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Coping with extremes: remarkably blunt adrenocortical responses to acute stress in two sympatric snow finches on the Qinghai-Tibet Plateau during winter relative to other seasons.

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ABSTRACT

The extreme climatic conditions (ECCs) of the Qinghai-Tibet Plateau impose strong selective pressures on the evolution of phenotypic traits in free-living animals. It is not well understood how animals on the Qinghai-Tibet Plateau modify their adrenocortical functions in response to both predictable and unpredictable events of ECCs, especially when the available resources are lowest during the wintering life-history stage. To uncover potential physiological mechanisms, we studied the life history stage dependent features of morphology, the plasma corticosterone response to acute stress and brain glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA expression in two sympatric snow finches: the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF); and the rufous-necked snow finch, *Montifringilla ruficollis*, RNSF) in Qinghai Province, China. Our results showed that (a) baseline corticosterone and stressor-induced corticosterone levels significantly varied with life history stage, but not between the species; (b) in WRSF, GR mRNA expression in the paraventricular nucleus was higher in the wintering stage compared to the pre-basic molt stage. There were no differences in hippocampus MR mRNA expression between stages in either species; (c) in the wintering stage, the suppression of corticosterone secretion in both species was an unexpected strategy in free-living animals. Both convergent and divergent phenotypic traits of adrenocortical responses to acute stress in two sympatric snow finches contribute to our understanding of the coping mechanisms of closely related species in the severe winter on the Qinghai-Tibet Plateau.

43 **Keywords:** corticosterone, corticosterone receptor, extreme climatic conditions,
44 Qinghai-Tibet Plateau, snow finch, stress response

1. Introduction

Extreme climatic conditions (ECCs) are characterized by dramatic variations such as high frequencies and long durations of environmental climatic events. These can occur on a predictable schedule or as unpredictable perturbations (Van de Pol et al., 2017; Wingfield et al., 2017). For free-living animals, ECCs function as unique and critical driving forces on the evolution of both genotypic and phenotypic traits (Møller, 2011; Bailey and Pol, 2016; Natarajan et al., 2016). The relationships among life histories, physiology, and phylogeny are critical for understanding how genotypes and phenotypes evolve with ECCs (Roff, 1992; Laiolo et al., 2015). Currently little is known about the mechanisms by which free-living animals modify their phenotypic traits in response to both predictable life-history cycles and unpredictable, life-threatening, perturbations (Richardson et al., 2003; Li et al., 2008, 2011; Wingfield et al., 2017). Furthermore, there is little or no information on whether physiological traits necessary to cope with ECCs are highly conserved in closely related species (Richardson et al., 2003; Li et al., 2008, 2012; Wingfield et al., 2017). As such it is important to uncover the physiological coping mechanisms in free-living animals responding to ECCs (Bailey and Pol, 2016; Wingfield et al., 2017).

In vertebrates, the hypothalamic-pituitary-adrenal (HPA) axis, has been shown to be one of the fundamental mediators of coping mechanisms as hormones from the HPA orchestrate a suite of behavioral and physiological traits in response to both predictable and unpredictable environmental stimuli (Romero, 2002; Landys et al., 2006; Wingfield et al., 2017). Generally, baseline circulating glucocorticoid levels regulate, for example, metabolism and

osmoregulation *via* the mineralocorticoid receptor (MR) in response to predictable perturbations (Sapolsky et al., 2000; Romero, 2002; Landys et al., 2006). In free-living birds, baseline corticosterone levels vary with life-history stages, with peaks occurring during the breeding and nadirs in the pre-basic molt life history stage (Romero, 2002). Such fine-tuned variations in baseline corticosterone levels are associated with life history stage dependent daily life processes and physiological activities (reviewed by Romero, 2002; Romero et al., 2005; Romero and Wingfield, 2016).

In contrast, stressor-induced glucocorticoid levels are often involved in redirecting physiological and behavioral responses by activation of the emergency life history stage through binding the low-affinity glucocorticoid receptor (GR) in response to unpredictable life-threatening perturbations (Romero and Wingfield, 2016; Wingfield et al., 2017). Short-term increases in glucocorticoid levels can produce “leave it” or “take it” behavioral strategies through suppression of “unnecessary” functions so that all available energy resources can be devoted to self-preservation (Sapolsky et al., 2000; Wingfield and Kitaysky, 2002). Previous evidence had shown that the lowest amplitude of stressor-induced corticosterone levels tends to occur in the pre-basic molt life history stage, but the greatest amplitude of stress response occurs in other LHSs such as breeding and winter (Romero, 2002; Lattin et al., 2016; Romero and Wingfield, 2016). Furthermore, stressor-induced corticosterone levels can also vary with breeding sub-stages, reflecting substantial trade-offs between reproduction and immediate survival (Li et al., 2016). Therefore, both life history stage dependent baseline and stressor-induced corticosterone variations in free-living birds

provide quantifiable ways to identify coping mechanisms of physiological and behavioral responses to extreme environmental conditions (Wingfield et al., 2017).

