

# Feather corticosterone levels are not correlated with health or plumage coloration in juvenile house finches

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Stressful developmental conditions can have both short- and long-term effects on animal physiology and behaviour, but studies on this topic are rarely conducted in the wild and, if so, largely focus on only the first few weeks of life. To fill this gap, we tested developmental links between early-life stress and the physiology of wild-caught juveniles later during development. Specifically, we examined potential associations between feather corticosterone levels of hatchling house finches (*Haemorrhous mexicanus*) and several phenotypic and physiological traits measured several months later in juveniles. We assessed four indices of health (oxidative damage to lipids, innate immunity, intestinal parasite infection intensity and plumage colour) and two morphological traits (body mass and tarsus length) in juveniles. Feather corticosterone content was not related to any of the juvenile traits later in development. Our results suggest that physiological variables can change rapidly during ontogeny, such that stress hormone levels in juvenile feathers could be uncoupled from the real stress levels experienced by nestlings. Instead, juvenile physiology might be more dependent on current environmental conditions than on early-life conditions (i.e. environmental matching), and this may limit the effects on fitness of poor early-developmental conditions.

**ADDITIONAL KEYWORDS:** body size – carotenoid pigmentation – *Haemorrhous mexicanus* – immunity – oxidative stress – parasitism – steroids.

## INTRODUCTION

The conditions that individuals experience during early life can have effects on their growth, physiology and behaviour that can persist into adulthood (Roff, 1992; Mousseau & Fox, 1998; Metcalfe & Monaghan, 2001; Larcombe *et al.*, 2017). Many of these early-life experiences are stressful (e.g. parasite burden, sibling competition, food shortages), and these are often accompanied by an increased secretion of stress hormones released by the

hypothalamic–pituitary–adrenal axis of the endocrine system (e.g. glucocorticoids; Harris & Seckl, 2011; Crespi *et al.*, 2012). These stress hormones can affect short-term behaviour and physiology in developing animals (Blas, 2006; Grava *et al.*, 2013) and can alter the organization of morphological, physiological, neurological and behavioural traits throughout life (Love & Williams, 2008; Spencer & MacDougall-Shackleton, 2011; Farrell *et al.*, 2015). For example, developmental stress has been suggested to increase oxidative stress and affect ageing in a wide variety of organisms, including mice (Gibson, Garratt & Brooks, 2015), fish (Kishi, 2014), snakes (Bronikowski & Vleck, 2010) and humans (de Rooij & Roseboom,

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2013). In addition, elevated levels of stress hormones or exposure to environmental stressors during development can have short-term effects on growth and immune responses (Saino *et al.*, 2003; Martin *et al.*, 2005; Loiseau *et al.*, 2008) and long-term effects on immunity (Berghänel *et al.*, 2016; Danese & Lewis, 2017) and parasite resistance (Devevey *et al.*, 2010).

In recent years, birds have emerged as popular subjects for investigating how the early developmental environment shapes the phenotype, mainly because the distinct egg stage in their development allows for relative ease in separating pre-hatch vs. post-hatch effects (Crino & Breuner, 2015). Although it is relatively easy to measure levels of stress hormones from eggs (i.e. Hayward & Wingfield, 2004), studies on long-term effects of post-hatch developmental stress have lagged behind, mainly because measuring glucocorticoid levels during development in wild animals can be challenging because of the rapid nature of stress responses (Romero & Reed, 2005) and the possible confounding effect of handling stress (Romero & Romero, 2002; Hamalainen *et al.*, 2014). A method for measuring levels of the stress hormone corticosterone (CORT) deposited in growing feathers was developed a decade ago to overcome this problem (Bortolotti *et al.*, 2008). Corticosterone is deposited into feathers throughout feather growth and thus its levels in plumage reflecting individual differences in stress level and adrenocortical response (i.e. integrating baseline level, magnitude and time course of stress responses, and number/types of stressors experienced) over several weeks (Bortolotti *et al.*, 2008; Lattin *et al.*, 2011). Prior avian studies have shown that feather CORT levels respond to experimental manipulations of food availability (Will *et al.*, 2014) and brood size (Lodjak *et al.*, 2015). Most studies of feather CORT have been done on adult birds; therefore, more studies on juveniles are needed to understand the long-term effects of developmental stress (Martinez-Padilla *et al.*, 2013).

