biology letters
Physiology

Biol. Lett. (2012) **8**, 312–315
doi:10.1098/rsbl.2011.0865
Published online 19 October 2011

Coping with the extremes: stress physiology varies between winter and summer in breeding opportunists

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Seasonal changes in stress steroid hormone secretions are thought to reflect investment in self-maintenance versus reproduction. The capricious conditions hypothesis (CCH) posits that reduced corticosterone (CORT) secretion during stress coincident with parental phases of breeding is necessary in harsh environments because a full response would otherwise trigger repeated nest abandonments. To test this hypothesis, we measured seasonal changes in stress physiology in free-living red crossbills (*Loxia curvirostra*), an opportunistically breeding songbird that regularly breeds in summer and winter. This species allows unique comparisons of breeding physiology under very different seasonal environmental conditions within locations. We found strong support for the CCH: red crossbills showed reduced CORT secretion only when in high reproductive condition in the winter, when compared with summer breeders and winter non-breeders. These data demonstrate that behavioural status and local environmental conditions interact to affect mechanisms underlying investment trade-offs, presumably in a way that maximizes lifetime reproductive success.

Keywords: corticosterone; reproduction; red crossbill; songbird

1. INTRODUCTION

A central question in biology is how animals partition investment in survival and reproduction to maximize fitness. Hormones are hypothesized to mediate investment by signalling broad-scale adjustments in physiology and behaviour in response to environmental change, thereby facilitating plasticity. Steroids involved in the stress response (cortisol, corticosterone, hereafter 'CORT') are known to shift adult behaviour towards self-maintenance [1–4]. Stress-induced hormone secretions are, however, modulated seasonally in many species [1,5], presumably to maximize fitness under different environmental pressures and species-specific life histories.

High levels of stress-induced CORT (hereafter 'induced CORT') can promote nest abandonment in birds, thus some species show reduced hormonal responsiveness to stressors during parental stages of breeding [6]. Selection favouring this type of reduced investment in self may be particularly strong when conditions limit future reproductive opportunities [7]. The capricious conditions hypothesis (CCH) predicts that species breeding under harsh conditions should secrete less CORT in response to stressors or be behaviourally insensitive to elevated CORT during parental phases of breeding to promote nest maintenance [3,7]. Most studies investigating this hypothesis compare differences across populations at different latitudes, elevations or locations that vary in degree of exposure to stress or breeding season length [8]. Some of these studies detected invariant induced CORT during parental phases [9,10], or even increased hormonal stress responsiveness in harsh conditions [11]. It is unknown if between-species or between-population comparisons generate this confound, or if conflicting results reveal a more complex story involving other regulatory hormones such as prolactin [9,10]. Alternatively, variation may reflect multiple evolved mechanisms for downregulating stress responsiveness (e.g. through corticosteroid-binding globulins, CBGs; [3,12,13]).

This study tests the CCH in an entirely novel way, using red crossbills (*Loxia curvirostra*), which are songbirds that can breed opportunistically both in summer and winter if conifer seeds are abundant. This flexible breeding behaviour allows a unique comparison of stress physiology in harsh versus benign conditions but at the same mid-temperate latitude locations and within the same species. The CCH predicts that breeding red crossbills will show (i) lower induced CORT and/or higher CBG during winter than during summer, and (ii) lower induced CORT and/or higher CBG than non-breeders within each breeding season. The CCH does not make specific predictions concerning baseline CORT levels unless birds are captured during an acute phase stress response. This prediction is not investigated here.

2. MATERIAL AND METHODS

One hundred and eighty-seven free-living adult red crossbills (Groth's types 3 and 5; see electronic supplementary material, table S1) were captured in mist nets from 2003 to 2007 in winter (January through to March) and summer (July through to September) in two regions: Washington coast (123°39′ W) between 46°23′ N and 46°27′ N, and Grand Teton National Park in northwest Wyoming (43°45′ N, 110°39′ W). Average monthly temperature was lower and wind speed and precipitation higher in winter at both locations (*t*-tests; $p < 0.05$; electronic supplementary material, figure S1).

Sampling methods are described in detail in the electronic supplementary material, methods section. Briefly, blood samples for hormone analysis were collected using a standard handling stress protocol (i.e. baseline within 3 min post-capture and induced at 30 min post-capture). Individuals were assigned to broad categories of high or low reproductive potential by cloacal protuberance length or brood patch stage [14,15], which significantly predicts testis size and ovary stage in red crossbills ($F_{2,334} = 40.3$, $r^2 = 0.20$, $p < 0.0001$ males; $F_{2,170} = 59.2$, $r^2 = 0.41$, $p < 0.0001$ females; electronic supplementary material, figure S2).