In recent years an increasing number of studies have focused on life history stage dependent strategies of adrenocortical responses to acute stress (Romero, 2002; Lattin et al., 2016; Romero and Wingfield, 2016). To date, there are no consistent patterns in stressor-induced corticosterone levels for those species breeding in ECCs (Wingfield et al., 1994, 1995, 2017). For example, several avian species nesting in the Arctic or desert areas suppress stressor-induced corticosterone levels relative to other LHSs but others do not (Wingfield et al., 1992, 1995; Walker et al., 2015; Krause et al., 2016; Romero and Wingfield, 2016). Indeed, similar patterns of stress response modulation determined in closely related species during specific life history stages regardless of environment, indicate that corticosterone responses may also be highly conserved and affected by phylogeny (Breuner et al., 2003; Li et al., 2008, 2011, 2012). To date no studies have investigated whether life history stage dependent corticosterone stress responses are similar in closely related co-occurring species that live year round in extreme environments, especially during the wintering stage when environmental conditions can be severe.

Actions of glucocorticoids mediated by binding to intra-cellular receptors (MR and GR) in target tissues (Proszkowiec-Weglarz and Porter, 2010; Krause et al., 2015). Previous studies in the arctic-breeding white-crowned sparrows (*Zonotrichia leucophrys gambelii*) during the pre-parental sub-stage of breeding showed higher brain MR mRNA expression, but not GR mRNA, compared to the parental sub-stage (Breuner et al., 2003; Krause et al., 2015).

To the contrary, temperate-zone breeding house sparrows (*Passer domesticus*) had the highest brain GR binding capacity for corticosterone but not MR binding capacity in the pre-breeding sub-stage compared to the breeding sub-stages (Lattin and Romero, 2013). How brain GR and MR levels vary with life history stage, and between closely related species in ECCs remains largely unknown.

The Qinghai-Tibet Plateau is one of the largest and harshest environments for life in the world (Wang et al., 2008), characterized by highly predictable and unpredictable climatic conditions. Unlike lowland temperate-zone areas, the alpine meadow ecosystem on the Qinghai-Tibet Plateau has a dramatic seasonal fluctuation of gross primary productivity (Kato et al., 2004). Environmental resources available to free-living birds are lowest during winter, and highest during summer. From the perspectives of energetic cost and perturbation resistance potential, determined by body condition, food resource availability, location etc. (Wingfield et al., 2011, 2017), increased corticosterone stress responses may come at a cost of consumption of energetic resources for self-preservation rather than for a LHS such as breeding (Sapolsky et al., 2000; Wingfield and Kitaysky, 2002). Under extreme conditions, a stronger corticosterone stress response may not be an optimal strategy and even may result in high mortality (Wingfield and Ramenofsky, 2011). On the contrary, a blunted corticosterone response may be a better strategy when the energetic costs of emergency life history stage exceed the current cumulative energetic resources available (McEwen and Wingfield, 2003; Wingfield et al., 2017). Therefore, it is important to explore how free-living animals on the Qinghai-Tibet Plateau adjust their adrenocortical responses to acute stress when the external

environment is at its most severe in winter, *i.e.*, reduced food resources, extreme cold, and increased energy-demands of interspecific and intraspecific competition. Here, we hypothesized that free-living birds on the Qinghai-Tibet Plateau in winter have lower stressor-induced corticosterone responses, when energetic costs are increasing, relative to those in summer. Furthermore, we predict that such a life history stage dependent coping style would be highly conserved among closely related species.

The white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) and the Rufous-necked snow finch (*Montifringilla ruficollis*, RNSF) are both native to the Qinghai-Tibet Plateau, and are distributed almost sympatrically year-round as typical resident species (Lu et al., 2009; the phylogenetic relationships in the snow finch complex are shown in Fig. S1, supplementary material). To evaluate our above hypothesis, we determined the life history stage dependent features of body mass, body condition, and adrenocortical response to capture-restraint stress in WRSF and RNSF across the early breeding, late breeding, pre-basic molt, and wintering life history stages. We further examined brain GR and MR mRNA expression in the two species during the breeding and wintering stages. We predicted that (1) both WRSF and RSNF would exhibit conserved features in stress physiology (baseline and stressor-induced corticosterone levels, expression of MR and GR) across life history stages because they share similar climatic and habitat conditions; (2) In the wintering life history stage, both WRSF and RSNF would also express suppressed corticosterone response-related features relative to other life history stages due to strong selective pressure of extreme environments on the coping mechanisms of physiology and behavior.

2. Materials and methods

2.1. Animals and study locations

Free-living adult WRSFs and RNSFs were studied at Qinghai Lake (37°02.216' N 99°44.293' E, Elevation, 3215 m), Wenquan (35°24.258' N 99°25.876' E, Elevation, 3946 m), Huashixia (35°06.345' N 98°51.390' E, Elevation, 4106 m), Maduo (34°54.821' N 98°12.598' E, Elevation, 4268m), and Nangqian (32°20.92' N, 96°45.761' E, Elevation, 4100m) Qinghai Province, China (Fig. S2, supplementary material). Samples were collected in the spring (April 17 to May 25; when birds were establishing territories, mating and egg-laying, i.e. the early breeding sub-stage), summer (July 2 to 17; the nestling period, i.e. the late breeding sub-stage), Autumn (August 1 to 26; the pre-basic molt stage), and winter (January 23 to February 10; also called the non-breeding stage) of 2006-2008. Sample sizes from each life history stage for the measurements of morphology, corticosterone response, and corticosterone receptors are summarized in Table S1 (supplementary material).

