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Breaking down seasonality: Androgen modulation and stress response in a highly stable environment



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ABSTRACT

Previous studies show that most birds inhabiting temperate regions have well defined life history stages, and they modulate the production of testosterone (T) and corticosterone (CORT) in response to changes in seasonality. In this study we aimed to examine baseline and stress-induced levels of CORT and circulating T in relation with life history stages in the rufous-collared sparrow, Zonotrichia capensis. We carried out this study for a year in a population inhabiting riparian habitats in the Atacama Desert in Chile, one of the most climatically stable and driest places in the world. This environment shows minimal yearly change in average temperature and precipitation is virtually zero. We found individuals breeding, molting and overlapping breeding and molt year round, although most individuals were molting during March and in breeding condition during October. T levels were not related to individual breeding condition, and at population level they were not significantly different across sampling months. Baseline levels of CORT did not vary across the year. Stress-induced levels of CORT were suppressed during March when most of the birds were molting. This phenomenon was also observed in birds not molting during this period suggesting a mechanism other than molt in determining the stress-response suppression. Our results strongly suggest that in this study site, long-term extremely stable conditions could have relaxed the selective pressures over the timing of life history stages which was evidenced by the breeding and molt schedules, its overlap and endocrine profiles.

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1. Introduction

Many vertebrate species experience a series of energetically costly events over the course of the annual cycle including reproduction, molt, and migration (Wingfield, 2005). These events require substantial investment of resources (Hoye and Buttemer, 2011), and are sufficiently costly that they cannot be undertaken simultaneously without fitness repercussions (Zera and Harshman, 2001). In non-migratory species breeding at mid- to high latitudes there is typically a pronounced temporal division of the most energetically demanding phases of breeding and molt, with minimal overlap between these activities at the individual level (Barta et al., 2006; Houston et al., 2005; Johnson et al., 2012). In fact, birds that overlap activities are observed to experience lower fitness than birds that, for example, finish breeding before initiating molt (Nilsson and Svensson, 1996). Partitioning of molt from other life history stages is therefore assumed to be an adaptation that minimizes physiological demands (allostatic load, McEwen and Wingfield, 2003) while maximizing the allocation of productive energy to enhance lifetime fitness (Hahn et al., 2008; Mitchell et al., 2012). In migratory birds, the schedule of migration, molting and breeding are even more constrained mainly because individuals which delay molting or breeding migrate later (Stutchbury et al., 2011; Mitchell et al., 2012), and the optimal arrival time on the breeding grounds is crucial to obtain the best territories (Kokko et al., 2006). In addition, in seasonal environments reproductive success is strongly linked to the appropriate timing of breeding, when hatching matches peaks in food supply (e.g. Brinkhof et al., 1993; Lambrechts et al. 1996).

The hormonal changes associated with the timing and duration of different life history stages are complex, but metabolic demands are considered linked to plasma levels of corticosterone (CORT) while testosterone (T) levels are associated with territory establishment and onset of breeding, at least in males. During regular

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life history stages, CORT regulates metabolic functions such as energy intake, storage, and mobilization (Sapolsky et al., 2000; Landys et al., 2006). In contrast, stress-induced elevation of CORT involves a variety of physiological and behavioral changes that allocate resources toward immediate survival (Wingfield et al., 1998; Landys et al., 2006). In this context, changes in plasma CORT levels can affect timing of breeding and molting directly as well as indirectly by promoting activities incompatible with molt and breeding (Murphy, 1996; Breuner et al., 1998). CORT may also indirectly increase energy expenditure with the potential to limit resources devoted to reproduction and feather growth (Romero et al., 2005). Consequently, despite high levels of variability in CORT plasma levels (Romero, 2002; Wingfield, 2005), a widespread pattern is the suppression of both baseline and stress induced CORT levels during the parental phase of breeding and the postbreeding (prebasic) molt relative to breeding (Cornelius et al., 2011).

