

Baseline corticosterone and stress response in the Thorn-tailed Rayadito (*Aphrastura spinicauda*) along a latitudinal gradient



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ABSTRACT

Glucocorticoids are essential for life and their secretion is regulated by the hypothalamic–pituitary–adrenal axis (HPA). The HPA axis is often divided into two components: baseline glucocorticoids levels and stress response glucocorticoids levels, which are affected by changes in ambient temperature and productivity among others factors. An approximation to evaluate how a species copes with these changes is to evaluate differences of this hormone amongst populations of the same species that inhabit places ideally presenting all the possible combinations of temperature and productivity. We aimed to evaluate whether environmental temperature or productivity, represent challenges in terms of stress in the Thorn-tailed Rayadito (*Aphrastura spinicauda*). We examined circulating baseline levels of CORT and stress responses from three populations, covering the whole geographic distribution of the species across large gradients in weather conditions. If low temperature influences baseline CORT levels, we expect higher levels of this hormone in the southernmost population (higher latitude). However, if productivity is the factor that influences baseline CORT levels, we expect the contrary pattern, that is, lower values of this hormone in the southernmost population (more productive environment). We observed that baseline CORT levels presented lower values in the southernmost population, supporting the environmental productivity hypothesis. Secondly, we tested the hypothesis that individuals breeding at higher latitudes should have a lower stress response than individuals breeding at lower latitudes. Contrary to our expectations, we found that stress response did not vary among populations in any of the three years. We concluded that low environmental temperatures did not represent a stress situation for the Thorn-tailed Rayadito if food abundance was sufficient to support energetic demands.

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1. Introduction

Responses to biotic and abiotic cues typically involve the hypothalamic–pituitary–adrenal cortex (HPA) axis, culminating in the release of glucocorticoids (e.g., Sapolsky et al., 2000; Wingfield, 2003; Romero et al., 2009). Glucocorticoids are essential for life as they regulate or support a variety of important cardiovascular, metabolic, immunologic, and maintain daily homeostatic energetic balance (Harvey et al., 1984; Dallman et al., 1993). The HPA axis is often divided into two components: baseline glucocorticoids levels and stress response glucocorticoids levels (Romero, 2004). The first is an approximation of the seasonal baseline level that the animal should maintain to be able to cope with the predictable demands of the current life-history stage (Landys et al., 2006; Bókony et al., 2009), so it reflects long-term adaptation. The former, (stress response: the increase in baseline glucocorticoids levels to the

level reached in 30 min) best reflect short-term plastic responses to environmental perturbations (Romero, 2002; Wingfield, 2013).

In birds, unexpected periods of inclement weather, such as storms, or low environmental temperatures can increase baseline corticosterone (CORT) (e.g., Passeriformes: Wingfield et al. 1983; Wingfield, 1985; Rogers et al., 1993; Breuner and Hahn, 2003; Ouyang et al., 2012; Apodiformes: Bize et al., 2010; Pelicaniformes: Smith et al., 1994; and Charadriiformes: Satterthwaite et al., 2012), the primary glucocorticoid in birds (Wingfield, 1994; Sapolsky et al., 2000; Romero, 2004). In addition, de Bruijn and Romero (2011) observed that small drops in ambient temperature (e.g., 3 °C) mimicking a natural storm, was sufficient to increase baseline CORT levels, in the European Starlings (*Sturnus vulgaris*), presumably due to the higher energetic cost demanded by low temperatures.

Not only low ambient temperatures, but also low habitat quality (i.e., low food availability or low productivity) can raise baseline CORT levels. Increased levels of this hormone, as a result of low habitat quality, has been registered in Black-legged Kittiwakes

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(*Rissa tridactyla* – Kitaysky et al., 1999; Lanctot et al., 2003; Buck et al., 2007), Common Murres (*Uria aalge* – Kitaysky et al., 2007), American Redstarts (*Setophaga ruticilla* – Marra and Holberton, 1998) and Barn Swallows (*Hirundo rustica* – Jenni-Eiermann et al., 2008). However difficulties arise when these two factors (temperature and productivity) co-vary and thus emerging patterns are equally consistent with alternative scenarios. For example, Ouyang et al. (2012) observed higher levels of baseline CORT levels in Great Tits, *Parus major*, in a year of low ambient temperatures that coincided with lower food availability. Separating the roles played by each of these factors is challenging, particularly in the field, because of the impossibility to manipulate ambient temperature and productivity. Although supplementary experiments can be performed, they present some disadvantages (see Desy and Batzli, 1989). So, an approximation to evaluate how a species copes with changes in temperature and productivity is to evaluate differences of this hormone amongst populations of the same species that inhabit places ideally presenting all the possible different combinations of temperature and productivity.