2.2. Capture-restraint stress protocol and sampling

All birds were captured opportunistically using mist nets followed by a standardized capture stress protocol to assess sensitivity of the HPA axis in response to acute unpredictable stressors (Wingfield et al., 1992; Li et al., 2008). To reduce variation in circulating corticosterone levels due to daily biological rhythms, all the birds were sampled from 0800 h to 1300 h during winter and spring (short day-length) and from 0700 h to 1200 h during

summer and autumn (long day-length). Within 3 min of capture, approximately 40 μ l of blood was collected aseptically by piercing the alar vein using a 26-gauge needle. The blood was transferred into heparinized microhematocrit capillary tubes and plasma assayed for baseline corticosterone levels. After initial blood sampling, the birds were then placed in opaque cloth bags, and subsequent blood samples were collected at 10 min, 30 min, and 60 min intervals after capture to create a plasma profile of acute corticosterone secretion during continued handling, and restraint. Blood samples were stored on ice in the field for 3–4 h before they were centrifuged at 855g for 10 min. Plasma samples were separated and stored at -20°C until assay.

After blood sampling, each bird was weighed to the nearest 0.1g, scored for furcular fat on a semi-quantitative scale of 0 (no fat visible) to 5 (bulging fat deposit; Wingfield and Farner, 1978). Wing, tarsus, and beak lengths were also measured. To estimate the absolute size of energy reserves, we calculated a body condition index for each species by using mass/size residuals (*i.e.*, the principal component of wing, beak and tarsus lengths). Since both WRSF and RNSF are monomorphic species, sex identification was unavailable for those individuals during the wintering and the early breeding stages. However, sex identification for both species in the late breeding and pre-basic molt life history stages was determined by the presence or absence of a brood patch (a female-specific trait). Finally, all birds captured were individually marked for field identification with a numbered metal leg band and then released.

Birds sampled for tissues were euthanized within 3 min of capture using isoflurane inhalation followed by decapitation. Whole brains were wrapped in aluminum foil and frozen

in liquid nitrogen until they could be moved to a -80 °C storage freezer. All protocols were approved by the Institutional Animal Care and Use Committees of the Institute of Zoology, Chinese Academy of Sciences, China, the University of Washington, Seattle and the University of California, Davis, USA, and were carried out under the auspices of scientific collecting permits issued by the Departments of Wildlife Conservation (Forestry Bureau) of Qinghai Province, China.

2.3. Corticosterone assays

The corticosterone assay followed the protocols of Wingfield et al. (1992) and Li et al. (2008). Briefly, 10-15 µl of plasma was equilibrated overnight with 2000 cpm of ³H-corticosterone to determine individual recoveries. Samples were extracted in 4 ml of freshly redistilled dichloromethane, dried under nitrogen and re-suspended in 550 µl phosphate-buffered saline with 1% gelatin. All samples were run in duplicate, and assay values were corrected for plasma volume and individual recoveries (ranged from 87.3% to 99.4%). Inter-assay and intra-assay coefficients of variation were 12.6% and 9.1%, respectively.

2.4. In situ hybridization histochemistry for MR and GR mRNA

The methodology for MR and GR *in-situ* hybridization procedures have been previously described in detail (Dickens et al., 2009). Whole brains were sectioned coronally at 15 µm on a cryostat and sections were thaw-mounted onto polysine, RNAase free, and pre-treated glass microscope slides. Marker slides were created by collecting every sixth section for staining using cresyl violet. Slides were stored at -80 °C with silica pellets as desiccant until *in-situ*

hybridization was performed. Slides were selected for *in-situ* hybridization after investigating marker slides in conjunction with the canary, *Serinus canaria*, stereotaxic atlas (Stokes et al., 1974) to identify regions of interest. Briefly, 500-bp fragments of the zebra finch GR (Genbank: XM_002186722) or MR (Genbank: DQ539433) were sub-cloned into PGEM-7. GR sense and antisense riboprobes in the presence of 35S-UTP, with SP6- and T7-RNA polymerase were generated by *in-vitro* transcription, after plasmid linearization with EcoRI or HindIII, respectively. Similarly, the MR sense and antisense riboprobes in the presence of 35S-UTP, with T7- and SP6-RNA polymerase were developed by *in-vitro* transcription after plasmid linearization with HindIII or ApaI, respectively. Both the MR and GR genes are highly conserved, with identities between chicken (galliform, *Gallus gallus*) and zebra finch (passerine, *Taeniopygia guttata*) of 90% and 88%, respectively (Dickens et al., 2009). Slides were dipped in the autoradiographic emulsion, air-dried, and left to expose in sealed boxes for 5 weeks. The slides were then developed and counterstained with hematoxylin-eosin, dehydrated, and cover slipped with DPX mountant (Sigma, St Louis, MO, USA). Hybridization of sections with GR or MR sense riboprobes, or pre-treatment with RNase-A prior to hybridization with the GR or MR antisense riboprobes, did not result in any detectable hybridization signal.