On a predictable, seasonal basis, environmental signals such as the annual changes in photoperiod, precipitation, and food abundance regulate the progression of life history stages through, for example, stimulation of the hypothalamic-pituitary-gonad (HPG) axis that in turn regulates T increases in males and acquisition of a territory and onset of breeding (Wingfield, 2008; Perfito et al., 2007). However, prolonged high levels of T can have detrimental effects such as decreases in body mass and fat stores, depressed immune activity and ultimately decreased survival (e.g., Dufty, 1989; Ketterson and Nolan, 1992; Hillgarth and Wingfield, 1997; Wingfield and Soma, 2002). In addition, high T levels can interfere with molt (Runfeldt and Wingfield, 1985; Schleussner et al., 1985). Nolan et al. (1992) showed that birds maintained at artificially elevated levels of T beyond the end of the breeding season, postponed or suppressed molt. Consequently, many avian species exhibit a marked breeding season where T increases and remains elevated for most of the breeding period (Farner and Wingfield, 1980; Wingfield, 1984). In environments where seasonality is less clearly delineated and typically reduced to two general seasons (i.e., wet and dry seasons), such as the tropics, year-round territorial species display continuously low levels of T, or T remains undetectable year round compared with species in seasonal environments (Levin and Wingfield, 1992; Goymann et al., 2004; Moore et al., 2004a). Despite studies in tropical environments, the hormonal regulation of life history stages in highly stable environments is poorly understood. In this context our goal was to assess life history stages and circulating levels of T and CORT in rufous-collared sparrow (Zonotrichia capensis peruviensis) in the Atacama Desert, the driest and oldest extant desert on Earth (Hartley et al., 2005; Supplemental materials Fig. 1). In this extremely stable climate the environmental cues to molt or breed are minimal. Despite variations in photoperiod are roughly 2 h, which eventually could be considered as a proximate cue since birds in the tropics can respond to differences <1 h (Hau et al., 2008), variations in temperature and precipitations are low. The annual average temperature is 18.75 ± 0.04 with minimal temperatures of 13.5 \pm 0.18 and maximal of 24.0 \pm 0.08 °C (Dirección Meteorológica de Chile, 2011). In terms of water and food availability, even though precipitation is extremely infrequent in the coastal desert region (i.e., roughly 0.5 mm per year), several streams flow from Los Andes and provide water and conditions for abundant riparian vegetation in the valleys. In our study site the valleys present similar climatic characteristics however San Jose River (Azapa Valley) can be classified as an endorheic basin where water is pumped to the surface to sustain agricultural activities, presenting a flow of 0.58 l/s. This flow can increase in 0.7 times between January and March every 4 or 5 years mainly associated with heavy rain in the Andes region during "El Niño" events. In contrast, the Lluta basin is exorheic and water naturally flows year round with a flow of roughly 600 l/s (Cade-Idepe Consultores, 2004). Flows are more regularly registered in Lluta, causing until 6-fold increase in discharge between January and March every year. In addition, Lluta presents higher levels of salinity than the San Jose River. These differences in salinity probably explain the higher levels of agricultural activities in Azapa valley where over the past few years the cultivable surface has increased 5-fold (Torres and Acevedo 2008). As a result of the increments of agricultural activity, semirural areas rich in vegetables, arthropods and seeds are common, creating habitats for some passerine birds, as rufous-collared sparrow (see Estades and Temple, 1999).

Our study model was the rufous-collared sparrow, a species distributed from southeastern Mexico to the Southernmost tip of South America, Cape Horn in Chile. Along this range, populations experience diverse habitats including urban areas, coastal and xeric environments, grasslands, chaparral and edges of tropical and temperate forests and alpine meadows (Estades and Temple, 1999; Class et al., 2011; Maldonado et al. 2012). Populations of rufous-collared sparrows through Central and South America exhibit different patterns of timing and duration of their life-history stages (Kalma, 1970; King, 1973; Moore et al., 2005; Class et al., 2011; Busch et al., 2010; Addis et al., 2010, 2011). Breeding and molting have been described to be unimodal seasonal and mostly mutually exclusive in most of Zonotrichia's distribution range in Latin America (Wolf, 1969; Kalma, 1970; Miller, 1959; Moore et al., 2005; Davis, 1971; King, 1973) with the exception of high-altitude populations in Colombia where birds were observed to breed and molt twice a year (i.e., bimodal schedule) but without overlapping between both stages (Miller, 1962; Miller and Miller, 1968). In all of these environments individuals experience some degree of seasonality or environmental cues that modulate timing of life history stages. Very few studies have examined life history stages in an organism that lives in an area with minimal environmental change. In this study, we explored the link between life history stages, T and CORT in a stable environment to better understand the trade-offs involved between molt and reproduction, and the relation between endocrine mechanisms and environmental cues that shape most of the life schedules in vertebrate species.

2. Material and methods

2.1. Species, study site and morphological measures

In our focal species, Z. capensis, males are territorial and socially monogamous, and both males and females exhibit parental care (Chapman, 1940; Miller and Miller, 1968). They are described as mainly granivorous, however they have been observed to consume insects and fruits depending on the environment that they inhabit and time of year (Novoa et al., 1996). To assess life history stages and endocrine profiles, and to determine the relationship between circulating hormone levels and breeding and molt conditions in this species, we carried out a field study in the Atacama Desert in Northern Chile. The extremely dry conditions in this place are maintained by several factors: (i) the Pacific Anticyclone along the coast generates a thermal inversion layer; (ii) the Andes Mountains act as a barrier blocking the humid influence of the Amazonian region, and (iii) the cold Humboldt marine current originating in Antarctica limits seawater evaporation (Cereceda et al., 2002). These conditions result in an extremely stable environment characterized by daily coastal morning fog and year round constant temperature (see Supplementary materials, Fig. 2). For example, in the year of this study, the maximum and minimal temperatures averaged 23.29 ± 2.63 °C and 18.79 ± 1.84 °C respectively. These temperatures are similar to those recorded in the last 50 years (Torres and Acevedo, 2008). We collected blood samples and phenology data for a week at

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Fig. 1. Body condition index of *Z. capensis* across sampling months in two localities. Sample size is shown on top. Mean ± se. Letters indicate significant differences (Tukey HSD, *P* < 0.05).

two month intervals from October 2010 to October 2011 at sites located in two valleys in the Atacama Desert, (i) Azapa valley (18°30'54.89"S, 70°11'35.09"W, 260 m above sea level) and Lluta valley (18°24'40.39'S, 70°14'10.79" W, 225 m above sea level) in the Atacama Desert.