Here we studied three populations of a passerine bird, the Thorn-tailed Rayadito (*Aphrastura spinicauda*) in Chile. This species is ideal to evaluate whether temperature or productivity represents challenges in terms of stress, because the distribution range of this species encompasses regions with strong differences in weather condition and productivity. Compared with the central and northernmost populations, the southernmost population (lower latitude) presents lower annual temperatures, higher annual precipitations and higher productivity (Table 1). Although the northernmost and the central population are similar in the aforementioned variables, they differed in the magnitude of annual variance, as is reflected in the coefficient of variations: the central population presents more seasonality in temperatures and precipitations (Table 1). In addition Thorn-tailed Rayadito presents larger clutch sizes (a life-history trait that can influence baseline CORT levels Criscuolo et al., 2006), and higher body weights towards the south. So, if low temperature influences baseline CORT levels, now called “low temperature hypothesis”, we expected higher levels of this hormone in the southernmost population in relation to the central and northernmost populations. However if productivity is the main factor influencing baseline CORT levels, we propose: “productivity environmental hypothesis”, we expected lower values of this hormone in the southernmost population because this population represents the most productive environment.

Although baseline CORT levels serve to maintain energy balance, high levels of this hormone results in the interruption of

the normal life-history stage (such as reproduction, migration, etc.) and triggers an emergency life-history stage that allows the individual to temporarily move away from the source of perturbation or endure it, while adopting many energy saving behaviors that allow it to cope (Wingfield, 2003). In birds, elevated levels of this hormone during breeding decreases reproductive behavior promoting nest abandonment (e.g., Astheimer et al., 1992; Love et al., 2004; Moore and Zoeller, 1985; Ouyang et al., 2012; Sapolsky, 1985; Silverin, 1986; Wingfield and Silverin, 1986; Silverin et al., 1989). These observations led to the formulation of the capricious conditions hypothesis (CCH) that predicts in species breeding in extreme and unpredictable environments and with restricted breeding periods (e.g., high latitude locations), that the stress response in general should be suppressed (Cornelius et al., 2012). And that this suppression would be observed especially during the parental phase of the reproductive cycle: the breeding environmental hypothesis (Wingfield, 1994; Wingfield et al., 1995a). Although some studies have supported the CCH hypothesis, with suppression of stress response in populations at higher latitudes (e.g., Cornelius et al., 2012; Silverin et al., 1997), others have detected invariant induced CORT during parental phases (e.g., Angeles et al., 2009; Breuner et al., 2003; Chastel et al., 2005), or even increased hormonal stress responsiveness in populations at higher latitudes (e.g., Wingfield et al., 1995b). Additionally, it has been proposed that the stress response is expected to depend upon parental care strategy, and individuals of the sex expressing more parental care should have a lower stress response than individuals of the sex expressing less, or no, parental care (Wingfield et al., 1995a). So, we tested the CCH hypothesis and the parental care hypothesis in Thorn-tailed Rayadito. If the stress response is down-regulated in the higher latitude population, presumably because of more severe environment due to unpredictable snowstorms and low environmental temperature, we should observe suppression of the stress response in southernmost populations. Because the Thorn-tailed Rayadito present a socially monogamous mating system where both members of the pair contribute to nest-building, incubation, and feeding of nestlings and fledglings (Moreno et al., 2007), the parental care hypothesis predicts absence of differences in stress responses between sexes.

Investigations of northern hemisphere populations reveals modulations of the adrenocortical responses to acute stress may have ecological bases related to gender, season, social status, climatic events and presence of predators. Such bases are also important in the southern hemisphere although few studies to date have tried to determine whether southern populations modulate the

Table 1
Geographical origin, climatic data, values of reproductive function (e.g., clutch size) and biometry (mass at fledgling, mass of adults) for three populations of rayaditos during two years in Chile^a. DMi = Martonne aridity index, Clutch size = number of eggs, Nestling mass (g) = weight of nestlings at 13 days old, Adult mass (g) = weight of adults when nestling were 13 days old.

| Population | Year | Precipitations (SD) | Coefficient of variation | Temperature (SD) | Coefficient of variation | DMi (SD) | Coefficient of variation | Clutch size | Nestling mass | Adult mass |
|------------|------|---------------------|--------------------------|------------------|--------------------------|-------------|--------------------------|-------------|---------------|--------------|
| Northern | 2010 | 134.70 (1.93) | 1.43 | 10.80 (1.88) | 17.41 | 0.56 (0.67) | 119.64 | 2.71 (0.68) | 12.74 (1.43) | 11.82 (0.92) |
| | 2011 | 186.90 (2.12) | 1.13 | 11.18 (1.90) | 16.99 | 0.79 (1.79) | 226.58 | 2.59 (0.74) | 12.73 (1.73) | 11.89 (0.85) |
| Central | 2008 | 160.23 (5.69) | 3.55 | 15.04 (5.01) | 33.32 | 0.73 (1.59) | 217.81 | 3.13 (0.72) | 14.34 (1.19) | 11.62 (0.91) |
| | 2010 | 193.10 (4.59) | 2.38 | 14.31 (5.04) | 35.22 | 0.97 (1.37) | 141.24 | 3.27 (1.00) | 14.05 (1.81) | 11.15 (1.00) |
| | 2011 | 144.40 (3.69) | 2.56 | 14.44 (4.94) | 34.21 | 0.66 (1.12) | 169.69 | 3.42 (0.90) | 13.57 (1.89) | 11.07 (0.96) |
| Southern | 2008 | 325.38 (2.50) | 0.77 | 4.58 (4.09) | 89.30 | 2.05 (1.15) | 11.00 | 5.00 (0.55) | 14.37 (1.49) | 11.90 (0.79) |
| | 2010 | 412.80 (2.69) | 0.65 | 6.49 (2.82) | 43.45 | 2.10 (1.13) | 22.22 | 4.68 (1.04) | 14.75 (1.25) | 12.58 (1.53) |
| | 2011 | 340.90 (2.25) | 0.66 | 5.92 (3.64) | 61.49 | 2.16 (1.08) | 50 | 4.85 (0.93) | 14.87 (1.1) | 12.3 (0.77) |

