample.

### (c) Statistical analyses

We performed linear mixed models with a stepwise backward procedure in R v. 2.10.1 (lme function) to determine whether the concentration of faecal glucocorticoids ( $\text{ng g}^{-1}$  dry weight) was influenced by sociality (territorial pairs versus gregarious non-breeders), sex, and presence and richness of parasites (protozoans, helminths, bacteria and viruses). To minimize pseudoreplication, we fitted as random factors: (i) the location ( $n = 18$ ) and (ii) the date of collection ( $n = 11$ ) nested within location. Because ravens walk around carcasses and trample the droppings, only the very fresh droppings were collected. Furthermore, as the number of samples collected was always lower than the number of ravens observed at carcasses and the roost during the field visit, we are confident that the probability of collecting two samples from the same individual in a given location and day was virtually null. In the case of territorial pairs, for every nest and day, we randomly chose one sample for the male and one for the female. Similarly, from the samples of territorial individuals detected in the flocks at carcasses (according to the discriminant function of the faecal concentration of steroids; see the electronic supplementary material), only one sample was chosen randomly for each sex for every location and day. The final number of faecal samples included in the analysis was 94 for non-breeders and 46 for territorial ravens ( $n = 140$ ).

## 3. RESULTS

Contrary to our expectations, non-breeders had significantly higher baseline corticosterone levels than territorial birds (table 1 and figure 1). Sociality and its interaction with sex were also highly significant: territorial males showed higher stress levels than territorial females. Consequently, the effect of sociality on the concentrations of faecal corticosterone metabolites was stronger for females. We did not detect significant effects of parasite presence and richness.

## 4. DISCUSSION

Our study provides new insight into stress levels in relation to group living versus territorial strategies. We found significant differences in faecal corticosterone metabolites between fractions of the same population with different life styles. At baseline concentrations, corticosterone primarily relates to metabolism and, by regulating energy intake, storage and mobilization, supports energetically demanding processes [1,17]. Higher stress levels in non-territorial ravens may be associated with higher energetic demands, perhaps through greater competition, more unpredictable environments, higher predation risk, food shortage or poor foraging skills. They may be also influenced by their younger age; however, most studies on long-lived birds have not found a significant effect of age on baseline corticosterone levels

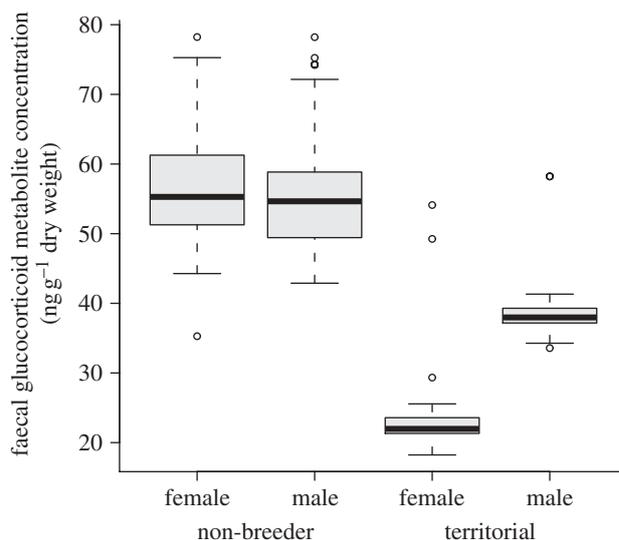


Figure 1. Boxplot of faecal glucocorticoid concentrations ( $\text{ng g}^{-1}$  dry weight) in males and females of non-breeding and territorial ravens ( $n = 140$ ).

Table 1. Linear mixed model explaining the variation in the levels of faecal corticosterone metabolites ( $\text{ng g}^{-1}$  dry weight) in the raven population.

fixed effects	estimate	s.e.	<i>p</i>
model corticosterone ~ sociality × sex			
intercept	39.296	3.167	<0.00001
sociality pair	-11.710	2.676	<0.00001
sex male	-0.356	0.944	0.707
sociality pair : sex male	15.256	1.740	<0.00001

(e.g. [18]). Raven flocks are unstable, non-cohesive groups where a dominance hierarchy—which determines the access to food—develops, and aggressive interactions are common [13]. This supports the idea that stress levels are elevated in unstable groups [6,8]. Additionally, elevated corticosterone concentrations may also denote adaptive responses to promote dispersal or movements and to improve foraging behaviour [7,19]. According to Goymann & Wingfield [1], our findings suggest that it may be more energetically demanding to live in groups than to maintain a territory.

We found an interaction between sociality and sex only in territorial birds, suggesting differences in resource competition and selection pressures between females and males in raven pairs, but not in non-breeders. The higher baseline corticosterone levels exhibited by territorial males in relation to females may be a response to higher energetic demands; males are most immediately involved in aggression associated with the establishment and maintenance of a territory, defence of food resources against raven flocks and mate-guarding behaviour [1,13]. Although the effects of parasites are energetically demanding, and can be better coped with by increasing baseline corticosterone concentrations (e.g. [7]), parasite burdens were not a prime factor mediating stress levels.

Because hormones mediate life-history trade-offs, including transitions between life stages and relationships with the environment [2,17], species with variable life histories, including breeding strategies and social structure, may show different hormonal patterns. For example, some studies demonstrated the absence of high levels of corticosterone in non-breeders of cooperatively breeding species [9,10,20], or no sex differences in baseline corticosterone concentrations in adult birds of colonial species [7,21]. Our study highlights the prevalent role of sociality in stress levels, and sheds light on the hormonal mechanisms underlying group living and territorial strategies within a population. Elevated corticosterone levels may simply help non-territorial birds maintain homeostasis in an unstable environment and social context; on the other hand, by affecting individual fitness, they may act as a selective filter to enter adulthood. High stress levels may also act as an important force pressing non-breeders to leave the group and acquire a territory. Our findings should inspire further research on the link between hormone levels and selective pressures modulating gregarious and territorial strategies in long-lived birds.

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## Expression of concern

*Biol. Lett.* **7**, 608–610 (23 August 2011; Published online 9 February 2011) (doi:10.1098/rsbl.2010.1204)

### **Stress associated with group living in a long-lived bird**

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Subsequent to publication of ‘Stress associated with group living in a long-lived bird, *Biol. Lett.* **7**, 608–610 (23 August 2011; Published online 9 February 2011, doi:10.1098/rsbl.2010.1204)’, the journal received an expression of concern from the authors (Nuria Selva, Ainara Cortés-Avizanda, Guillermo Blanco, Thomas Mueller, Bernd Heinrich and José A. Donázar) about the validity of some of the data based on laboratory analyses carried out by Jesús A. Lemus. While awaiting the outcome of further investigations, the Editor-in-Chief and the authors wish to notify readers of our concerns regarding this article.

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## Retraction

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## Retraction

# Stress associated with group living in a long-lived bird

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*Biol. Lett.* 7, 608–610 (23 August 2011; published online February 9 2011).  
(doi:10.1098/rsbl.2010.1204)

After careful examination, there is a need to question the validity of the results conducted by Jesús A. Lemus in the above paper, published in *Biology Letters*. Although the field and statistical procedures are correct, we have not been able to clarify in which external laboratories Dr Lemus conducted the hormonal and parasite analysis. Given the ephemeral nature of the material used (faeces), we are unable to repeat the analysis with the same samples employed in the original study. Therefore, we wish to retract the article. We sincerely apologize for any inconvenience to the readers of *Biology Letters*.

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An Expression of concern for this paper has previously been published and can be viewed at <http://dx.doi.org/10.1098/rsbl.2012.0777>.