Birds were passively captured using mist nets (i.e., no playback was used). For every bird captured we recorded body weight, and bill, tarsus, body, tail and wing length. Birds were weighed with a Pesola Micro-Line Spring Scale (capacity 50 g) and all linear measurements were collected with a Fowler (Brantford, Ontario) UltraCal Mark III Electronic Caliper. Body condition was calculated as the residuals of the regression between tarsus length and body mass. For individual identification, all birds were banded with numerical metal bands. Blood samples for endocrine analyses were collected during the sampling period from 7:00 am to 11:00 am (see below for details). All protocols were conducted according to Chilean laws, legal permits and the ethical committees of our universities.

2.2. Assessing sex, breeding condition, molting and their overlap

We determined the sex of the subjects if the bird was in breeding condition by visually examining the cloacal protuberance (CP) in males and brood patch in females to assign sex and breeding state. Males were classified as breeding if either the width or the height of the CP was greater than 5 mm (Miller, 1959). If the brood patch region of the female was defeathered, edematous, bare and wrinkled we considered the female to be in breeding condition (Addis et al., 2011). Females without brood patches (BP) were classified as non-breeding. For a subset of 100 birds the sex of each bird was molecularly determined following Fridolfsson and Ellegren (1999). Apart from this subset the sex of the birds in nonreproductive condition was classified as unknown. Seasonal patterns of breeding in males and females were determined based on the mean length of the CP or presence of BP, respectively, of adult birds only (N = 376 total number of birds).

Molt status was determined by the occurrence (yes/no) of flight feather molt (i.e., primary, secondary, coverts or tail) or more than 20% of body molt. In addition, we assessed the intensity of molt, scoring body molt from 0 to 4, where 0 are individuals not molting; 1, individuals molting 20% or more feathers in the back; 2, in the abdomen; 3, in the crown; and 4, individuals molting 20% or more feathers in more than one of the mentioned regions. We assessed flight feathers molt scoring subjects in categories from 1 to 6, where 1 represents individuals not molting; 2, individuals molting more than 20% of primaries; 3 of secondaries; 4 of coverts; 5 of tail; and 6, individuals molting 20% or more of the feathers in more than one of the mentioned regions. We also assessed the asymmetry of the molt, defined comparing each wing undergoing molt (Johnson et al., 2012).

We considered overlap between molting and breeding status to occur when an active brood patch or cloacal protuberance was observed simultaneously with molting flight or body feathers. To assess differences in the proportion of birds molting and breeding we assigned each bird to one of four categories: Not Molting, Not breeding (NM/NB), Molting (and not in a reproductive condition), Breeding (and not molting), and Overlap (i.e., breeding and molting).

2.3. Blood sampling

Blood was collected from the alar vein using a 26 gauge needle and heparinized microhematocrit tubes. Samples were collected within 3 min of capture for CORT baseline and within 10 min of capture for T. For birds that were sampled for CORT levels, after the CORT baseline sample was taken, we took morphological measures (see below) and then placed the bird in a small cloth bag for the next 30 min (i.e., handling time) to determine the responsiveness of the hypothalamic-pituitary-adrenal axis to stressful stimuli (Breuner et al., 1999). Blood was collected at the 30 min mark as previously described, and 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 4 h before centrifuging) and were centrifuged to separate the plasma from red blood cells. The plasma was aspirated and frozen (at -20 °C) until transported on dry ice to the University of California, Davis, for analysis.

2.4. Hormone analysis

T and CORT concentrations in plasma were determined using direct radioimmunoassays (Wingfield et al., 1992). To determine

the efficiency of hormone extraction from the plasma, $20 \ \mu$ l of 2000 cpm of tritiated testosterone or corticosterone were 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. Plasma volumes of the samples varied from 5 to 15 μ l for Cort and from 40 to 50 μ l for T. The detection limit of the assay ranged from 0.26 to 0.53 ng/ml for CORT and from 0.37 to 0.59 ng/ml for T, depending on the volume of the plasma samples. A total of three assays were run for T and seven for CORT. Intra-assay variation ranged from 8.4% to 13.3% for T and 7.2% to 11.8% for CORT. Inter-assay variation was 11.5% for T and 9.48% for CORT.

2.5. Statistical analyses

Differences between body condition index data from October 2010 and 2011 were assessed through Student's *t*-test. The effect of sampling month and locality on body condition index was assessed with a Two-way ANOVA. Distribution of data across life history stages from October 2010 and 2011 were tested for differences with a Kolmogorov-Smirnov test. The Bayesian information criterion (BIC; Link and Barker, 2006) and maximum-likelihood (Akaike information Criterion Corrected for sample size, AIC(c)) (Burnham and Anderson, 2002) estimation methods were used to evaluate the best of three models to explain timing of molting and breeding: (a) an ordinal logistic model considering month as the independent variable, (b) an ordinal logistic model considering location as the independent variable and (c) an ordinal logistic model considering month and location as independent variables. We calculated the weighted BIC (wBIC) for model probabilities to be the best model for the data given the linear and quadratic models (Link and Barker, 2006). Contingency analysis was used to analyze differences in the selected model. Similar analyses were used to assess variation in molt of body and flight feathers as functions of month and location. To assess differences between male and female breeding cycles a Kolmogorov-Smirnov test for two samples was performed.

Differences in the proportion of birds that were engaged in both molt and breeding (Overlap) between localities was assessed through a contingency analysis. To assess differences between sampling month in the proportion of birds that Overlap, we performed a Kolmogorov–Smirnov test. To assess differences in body condition index between Overlap birds and those in the other three lumped categories we performed a Student's *t*-test. To assess the relation between sex and overlapping we performed a contingency analysis analyzed with Likelihood Ratio Chi-square test.