The de Martonne (DMi) aridity index was calculated following di Castri and Hajek (1976), based on the average of monthly temperatures and precipitation. The index is low in hot, dry deserts (low productivity) and high in cool, wet areas (high productivity). The mean annual temperature (calculated as the average of the monthly temperatures) and total annual precipitation are reported. The variances of precipitation ($\sigma^2 P$) temperature ($\sigma^2 T$) and de Martonne index ($\sigma^2 DMi$) were estimated from the mean of monthly values.

^a Climatic data was collected from <<http://www.ceazamet.cl>, <http://www.meteochile.gob.cl/>>.

adrenocortical responses to stress in the same way as in the north, or whether completely novel responses may have evolved (e.g., Wingfield, 2008).

2. Methods

2.1. Biology of the Thorn-tailed Rayadito and the study populations

The Thorn-tailed Rayadito *A. spinicauda* (Furnariidae: Passeriformes) is an endemic species of the South American temperate forest (Remsen, 2003). Thorn-tailed Rayadito are small (11 g) and lay one clutch per breeding seasons during the austral spring (Moreno et al., 2005). The nest construction period lasts 6–15 days, and the incubation period is 9–15 days. Eggs are laid on alternate days and the Thorn-tailed Rayadito postpones incubation until after the clutch is complete. Because the Thorn-tailed Rayadito are secondary cavity nesters (Johnson and Goodall, 1967) they will adopt artificial boxes, that we have been monitoring, in three populations in Chile during three reproductive seasons (2008, 2010 and 2011) (Fig. 1): (1) Fray Jorge National Park (30°38' S, 71°40' W), the northernmost population (lowest latitude) of the species' distribution. In Fray Jorge National Park, there is a relic forest composed mainly of Olivillo (*Aextoxicon punctatum*), occurring in patches at the top of the coastal mountain range where fog-induced microclimatic conditions allow the forest to exist in this semiarid region (Villagrán et al., 2004). This fog-induced microclimate is reflected in the lower values of coefficient of variation in temperature and precipitation in relation to the central population located in Manquehue Hill; (2) Manquehue Hill (33°21' S, 70°34' W) is located at the edge of Santiago city and presents a xeric forest, composed mainly of Peumo (*Cryptocaria alba*), Quillay (*Quillaja saponaria*) and Litre (*Lithrea caustica*) which are characteristic of the Mediterranean climate semi-arid region of central Chile, where precipitation occurs only during the winter seasons and temperatures vary greatly during and between days; and (3) Williams Port in Navarino Island (55°4' S, 67°40' W), representing the southernmost (and thus highest latitude) limit of the species' distribution.

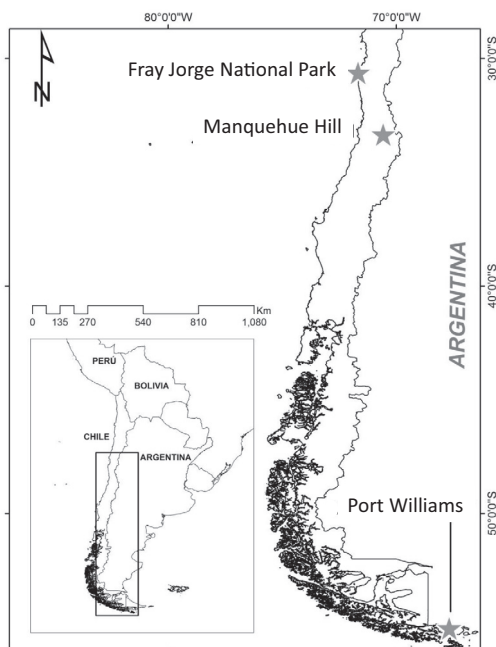


Fig. 1. Location of the three populations: Fray Jorge National Park the northernmost population (30°38' S, 71°40' W), Manquehue Hill, the central population at the edge of Santiago de Chile (33°21' S, 70°34' W) and Port Williams, Navarino Island, the southernmost population in Chile (55°4' S, 67°40' W).

At this site, the vegetation is characterized by deciduous Magellanic forest, whose characteristic species are Lenga Beech (*Nothofagus pumilio*), and Ñirre Beech (*Nothofagus antarctica*) (Rozzi et al., 2004).