2.5. Quantification of relative silver grain density for GR mRNA and MR mRNA

Slides were examined under bright field microscopy. Sections containing the paraventricular nucleus and hippocampus were determined in combination with the canary stereotaxic atlas (Stokes et al., 1974), and the marker slides containing hematoxylin-eosin counterstaining to locate brains regions and reveal neuroanatomical landmarks. Images were

captured at $\times 20$ magnification using a Nikon E600 microscope, Zeiss Axiocam 105 color camera, and Zen Capture Software.

Expression of GR or MR mRNA was quantified by determining the density of silver grains using ImageJ analysis software (NIH, Bethesda, MD, USA) over an area of known size (0.01260 or 0.01234 mm²). The proportion of the set area overlaid with silver grain was recorded as an average of 12 measurements per bird (taken over two consecutive sections) for both paraventricular nucleus (GR) and hippocampus (MR). The percentage of background silver grain was also noted over the same given area for each section and subtracted to correct for any inconsistencies. All slides were coded so that the identity of the groups was unknown during image analysis.

2.6. Statistical analysis

The maximal corticosterone level was calculated as the capture-restraint stressor-induced maximum level each individual achieved over the 60 minute restraint period. This level is generally much higher than baseline corticosterone level (i.e. the plasma level of corticosterone in the first sample collected – within 3 minutes of capture). The total integrated corticosterone values were determined as the area under curve calculated by trapezoid rule using Graph Pad Prism, *version 5.01* (Graph Pad Software Inc., San Diego, CA, USA; Li et al., 2008). Fold change was determined by using the ratio of maximal corticosterone and baseline corticosterone levels. To determine the potential effects of sex differences during the late breeding and pre-basic molt stages, we compared body mass, fat score, body condition and corticosterone stress response using independent sample *t*-tests between sexes in the

WRSF and the RNSF. Given that there were no significant differences in those measurements (Table S2, supplementary material), we then combined data from both sexes of each species during a specific stage in following analysis.

For those variables that met normality (body condition, maximal corticosterone, fold change, total integrated corticosterone, GR mRNA expression), we examined potential differences using a linear mixed model (LMM) fitted with the restricted maximum likelihood (REML) method in SPSS 21.0. Specifically, the fixed effects of species, life history stage and sub-stage, and the interaction between species and life history stage, with year and site as random factors were tested. For those variables that did not meet normality (body mass, fat score, MR mRNA expression), we examined life history stage differences in each species using Kruskal-Wallis tests with the exception of MR mRNA expression where we used a Mann-Whitney *U* test for RNSF. Species differences of these variables within a life history stage were also compared using a Mann-Whitney *U* tests. Differences between pairs of means were identified by Bonferroni-adjusted *post hoc* tests based on model-predicted estimated marginal means in either LMMs or Kruskal-Wallis tests. Effect sizes were estimated using *Cohen's d* for *U* tests and partial omega-squared (ω^2) for LMM or ANOVA to measure the strength of statistically significant differences between groups (Elis, 2010). Differences were considered significant at $p < 0.05$. All data are presented as means $\pm$ SEM.

3. Results

3.1. Variations of morphological traits across life history stages and between species

Body mass and body condition of both WRSF and RNSF varied with life history stages (Tables 1 and 2). WRSFs were lighter and in worse body condition while RNSFs were heavier and in better condition during the wintering relative to the pre-basic molt life history stages (Fig. 1A,B; Table S3, supplementary material). WRSFs were heavier than RNSFs during the early breeding, late breeding and pre-basic molt life history stages, in better condition during the pre-basic molt, but in worse condition during the winter than RNSFs (Table 1, Fig. 1A-B).

The fat score of both species varied across life history stages, with significantly more fat in winter and early breeding relative to late breeding and pre-basic molt (Table 2, Fig. 1C; Table S3, supplementary material). WRSF had significantly greater fat stores than RNSF during late breeding, but less fat in pre-basic molt (Fig. 1C; Table S3, supplementary material).

3.2. Variations of baseline and stressor-induced corticosterone levels across life history stages and between species

Baseline corticosterone levels of both species varied across life history stages (Table 1). *Post hoc* tests showed that WRSF had significantly higher baseline corticosterone levels during early breeding and winter relative to pre-basic molt, whereas RNSF exhibited higher baseline corticosterone levels during the winter compared to both late breeding and pre-basic molt (Fig. 2A; Table S3, supplementary material). However, baseline corticosterone levels did not vary between species at different life history stages (Table 1; Fig. 2A).

Stressor-induced maximal corticosterone levels, total integrated corticosterone levels and fold change also did not vary with species but were different across life history stages (Table 2;

Fig. 2B-D). *Post hoc* tests showed that both species had significantly lowered maximal corticosterone levels, total integrated corticosterone levels, and fold change during winter than those from other life history stages (Fig. 2B-D, F; Table S3, supplementary material). Maximal corticosterone and total integrated corticosterone levels of WRSF were significantly lower during pre-basic molt relative to both early and late breeding. Maximal corticosterone and total integrated corticosterone levels of RNSF did not vary across early breeding, late breeding, and pre-basic molt. Fold change also showed no differences with these life history stages in both species (Fig. 2B-D; Table S3, supplementary material).