The effect of handling time, sampling month and locality on CORT levels was assessed through two-way repeated measures ANOVA. In order to assess differences between baseline and stress-induced levels of CORT in a specific month we performed a Student's *t*-test for dependent samples. To assess the effect of life history stages and molt intensity on CORT levels we performed repeated measures ANOVAs. Tukey multiple comparisons (Honest Significant Differences) for different sample size were used as a post hoc tests. To assess the variation in absolute concentrations of stress-induced CORT during molt, we report the percentage of suppression using the following equation (Cornelius et al., 2011):% Suppression = (1 - CORT during molt/CORT premolt) × 100

We compared the percentages of baseline and stress induced suppression of CORT against 0% with an adjusted Wald confidence interval for one proportion. We used the same test for two proportions when we compared CORT suppression between localities (Agresti and Coull, 1998). To assess the relationship between body condition and baseline or stress induced CORT we performed a linear regression. To assess the effect of site (i.e. valley), sampling month and their interaction on testosterone levels we performed a two-way ANOVA. The effect of life history stages and sex on testosterone levels was assessed through one-way ANOVAs. Tukey multiple comparisons (Honest Significant Differences) for different sample size was used as post hoc test. The relationship between testosterone and body condition was assessed through linear regression. Statistical analyzes were performed with JMP 10.0, SAS Institute Inc. 2012.

3. Results

3.1. Body condition, molt and breeding phenology

We captured a total of 343 individuals, 7 of which were recaptured during the study. For recaptured birds, we only used data from the first capture in the analyses. Body condition index data from October 2010 (-0.52 ± 0.96 , mean \pm se, N = 32) and 2011 (-0.17 ± 2.25 , N = 39) were grouped since they were not significantly different (t = -0.82, df = 69, P = 0.42). Body condition was not significantly different between localities (F = 3.91, df = 1,332, P = 0.39) but it varied significantly across sampling months (F = 7.34, df = 4, 332, P < 0.001) and the interaction was also significant (F = 3.91, df = 4,332, P < 0.001, Fig. 1).

Distribution of data of life history stages from October 2010 and 2011 did not differ and were lumped (Kolmogorov-Smirnov d_{max} = 0.03, df = 4,32, *P* = 0.5). The Bayesian and maximum-likelihood estimation methods did not perform similarly when we analyzed the timing of life history stages. The Bayesian approach favored the ordinal logistic model that included just month and the maximum-likelihood favored the ordinal logistic model that included month and locality. Following the weighted BIC, the model including just month as independent variable had 2.8 times higher probability to be the best model for the data in comparison with the model including month and locality and 146 times higher than the model including just locality (Table 1). Paired contingency analysis showed significant differences among the proportions of bird's activities across all months (Fig. 2). Breeding condition was significantly associated with month (Likelihood ratio Chi-square, G^2 = 91.45, df = 4, *P* < 0.001) but not with locality (Likelihood ratio Chi-square, $G^2 = 1.677$, df = 1, P = 0.20). Breeding condition in both localities reached a peak during October and December when approximately 70% of individuals where breeding. In contrast, during March 7% of the individuals were breeding (Fig. 2). Male and female breeding cycles estimated through the length of cloacal protuberance for males and presence of breeding patch for females were coincident (Kolmogorov–Smirnov $d_{max} = 0.2$, df = 2,20, P = 0.1, Table 2). Although we found an effect of month in breeding condition we observed breeding and non-breeding birds every month.

Both estimation methods, maximum-likelihood and Bayesian, did perform similarly when we analyzed body molt, favoring the model that included just month. In addition, the weighted BIC show that the model including just month as an independent variable had 3.2 times higher probability to be the best model for the data in comparison with the model including month and locality and 11.8 times higher than the model including just locality (Table 1). Contingency analysis showed an association between body molt and sampling months (Likelihood ratio Chi-square, $G^2 = 120.24$, df = 16, P < 0.001, Fig. 3) with a peak during March when roughly 70% of individuals were molting one or more areas of the body. In contrast, roughly 7% of the individuals were molting body feathers in October. Despite these differences, we found birds molting body feathers in every sampling month.

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Table 1

Parameter estimates (Bayesian posterior modes) for the three models of timing, body and flight feathers molt in rufous-collared sparrow.

	Log likelihood	df	Chi-square	Р	AICc	BIC	BIC weight
Timing of life history stages							
Month (ordinal logistic model 1)	454.87	1	6.049	0.014	917.86	933.081	0.0005
Locality (ordinal logistic model 2)	434.88	4	46.01	0.0001	884.11	910.621	0.7352
Month + Locality (ordinal logistic model 3)	433.506	5	48.77	0.0001	883.44	913.69	0.2643
Body molt							
Month (ordinal logistic model 1)	228.02	1	1.45	0.228	731.064	750.06	0.0609
Locality (ordinal logistic model 2)	308.53	4	104.78	0.0001	632.73	662.971	0.7191
Month + Locality (ordinal logistic model 3)	307	5	107.07	0.0001	632.54	666.514	0.22
Flight feathers							
Month (ordinal logistic model 1)	228.023	1	1.45	0.22	468.297	491.056	0.002
Locality (ordinal logistic model 2)	207.99	4	41.51	0.0001	434.53	468.502	0.789
Month + locality (ordinal logistic model 3)	207.064	5	43.37	0.0001	434.79	472.476	0.209



Fig. 2. Life history stages of *Z. capensis* captured throughout the calendar year in two localities, Azapa (upper panel) and Lluta (lower panel). Percentages of captured birds assigned to Non-Molting/No Breeding (NM/NB), Molting, Breeding, Overlap are shown in each bar. Sample sizes are shown at the top of each bar.