Plasma CORT was determined using enzyme-immunoassay kits (Assay Designs, no. 901-097; Ann Arbor, MI, USA) as described previously [16]. Samples were run in duplicate on 32 plates, each with a separate standard curve and hormone standard. Inter-plate variation was 12 per cent, intra-assay variation was 7.3 per cent

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2011.0865 or via http://rsbl.royalsocietypublishing.org.

Received 2 September 2011
Accepted 26 September 2011

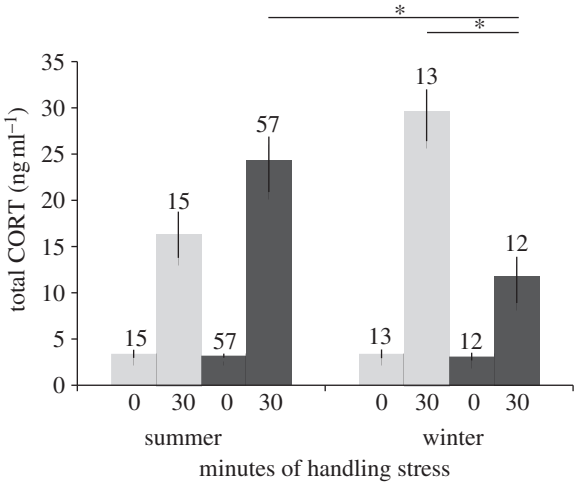

Figure 1. Seasonal differences in total corticosterone (CORT) secretion in free-living red crossbills of varying reproductive potential. Birds in high reproductive potential showed lower induced CORT secretion in the winter when compared with summer and when compared with non-breeders in the winter. $*p < 0.05$ by Tukey's test. Sample sizes and s.e.m. given (grey bars, low reproductive potential; black bars, high reproductive potential).

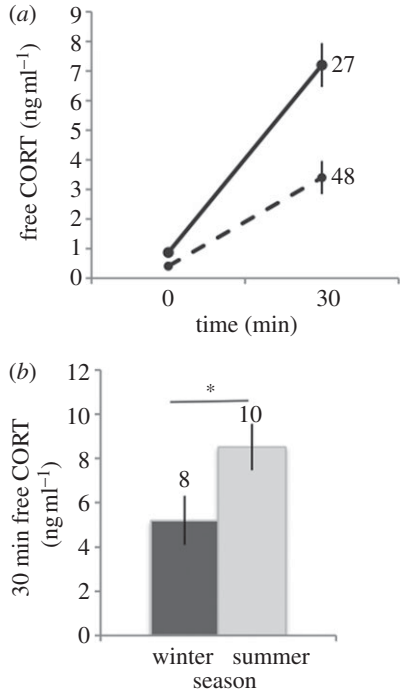

Figure 2. Free corticosterone (CORT) plasma concentrations in free-living red crossbills. (*a*) Changes in free CORT during a handling stress protocol differ by region/ecotype ($F_{1,57} = 23$, $p < 0.0001$). Solid line represents Grand Teton (type 5) and dashed line represents Washington coast (type 3). (*b*) Free CORT at 30 min of handling stress is lower in winter birds with high breeding potential when compared to the same group in the summer in the Grand Teton region. $*p < 0.05$ by Tukey's test. Sample sizes and s.e.m. are given.

and detectability was 1.9 ng ml^{-1}. CBG capacity was measured using a tritiated-corticosterone ligand-binding assay as described by Breuner & Orchinik [17] and was optimized for red crossbills as described by Cornelius *et al.* [18]. Samples were run in triplicate on 33 filters. Inter-filter variation was 14 per cent and between-triplicate variation was less than 7 per cent. Free CORT concentrations were estimated using the equation of Barsano & Baumann as in Lynn *et al.* [19].

All hormone levels and CBG capacities were square root transformed. Planned pairwise comparisons in total and free CORT levels and CBG capacity were performed by repeated-measure ANOVA with time since capture as the within-subjects variable and sex, region, season, reproductive potential and a season by reproductive potential interaction as between-subjects variables.