2.2. Monitoring, capture procedures, blood sampling and hormone analyses

To check for nest box occupation, nest boxes were monitored on a weekly basis. When occupied, the frequency of monitoring was increased in order to detect laying dates (date of first egg), and hatching dates. Adults were captured in their nest between 0800 and 1200 h with a manually triggered metal trap that sealed the entrance hole when adults entered to feed their 12–14 day old nestlings. Blood samples (ca. 50 μ L) were obtained by puncturing the brachial vein with a sterile needle and collecting blood into heparinized micro-hematocrit capillary tubes. In our study, samples were collected within 3 min of capture. After this baseline sample, we took morphological measures (see below) and then placed the bird in a small cloth bag for the next 30 min to stimulate a stress response from capture and restraint. After that we took a second blood sample from the other brachial vein to determine the responsiveness of the hypothalamic–pituitary–adrenal axis to stressful stimuli (total blood collected was less than 1% of the bird's mass). Samples were stored on ice until the end of the sampling period (maximum of 5 h) and were then centrifuged for 5 min at 8000 rpm to separate the plasma from red blood cells. The plasma was aspirated with a Hamilton syringe and stored (at -20° C) until assayed for total CORT content (University of California, Davis). The red blood cells were stored in FTA Classic Cards (Whatman[®]) for subsequent molecular sex determination. We measured tarsus length and weight of each bird. We banded nestling and adults with individual metal bands (National Band and Tag Co., Newport, Kentucky, USA and Split Metal Bird Rings, Porzana Ltd, UK) or with a numbered band provided by the Servicio Agrícola y Ganadero (SAG), Chile under the authority of SAG, and Corporación Nacional Forestal (CONAF), Chile.

CORT levels (i.e., concentrations) in plasma were determined using direct radioimmunoassays. To determine the efficiency of hormone extraction from the plasma, 20 μ L of 2000 cpm of tritiated CORT was added to all samples and incubated overnight. Hormones were extracted from the plasma using freshly re-distilled dichloromethane. The aspirated dichloromethane phase was evaporated using a stream of nitrogen at 45° C. Samples were then reconstituted in phosphate-buffered saline with gelatin. All samples were run in duplicate, intra-assay variation for CORT ranged from 11.2% to 14.7%, inter-assay variation was 12.73%. Plasma volumes of the samples varied from 5 to 15 μ L.

In total, 274 blood samples were collected from 155 adults. In 2008, baseline CORT and stress responses were measured in the central and southernmost populations. In 2010 baseline CORT was estimated for the northernmost, central and southernmost populations and stress responses were estimated from the northernmost and central populations. In 2011, baseline CORT and stress responses were estimated from the northernmost, central and southernmost populations (Table 2).

2.3. Molecular sexing

Because the Thorn-tailed Rayadito presents an absence of clear visual sexual dimorphism, we used molecular methods to sex the adults. Details of the protocol and validation of the method are described in Quirici et al. (in revision). Briefly, DNA was extracted using a commercial kit (QIAGEN Inc., Valencia, CA). The sex of adults was determined using 2550F and 2718R primers (Fridolfsson and Ellegren, 1999). PCR products were run in 1% agarose gels,

Table 2
Sample sizes of hormone samples of *Aphrastura spinicauda*.

| Population | Year | Log CORT (ng/ml) 3 min | | Log CORT (ng/ml) 30 min | |
|------------|------|---------------------------|-------------|----------------------------|-------------|
| | | N | Mean (SE) | N | Mean (SE) |
| Northern | 2008 | – | – | – | – |
| | 2010 | 15 | 0.79 (0.11) | 10 | 1.22 (0.10) |
| | 2011 | 23 | 0.92 (0.05) | 15 | 1.46 (0.06) |
| Central | 2008 | 22 | 1.41 (0.04) | 22 | 1.89 (0.09) |
| | 2010 | 15 | 0.91 (0.12) | 11 | 1.20 (0.05) |
| | 2011 | 26 | 1.11 (0.04) | 22 | 1.48 (0.03) |
| Southern | 2008 | 29 | 1.09 (0.04) | 29 | 1.63 (0.08) |
| | 2010 | 8 | 0.49 (0.12) | – | – |
| | 2011 | 17 | 0.83 (0.09) | 10 | 1.24 (0.12) |

pre-stained with ethidium-bromide, and detected in a Fluorimager (Vilber Lourmat). Birds were sexed as females (heterogametic: WZ) when the CHD1W of 450 bp and CHD1Z of 600 bp fragments were amplified, and identified as males (homogametic: ZZ) when only the CHD1Z of 600 bp fragment was present.

2.4. Data analysis

We estimated primary productivity of each locality using the Martone aridity index (DMi) as has been used in other passerine studies in Chile (Cavieres and Sabat, 2008; Maldonado et al., 2012). DMi was calculated monthly as $DM = P/T + 10$, where P is the monthly rainfall (mm) and T is the average monthly temperature (°C). This index was empirically derived to describe primary productivity in arid and semi-arid areas and as being low in hot, dry deserts and high in cool, wet areas (see Table 1). Daily rainfall (mm) and temperature (°C) was collected from local meteorological stations; in the northernmost and southernmost population via <http://www.ceazamet.cl> and for the central population via <http://www.meteochile.gob.cl/>.

We examined the effects of year (three levels), population (three levels), and sex (two levels) on baseline CORT (ng/ml) and stress responsiveness (difference between samples taken at 30 min and baseline CORT) using factorial analysis of variance (ANOVA) for an incomplete design (type III sum of squares). Because baseline CORT level could be correlated with body weight (at lower body mass, higher baseline CORT level – Breuner and Hahn, 2003; Heidinger et al., 2006), we include body weight as a covariate. We chose weight instead of the residuals between tarsus length and body weight (Jakob et al., 1996), because it has been proposed as a better predictor of body condition (Green, 2001). CORT levels and body weights were log transformed prior to analysis to fit assumptions of normality for parametric tests.