Table 2

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Percentages of males (upper panel) and females (lower panel) rufous-collared sparrows with cloacal protuberance 6–10 mm or brooding patch.

Month	Location	No. of adults	Cloaca 6-10 mm	%
Male cycle				
January	Azapa	38	5	0.13157895
	Lluta	8	2	0.25
March	Azapa	28	1	0.03571429
	Lluta	40	3	0.075
May-June	Azapa	34	5	0.14705882
	Lluta	34	7	0.20588235
October	Azapa	46	13	0.2826087
	Lluta	25	4	0.16
December	Azapa	47	14	0.29787234
	Lluta	42	16	0.38095238
Female cycle				
Month	Location	No. of adults	Brood patch	%
January	Azapa	38	7	0.18421053
	Lluta	8	5	0.625
March	Azapa	28	1	0.03571429
	Lluta	40	1	0.025
May-June	Azapa	34	4	0.11764706
	Lluta	34	5	0.14705882
October	Azapa	46	10	0.2173913
	Lluta	25	4	0.16
December	Azapa	47	16	0.34042553
	Lluta	42	13	0.30952381

When we analyzed flight feather molt, the Bayesian and maximum-likelihood estimation methods performed similarly, favoring the ordinal logistic model that included only month as independent variable. According to the weighted BIC this model had $3.8 \times$ higher probability of being the best model in comparison with the model including month and locality and over 300 times higher than the model including just locality (Table 1, Fig. 4). Flight feathers molt reached a peak during January and March in which roughly 35% of individuals were molting flight feathers. In contrast, during October 1% of individuals were molting flight feathers (Likelihood ratio Chi-square, $G^2 = 40.61$, P < 0.001). Although we observed the mentioned differences between the number of birds molting, we found birds molting flight feathers in each month. Of the 70 birds molting wing feathers, 43% (Azapa N = 25, Lluta N = 15) showed asymmetrical molt patterns between the wings. We did not find a significant association between asymmetry and locality (Likelihood ratio Chi-square, $G^2 = 1.82$, df = 1, P = 0.18). In addition, of the 158 birds molting body or flight feathers, 33% of the individuals showed an irregular sequence of molt progression (e.g., primary 1 and 9, see Miller, 1961) instead of the regular pattern (i.e., inner to outer wing feathers). This pattern was not significantly associated with locality (Azapa N = 28, Lluta N = 27, Likelihood ratio Chi-square, $G^2 = 0.81$, df = 1, P = 0.37).

The proportion of birds overlapping molt and breeding with respect to the total number of birds in breeding condition was five times higher in Azapa than in Lluta (Likelihood ratio Chi-square $G^2 = 18.84$, df = 1, P < 0.001, Fig. 5). Overlapping was not significantly different across sampling month (Kolmogorov–Smirnov $d_{\text{max}} = 0.5$, df = 5,10, P = 0.5). The body condition of birds overlapping molt and breeding (1.045 ± 0.26, mean ± se, N = 52) was $5.8 \times$ higher than the birds that were molting or breeding (-0.07 ± 0.12 , N = 221, t = -3.86, df = 271, P < 0.001). We did not find a significant association between sex and overlapping (Likelihood ratio Chi-square $G^2 = 2.00$, df = 1, P = 0.15).

3.2. Corticosterone

CORT levels differed among months (F = 5.08, df = 4,122, P < 0.001) and there was a significant effect of handling time on CORT levels (F = 78.58, df = 1, 122, P < 0.001). There was also a significant interaction effect between handling time and sampling month on levels of CORT (F = 3.48, df = 1,4, P < 0.001, Fig. 6). CORT did no differ between locations (F = 2.44, df = 1,122, P = 0.12) or among sampling months (F = 0.57, df = 1,4, P = 0.68) and there was no interaction effect between handling time and location (F = 0.57, df = 1,1, P = 0.45).

During March, when a significant proportion of birds were molting, we did not find a significant difference between baseline and stress-induced levels of CORT (t = -1.39, df = 31, P = 0.18). Remarkably, even the birds that were not molting during March showed the same pattern (t = -1.48, df = 9, P = 0.17). Across the year in birds that were molting, stress-induced CORT levels were significantly lower than in birds that were non-molting/non-breeding, breeding or overlapping (F = 2.77, df = 3,128, P < 0.05, Fig. 7). In addition we found a significant effect of handling time on CORT levels (i.e., higher stress-induced levels were higher than



Fig. 3. Body molt of Z. capensis across sampling months. Body molt categories are shown in each bar. Sample sizes are shown at the top of each bar.

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Fig. 4. Flight feathers molt of Z. capensis across sampling months. Molt categories are shown in each bar. Sample sizes are shown at the top of each bar.