3.3. Variations of corticosterone receptors across life history stages and between species

GR mRNA varied with life history stage, but MR mRNA did not (Tables 1 and 2). *Post hoc* results revealed no differences in GR mRNA between early breeding and winter in both species. However, WRSF had significantly higher levels of GR mRNA in the wintering life history stage compared with those during the pre-basic molt life history stage (n.a. for RNSF because we had no comparable data during the pre-basic molt life history stage; Figs. 3-4; Table S3, supplementary material). Both GR mRNA and MR mRNA did not vary with species (Tables 1 and 2).

4. Discussion

We determined life history stage dependent variations in baseline and stressor-induced corticosterone levels, and brain MR and GR mRNA expression of both WRSF and RSNF. As

predicted, we identified a highly conserved adrenocortical response to acute stress in the two closely related species. Notably, both WRSF and RSNF during the wintering life history stage exhibited markedly increased baseline, blunted stressor-induced corticosterone levels (a significantly lowered fold change) relative to other life history stages (Fig. 2). As far as we are aware, this is a unique coping strategy in response to one of the most extreme environmental exposures.

4.1. Annual changes of morphological traits in response to predictable perturbations

In birds, increased fat deposition in the non-breeding life history stage such as wintering is a physiological strategy for maintaining higher energetic reserves to cope with both the predictable and unpredictable events at that time (Witter and Cuthill, 1993; Rogers and Reed, 2003). Consistently, we found that both WRSF and RSNF exhibited similar patterns in annual changes of fat deposition as observed for other passerines (Pravosudov and Grubb, 1997; Cresswell, 1998). However, we found markedly lowered body mass in WRSF, whereas mass increased in RSNF during the wintering life history stage relative to others. Such species-dependent differences may result from the divergences in both behavioral and ecological traits in relation to life history stages. For example, in the wintering life history stage, WRSFs outcompeted RSNFs in rural areas (away from the buildings of human beings) ate more seeds. By contrast, RSNFs moved to urban areas where resources and shelters were less intensely competitive and fed on starchy materials from human food waste (Li et al., under review). Overall, the underlying physiological mechanism of such distinct differences in morphology warrants for further investigation.

4.2. Annual changes of corticosterone response traits in relation to predictable and unpredictable perturbations

The nadirs of baseline circulating corticosterone levels in both species occurred in the pre-basic molt life history stage, which is in line with previous findings in several other avian species (Romero, 2002; Cornelius et al., 2011; Romero and Wingfield, 2016). The reduced baseline corticosterone levels at this period can prevent protein catabolic activity, resulting in higher quality of feathers (Romero, 2002; Romero et al., 2005; Cornelius et al., 2011). Surprisingly, there were no significant differences in baseline corticosterone levels between the late breeding and pre-basic molt life history stages, which is in line with an invasive species - Eurasian tree sparrows (*Passer montanus*) on the Qinghai-Tibet Plateau (Li et al., 2008; Qu et al., 2019). Corticosterone can promote energy catabolism through several metabolic pathways to support energy demand for physiological and behavioral processes (Romero, 2002; Romero et al., 2005). Whether such a strategy of lowered baseline corticosterone levels after raising young successfully can divert resources for energy accumulation when the environmental resources are in the most abundant periods remains to be clarified. On the other hand, peak baseline corticosterone levels occurred both in the wintering and early breeding life history stages of WRSF, and in the wintering life history stage for RNSF. Our results differ from those findings showing upregulated baseline corticosterone levels are necessary for providing the extra energy needed for mating and territory defense during breeding (Romero, 2002; Goymann et al., 2006; Wada et al., 2006; Love et al., 2014). Generally, increased baseline corticosterone levels are associated with

poorer body condition, contributing to resource allocation towards immediate survival when the environment becomes more challenging (Romero, 2002; Bonier et al., 2009; Wingfield and Ramenofsky, 2011). Up-regulation of baseline corticosterone in WRSFs and RNSFs during the wintering and early breeding life history stages might be explained as a unique strategy for coping with the most challenging period of their environment on the Qinghai-Tibet Plateau.

The nadirs of all the stressor-induced corticosterone levels (maximal corticosterone, total integrated corticosterone, and fold change) in both WRSF and RNSF occurred in the wintering life history stage but not in the pre-basic molt stage (Fig. 2). This differs from previous findings in both migratory and non-migratory passerines (Romero, 2002; Li et al., 2008, 2012; Romero and Wingfield, 2016). Earlier studies have shown that those migratory species wintering in much less severe environments exhibited significantly stronger corticosterone response. This may be because environmental resources available in those environments can maximize perturbation resistance potential in the face of life-threatening and unpredictable perturbations (Wingfield et al., 1994, 1995, 2017; Romero, 2002). Increased stressor-induced corticosterone levels could contribute to optimizing individual survival by triggering the emergency life history stage and mobilize energy reserves to cope with the perturbation (see Landys et al., 2006; Wingfield et al., 2017). In contrast, suppressed stressor-induced corticosterone responses in non-migratory, wintering, snow finches in the severe environment of the Qinghai-Tibet Plateau may be an energy-saving strategy to resist predictable extreme winter conditions. To what extent this may increase the vulnerability of

snow finches to additional unpredictable events superimposed on winter conditions remains to be determined but such a scenario could quickly reduce perturbation resistance potential to zero or even become negative (allostatic overload type 1; see Wingfield et al., 2017). This is a concern especially in the light of global climate change. Considering that higher energetic requirements are necessary for immediate survival in such low ambient temperature during winter, whether an increase in baseline corticosterone levels and suppression in corticosterone response to a standardized stressor is a unique physiological and ecological strategy of avoiding extra costs in relation to a severe environment also remains to be determined.