We examined the effect of year (three levels), population (three levels), and sex (two levels) on stress response (i.e., increase in CORT level after 30 min) using ANOVA for repeated measures for an incomplete design (type III sum of squares).

To explore intra-population variation in baseline CORT levels and weather condition, we firstly performed multiple regression analysis with weather variables of the day prior (daily mean temperature: T1, daily precipitation: P1), and five days prior (the average of daily mean temperature: T5 and the average of precipitation: P5) blood samples were taken (Lobato et al., 2008). We tested four possible models for the weather the day before and five days before: (1) temperature by precipitation interactive model, (2) temperature and precipitation main effects model, (3) temperature only model, and (4) precipitation only model. The best-fit model was selected based on Akaike Information Criterion (AICc) (Akaike, 1974). The model selected had the lowest AICc value, Akaike weight approaching 0.90 or higher, evidence ratios

close to 1, models were assumed to be equivalent if delta AICc values <2 (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). Once we determined which of the weather variables influence baseline CORT, we correlated weather condition, body weight and baseline CORT levels performing a path analysis (Sokal and Rohlf, 1995). Because the Thorn-tailed Rayadito are an insectivorous species, and insects productivity is affected by weather condition (Dunn and Winkler, 2010), it is very likely that weather acts on the weight of an animal, and that changes in animal weight trigger the changes in CORT levels (i.e., “productivity environmental hypothesis”: weather \geq weight \geq CORT) (e.g., Jenni-Eiermann et al., 2008). All statistical tests were two-tailed and conducted using the R 3.0.1 software (R Development Core Team). Data are reported as mean \pm SE.

3. Results

3.1. The effect of population, year and sex on baseline CORT levels

We tested for the effect of year, population, and sex on baseline CORT levels with factorial ANOVA with body weight as a covariate. We observed no second order interaction amongst population, year and sex, or interaction between population by sex, population by year and sex by year (Table 3). There was a significant effect of year on baseline CORT (Table 3), 2008 presenting the highest values, followed by 2011 and 2010 (all Tuckey post-hoc comparisons <0.05) (Fig. 2). There was a significant effect of population on baseline CORT (Table 3), with the south populations presenting the lowest values (Tuckey post-hoc comparisons: north vs. south $P = 0.04$, central vs. south $P < 0.01$, central vs. north, $P = 0.17$). Finally, there

Table 3
Factorial analysis of variance (ANOVA) for incomplete design, with population (three levels), year (three levels) and sex (two levels) explaining baseline CORT levels (ng/ml). Body weight (gr) was considered as a co-variable.

| | Sum of square | Degree freedom | Mean square | F-test | P value |
|---------------------|---------------|----------------|-------------|----------|---------|
| Intercept | 151.40 | 1 | 151.40 | 1924.103 | <0.001 |
| Weight | 0.02 | 1 | 0.02 | 0.305 | 0.58 |
| Population | 2.12 | 2 | 1.06 | 13.261 | <0.001 |
| Year | 4.76 | 2 | 2.38 | 29.783 | <0.001 |
| Sex | 0.00 | 1 | 0.00 | 0.003 | 0.96 |
| Population*year | 0.15 | 3 | 0.05 | 0.654 | 0.58 |
| Population*sex | 0.38 | 2 | 0.19 | 2.478 | 0.09 |
| Year*sex | 0.44 | 2 | 0.22 | 2.778 | 0.07 |
| Population*year*sex | 0.60 | 3 | 0.20 | 2.550 | 0.06 |
| Error | 10.31 | 131 | 0.08 | | |

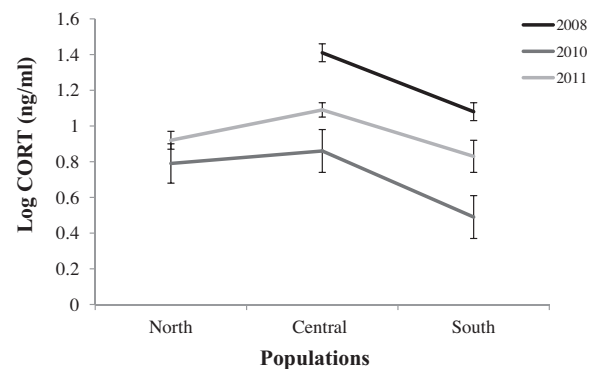


Fig. 2. Average baseline CORT (ng/ml \pm SE) in the northern (Fray Jorge National Park), central (Manquehue Hill) and southern (Port Williams) populations during 2008 (black line), 2010 (dark gray line) and 2011 (light gray line). Because there was no effect due to sex, samples were pooled together. Sample sizes are reported in Table 2.

was no effect for sex alone (Table 3) or effect from body weight (Table 3).