Fig. 5. Proportion of individuals overlapping molt and breeding respect of total of birds in breeding condition across sampling months in two localities. Sample sizes are shown at the top of each bar.

baseline levels of CORT) (F = 70.44, df = 1,128, P < 0.001) and the interaction between handling time and life history stages (F = 3.75, df = 1,3, P < 0.05). Although stress-induced CORT levels were lower in birds molting, these levels were significantly higher than baseline CORT (t = -3.13, df = 48, P < 0.01, Fig. 7).

In terms of CORT suppression, although most birds were molting in January, birds in March (the next sampling period) had higher suppression of stress-induced CORT levels (66%) than baseline levels of CORT (27.5%, P < 0.05, $\beta = 0.99$). This effect was higher in Azapa (44%) than in Lluta (22%) for baseline CORT (P < 0.05, $\beta = 0.98$) and it was higher in Lluta (85%) than in Azapa (39%, P < 0.001, $\beta = 0.99$) for stress-induced levels. When we compared birds exclusively molting across the year versus other activities (no-breeding/non-molting, breeding, overlap, all categories lumped), we observed the same pattern described for month effect. Birds suppressed stress-induced levels (47%), but did not suppress

baseline levels of CORT (-24%). This effect was higher in Lluta (-67%) than in Azapa (-7%, P < 0.001, $\beta = 0.99$) and it was not different between localities for stress-induced levels (Lluta 60%, Azapa 42%, P = 0.5, $\beta = 0.07$). Although both comparisons showed stress-induced suppression, the sampling month had a higher effect in the suppression of the stress-induced levels than the activity (P < 0.05, $\beta = 0.95$).

CORT levels were not significantly associated with molt intensity of flight feathers (F = 1.03, df = 5, 126, P = 0.40), the interaction between molt intensity of flight feathers and handling time (F = 1.76, df = 1, 5, P = 0.13), body molt (F = 1.16, df = 4, 127, P = 0.33) or the interaction between body molt and handling time (F = 1.89, df = 1, 4, P = 0.114). Body condition index was not significantly associated with baseline CORT ($R^2 = 0.001$, F = 0.07, df = 1, 128, P = 0.79) or stress induced CORT ($R^2 = 0.002$, F = 0.02, df = 1, 128, P = 0.86).

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Fig. 6. Stress-induced and baseline levels of CORT through sampling month in two localities. Mean ± se, sample sizes are shown at each month. Letters indicate significant differences (Tukey HSD, *P* < 0.05).



Fig. 7. Stress-induced and baseline levels of CORT (mean ± se) at different life history stages of *Z. capensis*. Sample size is shown at the bottom of each bar. Letters indicate significant differences (Tukey HSD, *P* < 0.05).

3.3. Testosterone

T data from October 2010 $(1.87 \pm 1.34 \text{ ng/ml}, \text{mean} \pm \text{ee})$ and 2011 $(3.27 \pm 1.16 \text{ ng/ml})$ were not significantly different and were grouped (t = -0.76, df = 34 P = 0.45, N = 36). T levels were not significantly different between localities (F = 1.02, df = 1, 147, P = 0.31) or among sampling months (F = 1.72, df = 4, 147 P = 0.15) or the interaction between month and location (F = 0.38, df = 4, 147, P = 0.81). Testosterone levels were not significantly different across life history stages (F = 0.38, df = 3, 149, P = 0.76) across sampling months for males (F = 1.50, df = 4, 56, P = 0.2, Fig. 8) or females (F = 0.80, df = 4, 33, P = 0.531, Fig. 8). Males had significantly higher

T levels $(2.17 \pm 0.43 \text{ ng/ml}, N = 61)$ than females $(0.63 \pm 0.58 \text{ ng/ml}, N = 38, t = -3.80, df = 97, P < 0.01)$. Testosterone was not significantly associated with body condition $(R^2 = .002, F = 0.31, df = 1,155, P = 0.58)$.

4. Discussion

We explored the role of season, locality and life history stages on plasma levels of testosterone and corticosterone in two desert populations of rufous-collared sparrows. We found that while reproductive condition and molt at the population level did vary significantly with time of year, there were at least some birds in

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Fig. 8. Testosterone plasma levels of males and females of Z. capensis across sampling months. Sample size is shown at the bottom of each bar.

each life history stage at all times of year, unlike in other northern and southern populations of *Z. capensis*. There was also considerable overlap of molt and breeding. We detected anomalous molt patterns. These factors correspond to CORT and T differentially, providing evidence of a possible breakdown in the distinct seasonal patterns seen in this species at other regions and may be related to the highly stable conditions that they experience yearround.

4.1. Body condition, molt and breeding phenology

The annual cycle of body condition in our study shows an important low during March, a month when most of the birds were molting. This is coincident with the annual fat storage cycle in migratory *Zonotrichia* species in the Northern hemisphere which includes rapid gains in mass/fat in early spring and summer, closely following periods of molt and immediately preceding migratory movement (King et al., 1965; King, 1968; Morton and Welton, 1973). In addition, in the northern wintering populations there is a midwinter peak of fat storage that is correlated inversely with mean air temperature (King and Farner, 1966). Although in our study site these climatic cues can be subtle, we observed an increase in body condition during May–June suggesting that minimal changes in ambient temperature may be associated with influence energy storage.