To explore changes of downstream glucocorticoid receptors, we found that brain MR mRNA did not vary across life history stages, and may have a permissive effect for changing baseline corticosterone titers (Sapolsky et al., 2000; Lattin et al., 2012; Lattin and Romero, 2015). Our results are in line with those findings in other passerines indicating no significant differences in brain MR mRNA between the breeding and pre-basic molt life history stages (Breuner and Orchinik, 2001; Lattin and Romero, 2013). However, WRSFs had significantly higher levels of GR mRNA in the paraventricular nucleus of the hypothalamus during the wintering life history stage relative to the pre-basic molt stage. GR mRNA density varied seasonally (Lattin and Romero, 2015) although the exact mechanism remains to be delineated (Herman and Tasker, 2016). Therefore, wintering WRSF exhibited a remarkable suppression of the corticosterone response at the adrenal level, but a significantly increased sensitivity of negative feedback for corticosterone at the hypothalamus level. Future investigations on the

regulatory mechanisms of MR and GR gene expressions are needed for understanding the coping strategy of WRSF and RNSF in the harsh environments of the Qinghai-Tibet Plateau.

4.3. Variations of morphological and hormonal traits between species

WRSFs and RNSFs had different patterns of body mass change and body condition, *i.e.*, RNSFs were heavier and had better body condition during the winter relative to other stages, but WRSF did not. The underlying explanation may be because WRSF remained in the plateau areas often distant from human-occupied sites, while RNSF shifted their winter range to human-occupied sites so as to take advantage of human resources and shelters (Li et al., under review). However, there were no significant differences in baseline and stress-induced corticosterone levels, brain GR and MR mRNA between WRSF and RNSF at any point indicating that coping strategies of stress physiology may be highly conserved traits in the harsh environment of the Qinghai-Tibet Plateau. Such a conserved strategy of stress responses in relation to phylogeny also occurs in other passerines, e.g., the genus *Passer* (Li et al., 2008) and *Carduelis* (Li et al., 2012).

5. Conclusions

Our studies revealed that peak baseline corticosterone levels occurred in the wintering and early breeding life history stages for WRSF and in the wintering life history stage for RNSF. The two closely related species also showed similar changes in stressor-induced corticosterone levels, fold change, and seasonal changes in GR and MR mRNA expressions. In the wintering life history stage, the increase of baseline corticosterone levels and the suppression of corticosterone secretion in response to acute stress in both species may be a

unique coping mechanism and specific energy-saving life history stage-dependent strategy because they have limited perturbation resistance potential at this time. Therefore, the convergent pattern of the life history stage-dependent adrenocortical response to acute stress in these two closely related species may reflect highly conserved mechanisms of hormonal regulation in response to both predictable environmental conditions and potential additional unpredictable environmental perturbations. This unique strategy may be derived from strong selective pressure of extreme environments of the Qinghai-Tibet Plateau. Our work in snow finches native to the Qinghai-Tibet Plateau contributes to our understanding of the coping mechanisms in severe environments, especially in the most challenging season when there are limited cumulative energy resources. Species differences, even when closely related, reveal how adjustments in morphology physiology and behavior may change according to local conditions such as favoring human-occupied sites versus remaining in rural habitats. Furthermore, measuring changes in down-stream components of the adrenocortical response to acute stress such as MR and GR also reveal novel potential mechanisms of coping with environmental change.

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Author contributions

F.L. and J.C.W. conceived the ideas and design methodology, and D.L., J.E.D., G.W., V.R.B., and S.L.M. contributed to data collection and laboratory assays; D.L. analysed the data and led the writing of the manuscript with the help of G.N. and Y.S. All authors contributed critically to the drafts and gave final approval for publication.

Additional information

Competing Interests: The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data may be found online in the Appendix A section at the end of the article.

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Figures

Fig. 1. Comparisons of morphological variables during the early breeding, late breeding, pre-basic molt, and wintering life history stages in the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) and the rufous-necked snow finch (*Montifringilla ruficollis*, RNSF). A) Body mass, B) body condition, C) fat score. Groups with different letters are significantly different from one another by life history stages or by species ($p < 0.05$).

Fig. 2. Comparisons of corticosterone (cort) responses during the early breeding, late breeding, pre-basic molt, and wintering life history stages in the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) and the rufous-necked snow finch (*Montifringilla ruficollis*, RNSF). A) Baseline corticosterone levels, B) stressor-induced maximal corticosterone levels, C) stressor-induced total integrated corticosterone levels, D) stressor-induced fold change of corticosterone, E) stressor-induced maximal free corticosterone levels (n.a. in the wintering life history stage because samples were unavailable), F) adrenocortical responses to capture stress in WRSF and RNSF during the wintering life history stages. Groups with different letters are significantly different from one another by life-history stages or by species ($p < 0.05$).