Here, we collected feathers grown during the first few weeks of life from young male and female house finches (*Haemorrhous mexicanus* Müller, 1776) during their first adult moult to study retrospectively the stress levels that these birds experienced during development, while assessing the physiological condition of the birds at this time (i.e. several months after hatching). We tested for relationships between feather CORT levels and four indices of current health state (oxidative damage to lipids, innate immunity, intestinal parasite infection intensity and plumage colour intensity) and two morphological traits (body mass and tarsus length).

As stressful conditions experienced during the first few weeks of life can have long-term negative effects on parasite resistance and immunity (Stjernman, Raberg & Nilsson, 2008; Kriengwatana *et al.*, 2013), we predicted that birds with higher feather CORT levels

would show decreased innate immunity and resistance to intestinal parasites. Developmental stress can also lead to accelerated ageing (Bronikowski & Vleck, 2010; Gibson *et al.*, 2015); therefore, we predicted that birds with higher feather CORT levels would have more oxidative damage. If developmental stress levels affect condition during the first adult moult, we would expect associations between the ornamental colour of a bird's first adult plumage and juvenile feather CORT levels, because sexual coloration is hypothesized to reflect individual quality (von Schantz *et al.*, 1999; Garratt & Brooks, 2012). This association could be either positive (a recent study in house finches indicated that redder adult male house finches had higher levels of feather CORT; Lendvai *et al.*, 2013) or negative (if higher stress levels interfere with feather pigmentation). Likewise, for morphological measurements (body mass, tarsus length and body condition), we could expect either positive or negative relationships with feather CORT levels, because elevated stress levels during development impair growth in some organisms (reviewed by Crino & Breuner, 2015), but accelerate growth in others (Coslovsky & Richner, 2011; Berghänel *et al.*, 2016).

## MATERIAL AND METHODS

### FIELD METHODS

A full description of the methods is available as [Supporting Information](#). From 13 to 20 September 2012, we used hanging basket traps and ground Potter traps baited with sunflower seeds (Giraudeau, Toomey & McGraw, 2012) to capture 74 moulting hatch-year house finches (37 females and 37 males) on the Arizona State University campus (Tempe, AZ, USA). At capture, birds were completing their first pre-basic plumage moult. Juvenile plumage was distinguished from basic female plumage by the broad buffy proximal edges of upper surfaces of secondaries, tertials and greater and median secondary coverts (Badyaev, Belloni & Hill, 2012). We determined body mass (to the nearest 0.1 g with a digital scale), tarsus length (to the nearest 0.1 mm with digital callipers) and body condition (calculated as mass/length residuals). We also collected 150 µL of whole blood from the alar vein with heparinized capillary tubes. Blood was centrifuged (10 000g for 3 min) and the plasma saved at -80 °C for later analysis of innate immunity and oxidative damage to lipids (see below). Source data are available as [Supporting Information](#).

### MEASUREMENT OF FEATHER CORTICOSTERONE CONTENT

We collected the four outer wing covert feathers of the juvenile plumage to quantify corticosterone levels. The CORT content were measured using

radioimmunoassay (RIA), following the method described by [Lendvai \*et al.\* \(2013\)](#). The results were corrected by feather mass (as described by [Lendvai \*et al.\*, 2013](#)), because the length of the covert feathers could be determined less reliably than their mass, and because the four feathers from the same individuals were pooled before extraction. Feather CORT corrected by feather mass was unrelated to sample mass. The intra-assay coefficient of variation was 4.27%.