As mentioned before, other subspecies of rufous-collared sparrows have been reported to have different schedules for the timing and duration of their life-history stages throughout the species' range (Class et al., 2011; Class and Moore, 2011), showing unimodal or bimodal schedules for molt and breeding (Wolf, 1969; Miller, 1959; Moore et al., 2005; Davis, 1971; Miller, 1962; Addis et al., 2011). The annual cycle of rufous-collared sparrows in this study differs remarkably from these previous reports. As far as we know this is the first study of timing of life history stages of birds in the Atacama Desert. The nearest previous study was carried out 1500 km north of our study site, in Chilca (Lima, Peru, 12°03'S, 77°03'W) (Davis, 1971). In that site, rufous-collared sparrows have two molts; the breeding period is preceded by a partial molt in some birds (i.e., prenuptial molt) and followed by a complete molt in all (i.e., postnuptial molt). The prenuptial molt lasts from late September to the end of November and involves primary

feathers usually accompanied by light to heavy molt of the crown feathers, while body molt is uncommon. In our study site we observed molt year round, although there was a period of high molt rate in which \sim 70% of the birds were molting (i.e., March). In fact, primary feather molt was present in each sample period, crown feather molt had no well-defined molting period and we observed individuals undergoing heavy body molt from January to June. On the other hand, the postnuptial molt described by Davis (1971) in Lima is consistent with the pattern described by Miller (1961) in Colombia and by Wolf (1969) in Costa Rica, with a peak between May and June. In our case the proportion of birds molting during May-June is lower than in March and not significantly different from January. In terms of anomalous molt, Miller (1961) and Davis (1971) reported a few birds molting out of sequence in both wings or numerous primaries (i.e., 3) in both wings at the same time. In this study we observed a remarkable proportion of the population with asymmetrical molt patterns between wings (\sim 40%) and/or irregular sequence of molt progression (~30%). Molt patterns in central Chile where seasons are marked, are regular and well-defined between January and March (González-Gómez unpublished data). On the other hand, Z. capensis in Tucuman, Argentina (26.9°S, 65.4°W) show only a postnuptial molt between January and February (King, 1972; Handford, 1985). The molt observed in this locality was reported symmetric and in a regular sequence (King, 1972). The anomalous observed molt pattern in our study could be a result of a variety of variables, such us intensive pesticide use in our site, especially in Azapa where the agricultural activities are intensive. Although it is necessary to perform further research to role out this hypothesis, none of the pesticides used in these localities have been shown to cause endocrine disruption (David Baston, pers. com.). In addition, we did not find significant differences between anomalous molt patterns between localities. A second possibility to explain the anomalous molt, could be low thermoregulatory demands at our site based on comparisons with other localities in Z. capensis distribution. In fact, in Chile, Peru there is a pronounced difference between the dry and wet seasons, which could explain why molt patterns there were more similar to tropical locations than to desert locations such as Arica.

The breeding schedule in our study site is similar to the schedule described by Class et al. (2011) at Las Caucheras (Ecuador) where *Z. capensis* appear to breed year round. In contrast, in the coastal desert of Peru (Davis, 1971), birds had a breeding season from October to early April with individual birds having breeding seasons lasting at least four months. Between May and October the gonadal activity was reduced and breeding activity was depressed. Although we were unable to follow individual bird's schedules at our study site, we found breeding activity year round which overlapped with molt in 44% of individuals in breeding condition (N = 167). Our results support the idea proposed by Class et al. (2011) suggesting that specific life-history characteristics, in combination with seasonality of climate, could characterize reproductive seasonality more precisely than only latitude (Stutchbury and Morton, 2008).

Other species in the genus Zonotrichia in the northern hemisphere show a high seasonality and synchronicity in the breeding and molt schedules (Wingfield and Hahn, 1994; Wingfield, 2005), supporting the idea that latitude as a proxy of photoperiodic conditions and climate characteristics predicts how costly life history stages are and therefore result in narrower and well defined schedules with changing in latitude (Stutchbury and Morton, 2001; 2008). Specific conditions in the Atacama Desert such as moderate high temperatures, absence of rain and wind, abundant food and water derived from natural and agricultural sources probably result in a low cost molt and breeding processes thus allowing overlap. However, it is remarkable that this pattern was exhibited in birds with a higher body condition, suggesting that there is individual variability in energy expenditure and/or in foraging abilities. We found significantly higher proportion of birds overlapping in Azapa than in Lluta, which could be explained, at least partially, by higher resource availability derived of a more intense agricultural activity in Azapa than in Lluta.

4.2. Corticosterone

The release of glucocorticoids, CORT, regulated by the hypothalamic-pituitary-adrenal (HPA) axis plays a major role in an animal's ability to cope with perturbations (Wingfield et al., 1983; McEwen and Wingfield, 2003) including those associated with harsher climatic conditions (Bonier et al., 2007; Landys et al., 2004; Wingfield, 2003; Wingfield et al., 1998). In this study we observed stress-induced CORT levels $4 \times$ lower than previous studies in rufous-collared sparrows (Wada et al., 2006; Busch et al., 2010) which could indicate less challenging physiological conditions in Atacama Desert valleys relative to more seasonal environments. Also, CORT plays an important role in metabolic regulation and energy expenditure (Sapolsky et al., 2000; McEwen and Wingfield, 2003). Given this, a possible explanation for the lower levels of CORT associated with this environment may be that these birds have lower metabolic needs than seasonal populations (Cavieres and Sabat, 2008). In fact, the absence of seasonality in the environment could explain the fact that baseline levels of CORT do not differ across sampling month in this study.