3. RESULTS

Total CORT increased following 30 min of handling stress ($F_{1,92} = 253$, $p < 0.0001$). The increase in total CORT during handling was significantly affected by the interaction between reproductive potential and season ($F_{1,92} = 15.1$, $p = 0.0002$), but not by sex ($p = 0.59$), reproductive potential ($p = 0.21$), season ($p = 0.6$) or sampling region ($p = 0.36$). Birds with high reproductive potential had lower 30 min total CORT in winter than in summer, and lower 30 min total CORT than birds in low reproductive potential in the winter but not in the summer (figure 1; Tukey's $p < 0.05$). Baseline CORT did not vary between reproductive potentials within or between seasons.

Free CORT plasma concentrations increased ($F_{1,57} = 62$, $p < 0.0001$) and there was a trend for CBG capacity to decline ($F_{1,59} = 3.3$, $p = 0.07$) following 30 min of handling stress. Neither CBG nor free CORT was affected by season ($p = 0.10$, $p = 0.51$), reproductive potential ($p = 0.08$, $p = 0.51$) and the reproductive potential-by-season interaction ($p = 0.55$, $p = 0.32$) during handling. By contrast, both measures were significantly affected by sampling region (CBG: $F_{1,59} = 6.2$, $p = 0.02$; free CORT: $F_{1,57} = 23$, $p < 0.0001$). Induced free CORT was higher in Wyoming than in Washington (figure 2*a*; Tukey's $p < 0.05$). Sample sizes for 30 min CBG capacity limited regional analyses to within the Grand Teton region: birds in high reproductive potential had lower free CORT levels in the winter than they did in the summer (figure 2*b*; Tukey's $p < 0.05$), similar to total CORT measures.

4. DISCUSSION

Red crossbills experience colder, wetter conditions during the winter breeding season when compared with the summer breeding season. As predicted by the CCH, red crossbills in high reproductive potential had lower induced total CORT secretion in the winter than in summer at the same mid-temperate breeding locales (figure 1). Further, birds with high reproductive potential showed reduced stress responsiveness relative to non-breeders in the winter (figure 1). This pattern is expected to enhance reproductive success by reducing CORT-induced nest abandonment in winter. CBG capacity and free CORT showed no relationship to season or reproductive potential but did vary between sampling regions (figure 2*a*). Region, however, cannot be separated from ecotype in this study (see electronic supplementary material, methods section). This effect may therefore represent either evolved differences between ecotypes or environment-dependent plasticity in CBG physiology. Sample sizes for free CORT preclude an overall analysis of a

region–season–reproductive potential interaction effect. However, winter breeders had significantly lower induced free CORT than summer breeders within the Grand Teton region (figure 2*b*), suggesting that CBG capacity does not change the overall pattern found in total CORT secretion.

These data support the CCH; however, other field data have suggested a more complex relationship. Alternative mechanisms of control such as CBG and receptor expression [7], proximity to refugia [20] and brood value [21,22] have all been invoked to explain variable results. In harsh desert environments, for example, individuals nesting far from refugia suppress the stress response whereas those near refugia maintain it, presumably because distant refugia cannot be used without abandoning the nest [20]. Similarly, some alpine species may make short trips to nearby feeding refugia at lower elevations with relatively low risk of nest failure, possibly allowing for maintenance of the stress response system [23].

Relative brood value may also affect degree of stress suppression. Species that are limited to a single nesting attempt may reduce investment in self (e.g. reduce hormonal stress responsiveness) to promote nest maintenance because brood value is high [22,24]. Red crossbills have one of the longest breeding seasons of any temperate zone songbird species. A very long breeding season reduces value of individual broods, yet crossbills still show suppression of the hypothalamic-pituitary-adrenal axis during winter breeding. Brood value in red crossbills, however, may be less related to breeding season length and more related to long-term unpredictability of cone crops (i.e. strong inter-annual variation in seed abundance inflates brood value). It is noteworthy, however, that crossbills breeding in the summer do not downregulate the stress response, suggesting that downregulation is specifically related to winter environmental conditions rather than to crossbills' general dependence on an unpredictable resource.

In conclusion, we found strong support for the CCH in free-living red crossbills. These data suggest that birds modulate hormonal stress responsiveness relative to environmental conditions but dependent on behavioural and physiological context, presumably in a way that maximizes fitness.

Data were collected under Animal Care and Use Protocol no. 15059 and all relevant state and federal permits.

We thank The Murie Centre, Grand Teton NP, Del Norte SP and the Columbia River Land Trust for access to field sites and Taylor Chapple, Kendra Sewall and Rodd Kelsey for assistance in the field. Funding was provided by NSF grants IBN-0235911 and IOS-0744705 to T.P.H. and P.E.O., A.R.C.S. and UC-Davis fellowships to J.M.C.

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