#### QUANTIFYING COCCIDIAN PARASITES

We assessed the coccidian infection intensity of each bird from faecal samples collected in the afternoon and evening (i.e. after 16:30 h), which is when coccidian oocysts are shed, via standard faecal-float and microscope-evaluation methods ([Brawner, Hill & Sundermann, 2000](#); [Giraudeau \*et al.\*, 2014](#)). Coccidians inhabit the gut lining of birds and are thought to disrupt nutrient uptake, thereby affecting the health and appearance of birds ([Brawner \*et al.\*, 2000](#); [Pap \*et al.\*, 2009](#)). The coccidian oocyst load was estimated with a light microscope on an integer scale from zero to five, as follows: 0, no oocysts present; 1, 1–10 oocysts present; 2, 11–100 oocysts present; 3, 101–1000 oocysts present; 4, 1001–10 000 oocysts present; and 5,  $\geq 10\,000$  oocysts present.

#### AGGLUTINATION AND LYSIS ASSAY

We used the haemolysis–haemagglutination assay to assess the strength of innate immune responses. Agglutination of foreign red blood cells is a measure of the concentration of natural antibodies (produced before antigen exposure) that assist in foreign particle removal and complement-mediated lysis. Lysis assesses the ability of the plasma to destroy foreign cells by rupturing them ([Matson, Ricklefs & Klasing, 2005](#)). We followed the protocol developed by [Matson \*et al.\* \(2005\)](#), which was then modified by [Moeller, Butler & DeNardo \(2013\)](#) and [Butler \*et al.\*, \(2013\)](#). Details of this method are available as [Supporting Information](#). Two persons independently scored the plates blind to sample identity. We previously showed that their lysis and agglutination scores were highly repeatable for both agglutination and lysis ([Davies \*et al.\*, 2015](#)).

#### MEASUREMENT OF LIPID PEROXIDATION

Following the description by [Giraudeau \*et al.\* \(2014\)](#), we used a commercially available kit (Oxi-Tek TBARS assay kit; ZeptoMetrix Corp., Buffalo, NY, USA) to assess oxidative damage to lipids in the form of the concentration of malondialdehydes (MDA) from plasma. The thiobarbituric acid reactive substances (TBARS)-based colorimetric method is widely used

in ecological studies because it is convenient, simple and low cost, but it is criticized for the inherent problems of data specificity (reactivity towards other compounds other than MDA) and variability ([Halliwell & Gutteridge, 2007](#)), suggesting caution in interpreting results obtained with this method. Details of this method are available as [Supporting Information](#). Sample concentrations are expressed as nanomoles per millilitre of MDA equivalents. Higher values correspond to greater oxidative damage.

#### CAROTENOID-BASED COLORATION

At capture, we digitally photographed each male to measure the expression of ornamental plumage coloration, following the methods published by [Giraudeau \*et al.\* \(2014\)](#). Using a Canon PowerShot SD1200S (Lake Success, NY, USA), we took two separate photographs of the breast of each bird against a neutral grey-board, using identical distance from camera to object, shutter, exposure and flash settings for each photograph. Digital images (JPEG, 3648 × 2736 pixels) were imported into Adobe Photoshop (San Jose, CA, USA) to determine the plumage hue.

#### STATISTICS

All statistical analyses were carried out using Statistica software (StatSoft, Tulsa, OK, USA). We used separate analyses of covariance (ANCOVAs) to test for the effects of feather CORT, sex and their interaction on the physiological and morphological traits measured. To eliminate the possible problem of collinearity, we tested whether feather CORT levels differed for sexes. This was not the case [mean for males  $6.99 \pm 0.73$  (SE), mean for females  $7.44 \pm 0.68$  (SE),  $t$ -test  $P = 0.66$ ,  $t = -0.44$ ]. We  $\log_{10}$ -transformed the TBARS and hue data to normalize them. No transformation could normalize the coccidia and immunity data, so we ranked them ([Conover & Iman, 1981](#)) before analyses. Given that CORT analyses were run on two plates, we included plate number as a cofactor in all analyses. This factor was significant for the hue model only, and was therefore removed together with the non-significant sex × feather CORT interaction from all other final analyses. Full models ([Table S1](#)) and source data ([Table S2](#)) are presented in [Supplementary Material](#).