Although we did not find significant differences across months, we observed a higher variation of baseline and stress-induced CORT levels during January, the month in which Altiplanic winter of the high Andes can affect the coastal zone. Although this phenomenon rarely affects this zone directly, it can cause a sudden rise in stream water levels. San José river (the river that goes through Azapa valley) has been defined by Campos et al. (2007) as an ephemeral river existing only between December and March when this river reaches its higher level. In contrast, at that time of the year, Lluta river has at least a $2.4 \times$ higher water level than San Jose river showing permanent exoreic draining (Campos et al., 2007). Thus, the possible floods derived from Altiplanic winter could be more important in this valley than in Azapa valley. Individuals with higher baseline levels of CORT may be able to have faster responses to environmental perturbations, such as storms and

flooding (Bonier et al., 2007; Romero et al., 2000). If there are a greater number of stressful perturbations during summer in that particular valley, higher baseline levels of CORT could be advantageous.

We observed no differences between baseline and stress-induced levels of CORT during March, when most birds were molting. The suppression of the stress-response during molt in seasonal environments has been widely documented (Astheimer et al., 1994; Romero et al., 2005) and several hypotheses such as deleterious effects of CORT on feather growth have been proposed to explain the negative relation between stress-response and molt (Cornelius et al., 2011). Notably in our study site, the suppression of the stress response cannot be explained exclusively by molt. We found a decrease in stress-induced levels in birds molting across the year compared to birds non-molting, but still a significant difference between baseline and stress-induced levels in birds molting across the year. On the other hand we found no difference between baseline and stress CORT plasma levels during March in both molting and non-molting birds. Coincidently, the percentage of suppression of stress-induced levels of CORT was significantly more associated with the sampling month than with molt or breeding activities across the year and that could explain why our pattern is coincident with patterns exhibited by seasonal breeders (Cornelius et al., 2011), an spurious phenomena derived from the association between month and molt, but not revealing a real 'seasonal breeder' pattern association. Wada et al. (2006) found a similar pattern in tropical populations of Z. capensis where birds undergoing postnuptial molt show a significant increase in stress-induced levels of CORT, although below breeding season levels. These results may be explained by the duration of molt in this species (~2 months), significantly longer than the 5 weeks described for the northern congeneric species (Wada et al., 2006). Although in our study site we were unable to follow individual birds, we observed birds undergoing molt year-round, suggesting that the molt duration could be even longer than rufous-collared sparrows in tropical locations such as Colombia (Miller, 1961). As proposed by Wada et al. (2006) the absence of suppression of stress-response during molt could be a byproduct of less energetically costly molt processes as in temperate Zonotrichia species because it occurs over a longer period of time and, in the case of our study site, overlapped with breeding.

4.3. Testosterone

In the Northern Hemisphere, seasonal modulation of T is observed in many socially monogamous species in which males are seasonally territorial and use territories for resources as well as mate recruitment (Wingfield et al., 1990). In these species, T levels are high at the beginning of the breeding season and it decreases during mid-breeding, when most of the parental care is displayed (Wingfield et al., 1990). Male rufous-collared sparrows are socially monogamous, provide parental care to and show high levels of male-male interactions (Miller and Miller, 1968). However, at our study site we did not observe significant seasonal variations in T levels which are consistent with the low degree of breeding seasonality. This pattern is consistent with many tropical species that maintain constant levels of T throughout the breeding season (Hau et al. 2008; Wikelski et al., 2000; Busch et al., 2008), but not all including subspecies of rufous-collared sparrows in Costa Rica (Addis et al., 2011) and Ecuador (Moore et al., 2004a,b). In this sense, our ranges of circulating testosterone levels in Z.c. peruviensis were within the same range as those observed for Ecuadorian and Costa Rican Z.c. costaricensis, between 2.5 and 6 ng/ml (Moore et al., 2004a,b; Addis et al. 2010). However, we did not observe a marked reproductive season which could be attributed to the benign climatic conditions year-round. On the other hand, social

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instability can be another factor for fluctuations of T levels (i.e., social modulation of T) as it has been shown in subspecies of white-crowned sparrow, *Z. leucophrys* (Wingfield and Wada, 1989; Wingfield and Hahn, 1994). However, tropical species with long breeding periods tend to not exhibit social modulation of T, as *Z.c. costaricensis* (Addis et al., 2011). In this context, further research is needed in order to elucidate individual annual cycles of T. The natural history of *Z.c. peruviensis* suggests that social modulation of T should be highly depressed in our study site.

5. Conclusions

In contrast with highly seasonal environments where the timing of behaviors may considerably affect an organism's chances of survival and reproduction (Wingfield, 2006), in aseasonal environments the order and timing of behaviors are not likely to be shaped by natural selection. In our study site, although river water level and photoperiod are seasonal, the most challenging cues such as temperature and rainfall are not. In this scenario, we observed the potential for birds to breed and molt at all times of year suggesting a lower seasonality of life history stages. Our results strongly suggest that in the Atacama desert, the long-term extremely stable and benign conditions could have relaxed the selective pressures over the timing of life history stages which was evidenced by the breeding and molt schedules, its overlap and relatively stable endocrine profiles.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ygcen.2013. 05.007.

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