3.2. The effect of weather condition on baseline CORT

In the north population, the best fit, although not a well supported model for variation in baseline CORT level, was the average in precipitation five days before blood samples was taken (P5, Table 4). In the central population, the best fit although no well supported model for variation in baseline CORT level was the mean daily temperature the day before blood samples was taken model (T1, Table 4). In the south population, the best fit although no well supported model for variation in baseline CORT level was the average of the mean daily temperature 5 day before blood samples was taken model (T5, Table 4).

Based on the aforementioned analysis, we choose the more plausible weather variable affecting baseline CORT (using AIC criteria) and using path analysis (Fig. 4), we analyzed the model that weather affects body weight, and how this affected baseline CORT levels (“productivity environmental hypothesis”). In the northern population, precipitation (P5) had a strong and negative effect on body weight (path coefficient $cp = -0.41$) and a considerable, but not significant negative effect on baseline CORT ($cp = -0.21$). The path coefficient between body weight and baseline CORT ($cp = 0.03$) represents the effect of body weight on baseline CORT level for a given precipitation, not the overall effect of body weight on baseline CORT level. In the central population, temperature (T1) had a strong and positive effect on baseline CORT level (path coefficient $cp = 0.44$) and a negative and very low effect on body weight ($cp = -0.07$). The path coefficient between body weight and baseline CORT was low as well ($cp = 0.13$). In the southern population, temperature (T5) had a strong and negative effect on baseline CORT level (path coefficient $cp = -0.54$) and a negative and low effect on body weight ($cp = -0.17$). The path coefficient between body weight and baseline CORT was low as well ($cp = 0.12$).

Table 4

AICc values of the possible models explaining differences in baseline CORT (ng/ml) levels in the Northern, Central and Southern populations.

| Model and variable | AICc | Delta AICc | AICc weight | Evidence ratio | Parameters |
|--------------------|-------|------------|-------------|----------------|------------|
| <i>Northern</i> | | | | | |
| P5 | 22.72 | 0 | 0.29 | 1 | 1 |
| P1 | 23.5 | 0.78 | 0.20 | 0.68 | 1 |
| T1 | 24.27 | 1.55 | 0.13 | 0.46 | 1 |
| T5 | 24.5 | 1.78 | 0.12 | 0.41 | 1 |
| T1+P1 | 24.64 | 1.92 | 0.11 | 0.38 | 2 |
| T5+P5 | 25.4 | 2.68 | 0.08 | 0.26 | 2 |
| T1*P1 | 26.64 | 3.92 | 0.04 | 0.14 | 3 |
| T5*P5 | 26.78 | 4.06 | 0.04 | 0.13 | 3 |
| <i>Central</i> | | | | | |
| T1 | 43.79 | 0 | 0.56 | 1 | 1 |
| T5 | 44.31 | 0.52 | 0.44 | 0.77 | 1 |
| <i>Southern</i> | | | | | |
| T5 | 10.61 | 0 | 0.36 | 1 | 1 |
| T5+P5 | 10.71 | 0.1 | 0.34 | 0.95 | 2 |
| T5*P5 | 11.12 | 0.51 | 0.28 | 0.77 | 3 |
| T1+P1 | 18.05 | 7.44 | 0.01 | 0.02 | 2 |
| T1 | 18.3 | 7.69 | 0.01 | 0.021 | 1 |
| P1 | 19.53 | 8.92 | 0.00 | 0.01 | 1 |
| T1*P1 | 19.82 | 9.21 | 0.00 | 0.01 | 3 |
| P5 | 21.3 | 10.69 | 0.00 | 0.00 | 1 |

Italicized values indicate the best-fit but not well supported model. A best fit model that was well supported had the lowest AIC value, delta AIC <2, Akaike weight approaching 0.90 or higher, and evidence ratios close to 1 (Burnham and Anderson 2002; Symonds and Moussalli 2011).

North, P = 0.18.
Central P = 0.48.
South P = 0.01.