Fig. 3. Representative photomicrographs of autoradiographs in bright field showing cells expressing glucocorticoid receptor mRNA in the paraventricular nucleus (PVN; A-E) of the hypothalamus and mineralocorticoid receptor mRNA in the hippocampus (F & G) of the white-rumped snow finch (*Onychostruthus taczanowskii*; A-C & F) and rufous-necked snow finch (*Montifringilla ruficollis*; D, E & G) during the pre-basic molt (A), early breeding (B, D, F & G), and wintering life history stages (C & E). Hybridisation is visible as silver grains (black dots) over cell bodies. 3V = Third ventricle. Scale bars = 100 µm.

Fig. 4. (A-B) Comparisons of glucocorticoid receptor (GR, A) mRNA expression in paraventricular nucleus (PVN) of the hypothalamus and mineralocorticoid receptor (MR,B) mRNA expression in hippocampus (Hp) in the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) and the rufous-necked snow finch (*Montifringilla ruficollis*, RNSF) during the early breeding, pre-basic molt (n.a. for RNSF), and wintering life history stages. Groups with different letters are significantly different from one another by life-history stages ($p < 0.05$).

Table 1

Comparisons of fat score, body mass, plasma baseline corticosterone (cort) levels, and hippocampus mineralocorticoid receptor (MR) mRNA expression in the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) and the rufous-necked snow finch (*Montifringilla ruficollis*, RNSF) among different life-history stages (the early breeding, late breeding, pre-basic molt, and wintering life history stages) by Kruskal–Wallis tests, and comparisons of those variables between species at certain life history stages in Mann–Whitney U test.

Response	Species	df	χ^2 value	n	ω^2	Life-history	df	Z	n	Cohen's d
Body mass	WRSF	3.55	20.949	<0.0	0.30	Early breeding	1.2	3.999	<0.00	5.613
	RNSF	3.50	10.009	0.01	0.27	Late breeding	1.1	3.776	<0.00	4.360
						Pre-basic molt	1.2	4.751	<0.00	6.510

						Wintering	1.2	-0.021	0.984	0.065
Fat score	WRSF	3.55	39.727	<0.0	0.42	Early breeding	1.2	-0.743	0.474	0.284
	RNSF	3.51	43.748	<0.0	0.56	Late breeding	1.1	3.373	0.007	1.886
						Pre-basic molt	1.2	-2.39	0.017	0.901
						Wintering	1.2	-1.384	0.166	0.575
Baseline cort	WRSF	3.48	16.074	0.00	0.12	Early breeding	1.2	1.919	0.057	1.041
	RNSF	3.40	32.722	<0.0	0.63	Late breeding	1.1	0.001	1.000	0.680

Variable	Body mass, fat score, and body condition		Corticosterone response		Mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) mRNA	
	WRSF	RNSF	WRSF	RNSF	WRSF	RNSF
Early breeding	25	7	25	6	5	5
Late breeding	9	13	8	13	n.a.	n.a.
Pre-basic molt	15	15	15	16	6	n.a.
Wintering	11	20	6	14	5	10

						Pre-basic molt	1.2	0.519	0.624	0.581
						Wintering	1.1	-1.228	0.219	0.725
MR mRNA	WRSF	2.13	0.781	0.67	-0.0	Early breeding	1.6	-0.447	0.786	0.627
	RNSF	1.13	18.000	0.44	0.05	Late breeding	n.a.	n.a.	n.a.	n.a.
						Pre-basic molt	n.a.	n.a.	n.a.	n.a.
						Wintering	1.1	1.269	0.230	0.800

Partial omega-squared (ω^2) and Cohen's d are measures of effect size for Kruskal–Wallis tests and Mann–Whitney U test, respectively. Significant factors ($p < 0.05$) and medium ($\omega^2 > 0.06$; Cohen's $d > 0.5$) or large size effects ($\omega^2 > 0.14$;

Table S1 Sample size of body mass, fat score, body condition, corticosterone (cort) response, brain mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) mRNA in the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) and the Rufous-necked snow finch (*Montifringilla ruficollis*, RNSF) during the early breeding, late breeding, pre-basic molt, and wintering life history stages.

Table S2 Comparisons of body mass, fat score, body condition, and corticosterone (cort) response in independent sample *t*-tests between sexes in the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) and the rufous-necked snow finch (*Montifringilla ruficollis*, RNSF) during the late breeding and pre-basic molt life history stages.

Variable	WRSF			RNSF								
	Late breeding			Pre-basic molt			Late breeding			Pre-basic molt		
	<i>t</i> value	df	<i>p</i> value	<i>t</i> value	df	<i>p</i> value	<i>t</i> value	df	<i>p</i> value	<i>t</i> value	df	<i>p</i> value
Body mass	-0.067	6	0.949	1.165	14	0.264	0.697	11	0.500	-0.280	13	0.784
Fat score	-0.509	7	0.626	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	2.902	9	0.018
Body condition index	-0.216	6	0.836	0.813	13	0.431	-0.417	11	0.685	-0.064	13	0.950
Baseline cort	-1.215	5.211	0.276	-0.725	13	0.481	0.841	8.743	0.423	0.128	9.299	0.901
Maximal cort	-0.943	6	0.382	0.876	14	0.396	0.013	10	0.990	-0.210	14	0.836
Fold increase	-0.832	6	0.437	0.848	12	0.413	-0.373	10	0.717	0.718	14	0.485
Total integrated cort	n.a.	n.a.	n.a.	0.081	9.822	0.937	-0.173	10	0.866	-0.792	14	0.441
There were not enough sample sizes for male WRSFs in the late breeding stage for total integrated cort levels.												