#### RESULTS

The mean SEM value for feather corticosterone was  $7.23 \pm 4.23$  pg/mg, minimum 0.70 pg/mg and maximum 22.21 pg/mg. Feather corticosterone levels were not significantly correlated with any of the

variables measured (Table 1, Fig. 1). There were no intercorrelations between variables (all  $P > 0.1$ ) except for agglutinations scores that were correlated with oxidative damage levels ( $F_{1,43} = 7.68$ ,  $P = 0.0082$ ) and lysis scores ( $F_{1,44} = 61.60$ ,  $P = 10^{-6}$ ) and hue values that were correlated with coccidian levels ( $F_{1,28} = 5.74$ ,  $P = 0.023$ ) and oxidative damage levels ( $F_{1,33} = 4.17$ ,  $P = 0.049$ ). The only trait that was affected by the sex of the bird was body condition, which was lower for females (Table 1, Fig. 1).

## DISCUSSION

Here, we examined possible relationships between CORT levels in juvenile feathers and morphological and physiological measurements several months after fledging in hatch-year house finches. Feather CORT content was not related to four indices of health (oxidative damage to lipids, innate immunity, intestinal parasite infection intensity and plumage color intensity) or to body mass, tarsus length or body condition.

Our negative results seem to suggest that house finches display a significant amount of developmental plasticity, growing out of the effects of varying stress hormone levels during the nestling period by the time they have moulted into their first adult plumage. Previous studies have shown that nestling feather CORT is related to condition when the latter is measured concurrently with CORT secretion in feathers. For example, strong associations between feather CORT and nestling body condition have been shown to exist in tree swallows (*Tachycineta bicolor*;

Harms *et al.*, 2010) and black kites (*Milvus migrans*; López-Jiménez *et al.*, 2016) and between fledging success and feather CORT levels in northern flickers (*Colaptes auratus*; Gow & Wiebe, 2014) and tree swallows (Fairhurst *et al.*, 2013).

Another possibility is that CORT levels might be uncoupled from the stress levels experienced by nestlings, so that the physiological condition of the birds would not be related to their feather CORT at the nestling age. For example, a recent study on European starlings (*Sturnus vulgaris*) showed that there was no difference in feather CORT between nestlings with unpredictable access to food and those with continuous access, indicating that feather CORT might not always detect ecologically relevant stressors in the nestling period (Fischer, Rao & Romero, 2017). Young birds may be limited in their abilities to perform many of the adult-like responses to overcome stressful situations and, as a consequence, an adult-like adrenocortical response to stress might expose chicks to chronic CORT elevations, with potentially deleterious consequences for development, without any rapid benefits in terms of survival (Sims & Holberton, 2000; Kitaysky *et al.*, 2003; Blas *et al.*, 2006).

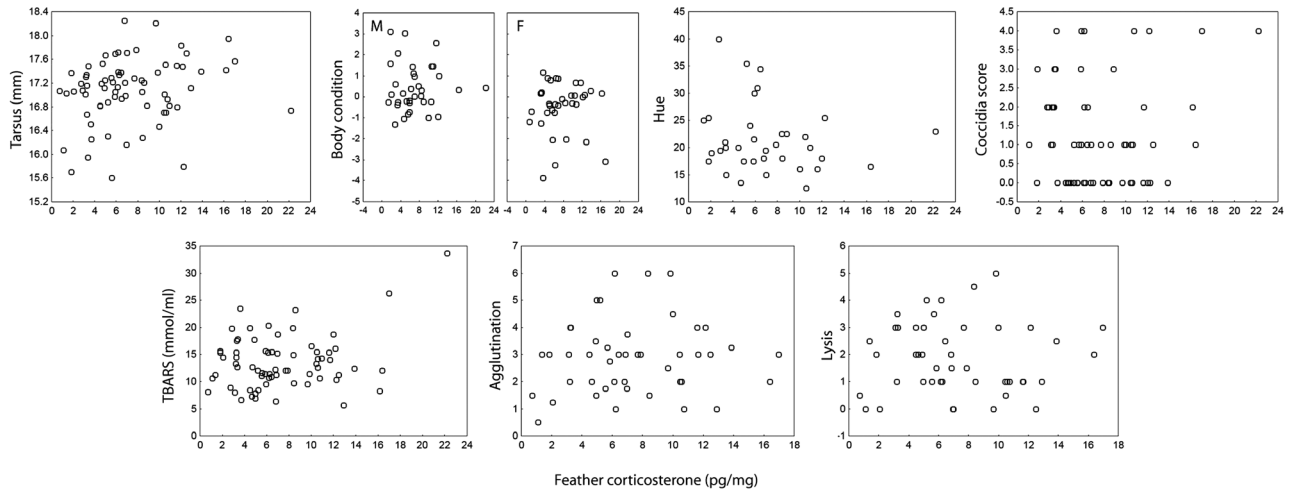
This lack of connection between condition and the level of stress responses could be mediated by, for example, plasma corticosteroid binding globulins, which can regulate the general availability of steroid to tissues (Malisch & Breuner, 2010), or the number of CORT receptors on the target cells (Lattin, Waldron-Francis & Romero, 2013). Accordingly, some non-precocial nestlings have been shown to display very low stress responses at the beginning of the nestling period, but the typical adrenocortical pattern of fully