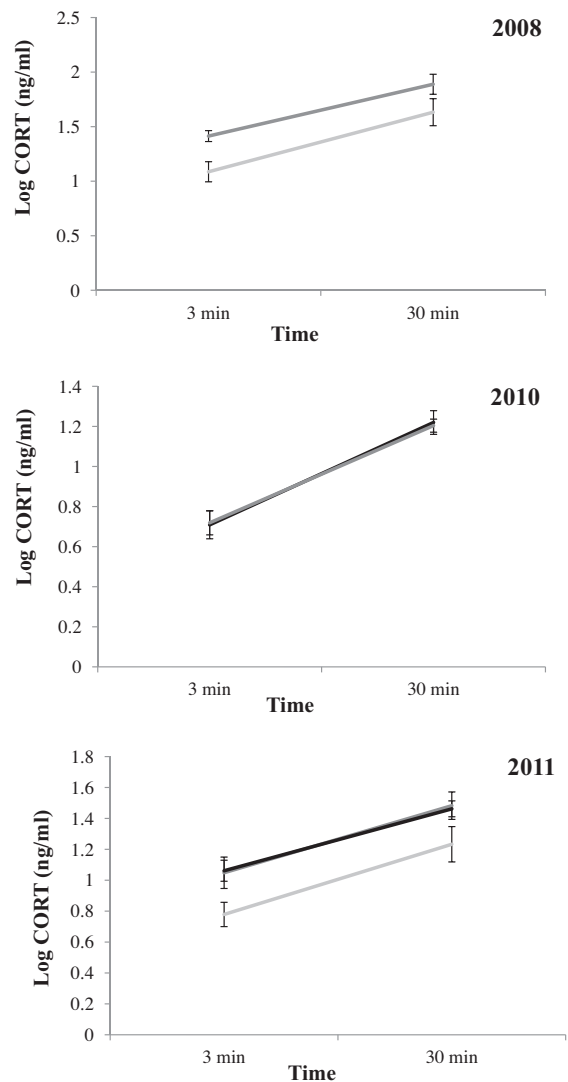


Fig. 3. Average baseline CORT (ng/ml ± SE) and stress-induced at 30 min during 2008 (upper panel), 2010 (middle panel) and 2011 (lower panel) in the northern (black line), central (dark gray line) and southern (light gray line) populations. Because there was no effect of sex, samples were pooled together. Sample sizes are reported in Table 2.

3.3. The effect of population, year and sex on stress response and stress responsiveness

We tested for effects of year, population, and sex on stress-induced CORT levels with repeated measures ANOVA. All groups of the Thorn-tailed Rayadito showed significant increases in CORT levels following capture, handling and restraint ($F = 319.76$, $df = 1,93$, $P < 0.001$). There was a significant interaction effect between year and population ($F = 8.81$, $df = 2,93$, $P < 0.01$) (Fig. 3). Because there was no effect determined by sex ($F = 3.63$, $df = 1,93$, $P = 0.06$) data was pooled together for comparisons. In 2008 (Fig. 3: upper panel), the central population showed higher levels compared with the southern population ($F = 32.60$, $df = 1,49$, $P < 0.01$). In 2010 (Fig. 3: middle panel), stress responses were similar between the northern and central populations ($F = 1.16$, $df = 1,17$, $P = 0.30$). In 2011 (Fig. 3: lower panel), stress responses differed amongst populations ($F = 8.26$, $df = 2,34$, $P < 0.01$), with the southern population presenting lower values.

We define stress responsiveness as the difference between baseline and 30 min CORT levels. Factorial ANOVA indicated there was no effect of year ($F = 0.65$, $df = 2,93$, $P = 0.53$), population

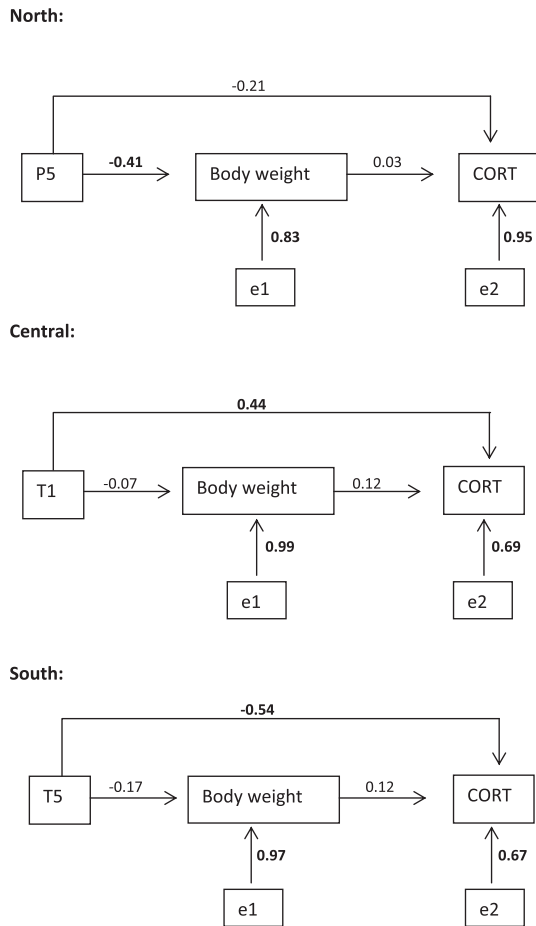


Fig. 4. Path analysis of the relationships between weather, body weight and baseline CORT levels in the northern, central and southern populations. Also shown are the residuals (e1, e2), which combine all unexplained effects and measurement errors. Indicated are the path coefficients (cp). The path coefficients give the strengths of the relationships between various pairs of variables when the influences of variables before are accounted for. Values in bold indicate path coefficients statistically significant ($P < 0.05$).

($F = 1.45$, $df = 2,93$, $P = 0.24$), sex ($F = 0.65$, $df = 1,93$, $P = 0.46$) or any interaction among these factors ($F = 0.51$, $df = 2,93$, $P = 0.60$).

4. Discussion

4.1. Baseline CORT levels

The first objective of this study was to examine patterns of variation in baseline CORT in three populations of the Thorn-tailed Rayadito with differences in climate, seasonality and productivity (Table 1). We predicted that if low temperature influences baseline CORT levels, we should observe highest levels of this hormone in the southernmost population in relation to the northern and central population, a “low temperature hypothesis”. We observed that Thorn-tailed Rayadito in the southernmost populations did not have higher values of baseline CORT in any of the three study years, which did not support that hypothesis. Alternative we proposed the “productivity environmental hypothesis”, which states that productivity is the main factor influencing baseline CORT. So, we expected lower values of this hormone in the more productive environment, the southern population. We observed that the southern population presents lower values of this hormone, supporting the aforementioned hypothesis.