Variable	Species	Life-history stage	Late breeding	Pre-basic molt	Wintering
Body mass [*]	WRSF	Early breeding	0.341	0.067	0.292
		Late breeding		0.001	1.000
		Pre-basic molt			0.001
	RNSF	Early breeding	1.000	1.000	0.208
		Late breeding		1.000	0.041
		Pre-basic molt			0.141
Body condition	WRSF	Early breeding	1.000	0.008	<0.001
		Late breeding		0.004	0.010
		Pre-basic molt			<0.001
	RNSF	Early breeding	1.000	0.662	0.013
		Late breeding		1.000	0.035
		Pre-basic molt			0.384
Fat score [*]	WRSF	Early breeding	0.029	<0.001	1.000
		Late breeding		0.535	0.046
		Pre-basic molt			<0.001
	RNSF	Early breeding	0.001	0.005	1.000
		Late breeding		1.000	<0.001
		Pre-basic molt			<0.001
Baseline cort [*]	WRSF	Early breeding	0.307	0.002	1.000
		Late breeding		1.000	0.419
		Pre-basic molt			0.030
	RNSF	Early breeding	0.285	0.030	1.000
		Late breeding		1.000	<0.001
		Pre-basic molt			<0.001
Maximal cort	WRSF	Early breeding	1.000	<0.001	<0.001
		Late breeding		0.008	<0.001
		Pre-basic molt			<0.001
	RNSF	Early breeding	0.849	1.000	<0.001
		Late breeding		0.341	<0.001
		Pre-basic molt			<0.001
Total integrated cort	WRSF	Early breeding	1.000	<0.001	<0.001

		Late breeding		0.006	<0.001
		Pre-basic molt			0.001
	RNSF	Early breeding	1.000	1.000	<0.001
		Late breeding		0.227	<0.001
		Pre-basic molt			0.001
Fold increase	WRSF	Early breeding	0.059	1.000	<0.001
		Late breeding		0.323	<0.001
		Pre-basic molt			0.001
	RNSF	Early breeding	0.131	0.618	<0.001
		Late breeding		1.000	<0.001
		Pre-basic molt			0.037
PVN GR mRNA	WRSF	Early breeding	n.a.	0.256	0.874
		Late breeding		n.a.	n.a.
		Pre-basic molt			0.020
	RNSF	Early breeding	n.a.	n.a.	0.920
		Late breeding		n.a.	n.a.
		Pre-basic molt			n.a.
Significant factors ($p < 0.05$) are shown in bold type.					

Table S3 Multiple comparison results of significant variables (see Tables 1 and 2) across different life-history stages (the early breeding, late breeding, pre-basic molt, and wintering stages) from Kruskal–Wallis tests (variable with asterisk) or linear mixed model (LMM) in the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) or the rufous-necked snow finch (*Montifringilla ruficollis*, RNSF).

Table 2 The comparisons of body condition index, plasma corticosterone (cort) response (maximal corticosterone levels, fold increase, total integrated corticosterone levels, baseline and maximal free corticosterone levels), and paraventricular nucleus glucocorticoid receptor (GR) mRNA expression in the white-rumped snow finch (*Onychostruthus taczanowskii*, WRSF) and the rufous-necked snow finch (*Montifringilla ruficollis*, RNSF) during the early breeding, late breeding, pre-basic molt, and wintering life history stages in a linear mixed model (LMM) by considering species, life-history stage (stage), and interaction of species and life history stage as fixed factors, and sampling site and year as random factors.

Response variable	Mixed model	df	F value	p value	ω^2	Response variable	Mixed model	df	F value	p value	ω^2
Body condition index	Intercept	1, 104	10.321	0.002		Maximal cort	Intercept	1, 92	642.926	<0.001	
	Species	1, 104	1.929	0.168	0.004		Species	1, 92	2.038	0.157	0.001
	Stage	3, 104	7.923	<0.001	0.067		Stage	3, 92	40.606	<0.001	0.479
	Species × stage	3, 104	18.700	<0.001	0.299		Species × stage	3, 92	1.271	0.289	0.007
Fold increase	Intercept	1, 98	1314.721	<0.001		Total integrated cort	Intercept	1, 91	1069.402	<0.001	
	Species	1, 98	0.772	0.382	-0.002		Species	1, 91	0.201	0.655	0.023
	Stage	3, 98	63.240	<0.001	0.584		Stage	3, 91	45.737	<0.001	0.604
	Species × stage	3, 98	1.922	0.131	0.003		Species × stage	3, 91	2.617	0.056	0.017
GR mRNA	Intercept	1, 23	113.738	<0.001							
	Species	1, 23	0.278	0.603	0.002						
	Stage	2, 23	4.491	0.023	0.244						
	Species × stage	1, 23	0.616	0.44	0.025						

	stage			0							
Partial omega-squared (ω^2) is a measure of effect size for LMMs. Significant factors ($p < 0.05$) and medium ($\omega^2 > 0.06$) or large size effects ($\omega^2 > 0.14$) are shown in bold type.											

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