**Table 1.** Results of ANCOVAs examining the effects of sex and feather corticosterone levels on various physiological and morphological variables

Dependent variable	Factor	d.f.	$F$	$\eta^2$	$P$ -value
Tarsus length	fCORT	1,68	2.99	0.04	0.088
	Sex	1,68	0.42	0.006	0.52
Body condition	fCORT	1,68	0.004	<0.0001	0.95
	Sex	1,68	10.26	0.13	0.002*
Oxidative damage (TBARS)	fCORT	1,66	3.43	0.007	0.07
	Sex	1,66	3.4	0.002	0.07
Immunity (agglutination)	fCORT	1,42	0.24	0.005	0.63
	Sex	1,42	0.68	0.01	0.41
Immunity (lysis)	fCORT	1,44	0.00008	0.0002	0.99
	Sex	1,44	3.22	0.09	0.08
Coccidia infection	fCORT	1,56	0.15	0.01	0.7
	Sex	1,56	0.12	0.0006	0.91
Colour (hue)	fCORT	1,32	0.02	0.0006	0.89
	Assay number	1,32	6.13	0.16	0.019*

Abbreviations: fCORT: feather corticosterone; TBARS, thiobarbituric acid reactive substances.  $\eta^2$  denotes effect sizes in the models. \*Statistically significant values.





**Figure 1.** Associations between the levels of corticosterone deposited in the feathers during the nestling age and traits related to physiological condition measured during first adult moult in male (M) and female (F) house finches. Feather hue was measured only for male birds, because females generally lack carotenoid-based coloration.

developed birds near fledging (Magellanic penguin, *Spheniscus magellanicus*, Walker, Wingfield & Boersma, 2005; white storks, *Ciconia ciconia*, Blas *et al.*, 2006; American kestrel, *Falco sparverius*, Love & Williams, 2008).

Given that we measured only birds that had reached the fledgling phase, selective disappearance of birds experiencing high levels of stress (and, as a result, a lower health state) during the nestling period cannot be ruled out. Additional data on feather corticosterone levels and survival until the juvenile stage are necessary to gain a full understanding of the ecological meaning of juvenile feather corticosterone levels.

In conclusion, we found that natural variation in feather CORT levels during development were uncoupled from physiological and morphological parameters measured several months later in life. This is one of the first studies to test the impact of developmental stress on the physiology later in life in wild animals in their natural environment. We propose the following hypotheses to explain these negative results: (1) birds in our study population might display high levels of developmental plasticity and, as they approach maturation, their physiological condition might be uncoupled from the levels of stress experienced during the nestling period; (2) stress hormone levels in nestlings might be uncoupled from the stress levels that the nestlings experience; and (3) natural conditions allow environmental matching that might limit the effects on fitness of poor developmental conditions. Future studies on the associations between nestling feather corticosterone levels, reproductive success, behaviour and lifespan in wild animals might considerably add to our

knowledge about the lifetime effects of developmental stress.

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### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Results of ANCOVAs examining the effects of sex and feather corticosterone levels on various physiological and morphological variables.  $\eta^2$  denotes effect sizes in the models.

**Table S2.** Source data.