Although Thorn-tailed Rayadito in the southern population were experiencing a cooler environment which, per se, additionally

contributed to increase baseline CORT levels because of high energy expenditure devoted to thermoregulation, as observed in the negative effect of the path coefficient (Fig. 4), this would not represent a stress situation if food was abundant (primary productivity directly influences the abundance of insects – Dunn and Winkler, 2010), as in the southernmost population.

Other studies, on longer living bird species, have identified indirectly (Buck et al., 2007; Kitaysky et al., 1999; Shultz and Kitaysky, 2008) or directly (Kitaysky et al., 2007) food availability in seabirds the principal factor influencing baseline CORT levels. To the best of our knowledge, in songbirds only the study of Marra and Holberton (1998) on American Redstarts and the study of Jenni-Eiermann et al. (2008) on Barn Swallows, identify food availability as a primary factor influencing baseline CORT. The sensitivity of Thorn-tailed Rayadito to differences in productivity is not surprising. In general, habitat specialist species are particularly vulnerable to the negative impacts of poor quality habitat (Tallmon et al., 2002) and in particular, forest specialist bird species are very sensitive, as they only inhabit this type of habitat (Burke and Nol, 1998; Zanette et al., 2000). In this study, Thorn-tailed Rayadito in less productive environments, as in the central and northernmost populations, may increase foraging effort in response to greater CORT secretion to compensate for lower food availability. Increased foraging effort accompanied by mobilization of energy reserves, together may help an individual through periods of low food availability or when opportunities to forage become limited.

Particularly, in addition to low environmental productivity, in the central populations, ambient temperatures are highest and rainfall do not occur during the reproductive seasons, which taken together, may represent a stressful environment. When we analyzed the correlation between weather, body weight and baseline CORT, we observed a significant effect of temperature on the central population (Fig. 4). In this population, and contrary to the southern population where an increase in temperature produced a decrease in baseline CORT, we observed that an increase in temperature produced an increase in baseline CORT, supporting the notion that endotherms living in a hot environment are at risk of hyperthermia and thus these environments might be challenging to small animals, like the Thorn-tailed Rayaditos (Cabrera et al., 2000; Carere et al., 2003).

Finally in the northern population, we did not detect any significant effect of weather on baseline CORT. This forest is quite particular as it is a patchy relic forest, from the Pleistocene period (Villagrán et al., 2004), and this population of Thorn-tailed Rayaditos is genetically isolated from the rest of the population in Chile (González and Wink, 2010) because xeric shrub matrixes that surround the forest represent a barrier to the bird’s dispersal (Cornelius et al., 2000). So, it is likely that habitat fragmentation and isolation, together with low environmental productivity, represent the main stress factors for Thorn-tailed Rayadito, as has been observed in Eurasian Treecreeper chicks (*Certhia familiaris* – Martínez-Mota et al., 2007) and American Redstarts (*S. ruticilla* – Marra and Holberton, 1998).

4.2. Invariant stress responses and responsiveness

The second objective was to examine how stress response and stress responsiveness varied among the three study populations. Based on previous studies (Cornelius et al., 2012; O’Reilly and Wingfield, 2001; Silverin et al., 1997) we tested the hypothesis that stress responses should be down regulated at higher latitudes, i.e., for the southernmost population (the CCH). Contrary to our expectations, and similar to other studies (Breuner et al., 2003; Lindström et al., 2005) we observed that neither stress responses nor responsiveness varied among the three populations. So in Thorn-tailed Rayadito there was the same magnitude of stress re-

sponses and responsiveness along the latitudinal gradient, although the southernmost populations had lower levels. This trend has also been observed, for example, in Bush Warblers (*Cettia diphone* – (Wingfield et al., 1995a)) amongst others. The difference between the study of Wingfield et al. (1995a) and our study is that they found higher values of baseline and stress response in the populations breeding at higher latitude.

When comparing stress responses and responsiveness between sexes, we found no differences in any of the three populations and not during the 3 years. The lack of differences between sexes was what we expected for the monogamous mating system of Thorn-tailed Rayadito and principally because both sexes of the pair care for the offspring (Moreno et al., 2007). The parental care hypothesis proposes that the sex investing most in the care of the offspring should down regulate the stress response in order to avoid brood abandonment. For example, a reduced stress response has been observed in the sex that invests more offspring care in Arctic birds (Holberton and Wingfield, 2003; O'Reilly and Wingfield, 2001; Wingfield et al., 1995a) and (Bókony et al., 2009) in a comparative phylogenetic analysis demonstrated that females in species with more female-biased parental care had weaker stress responses.

4.3. Conclusions

Because baseline CORT levels were lower in the southern population and stress response did not vary amongst populations, we concluded that low environmental temperatures did not represent a stress situation for the Thorn-tailed Rayadito if food abundance is sufficient to support energetic demands. Secondly, we suggest that primary productivity is the main factors influencing baseline CORT, and that in the case of the central population it is affected at the same time by high environmental temperature and in the case of the northern population it is affected presumably by the isolated and fragmented characteristic of this relic forest.

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