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Contrasting seasonal and aseasonal environments across stages of the annual cycle in the rufous-collared sparrow, *Zonotrichia capensis*: Differences in endocrine function, proteome and body condition

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Abstract

1. The timing and duration of life-history stages (LHSs) within the annual cycle can be affected by local environmental cues which are integrated through endocrine signalling mechanisms and changes in protein function. Most animals express a single LHS within a given period of the year because synchronous expression of LHSs is thought to be too costly energetically. However, in very rare and extremely stable conditions, breeding and moult have been observed to overlap extensively in rufous-collared sparrows (*Zonotrichia capensis*) living in valleys of the Atacama Desert—one of the most stable and aseasonal environments on Earth.
2. To examine how LHS traits at different levels of organization are affected by environmental variability, we compared the temporal organization and duration of LHSs in populations in the Atacama Desert with those in the semiarid Fray Jorge National Park in the north of Chile—an extremely seasonal climate but with unpredictable droughts and heavy rainy seasons.
3. We studied the effects of environmental variability on morphological variables related to body condition, endocrine traits and proteome. Birds living in the seasonal environment had a strict temporal division of LHSs, while birds living in the aseasonal environment failed to maintain a temporal division of LHSs resulting in direct overlap of breeding and moult. Further, higher circulating glucocorticoids and androgen concentrations were found in birds from seasonal compared to aseasonal populations. Despite these differences, body condition variables and protein expression were not related to the degree of seasonality but rather showed a strong relationship with hormone levels.

4. These results suggest that animals adjust to their environment through changes in behavioural and endocrine traits and may be limited by less labile traits such as morphological variables or expression of specific proteins under certain circumstances. These data on free-living birds shed light on how different levels of life-history organization within an individual are linked to increasing environmental heterogeneity.

KEYWORDS
life-history stages, proteomics, seasonality, stress, testosterone, thyroid

1 | INTRODUCTION

Organisms arrange the temporal expression of each life-history stage (LHS) of the annual cycle according to environmental cues such as photoperiod, rainfall, food availability and changes in ambient temperature (Wingfield, 2008). The underlying physiological mechanisms such as endocrine traits and protein expression that coordinate the development, mature capability and termination of each stage of the annual cycle are reliant upon the predictability of these local factors (Wingfield et al., 2017). Thus, temporally appropriate expression of the annual stages allows animals to match their energetic expenditures with resource availability (Lack, 1954; Ashmole 1963, #712; Ricklefs, 1980). For example, in seasonal environments birds coordinate migration and breeding to utilize seasonal peaks in primary productivity (Cornelius, Boswell, Jenni-Eiermann, Breuner, & Ramenofsky, 2013; Ramenofsky, Campion, Perez, Krause, & Nemeth, 2017; Slagsvold, 1975). Considering that some stages of the annual cycle are associated with high energy costs, minimal overlap of LHSs of the annual cycle avoids detrimental energetic fitness costs (Echeverry-Galvis & Hau, 2012; Zera & Harshman, 2001;). In this study, we aimed to describe the effects of highly seasonal and aseasonal environments (i.e., variations on environmental heterogeneity) on physiology, morphology and the timing and duration of their three LHSs (breeding, moult and winter). We were particularly interested in the roles of the endocrine system in regulating the breeding LHS as assessed by circulating androgen concentrations and the ability of birds to cope with acute challenges indicated by fluctuations in plasma corticosterone concentrations. We also studied proteome dynamics by investigating chaperone and metabolic proteins. Finally, we investigated morphological traits such as body condition, pectoralis muscle profile, parasite load, fat storage and haematocrit. This broad approach allowed for the identification of underlying themes associated with living in either a seasonal or an aseasonal environment across three major LHSs of the annual cycle. It also provided a better understanding of the integration and consistency in the response of multiple traits at different levels under different environmental conditions across the annual cycle in free-living birds.

Rufous-collared sparrows (*Zonotrichia capensis*) live both in the fertile aseasonal valleys of the Atacama Desert—the driest and oldest desert on Earth (Hartley, Chong, Houston, & Matter, 2005)—and in the semiarid highly seasonal region of Fray Jorge National Park in the north of Chile. These sites provide a natural study system to examine the functions and mechanisms associated with seasonality within the same study species. In the Arica region of the Atacama Desert (hereafter AR), populations of *Z. capensis peruviensis* are resident in year-round green riparian habitats along river valleys. Based on temperature, humidity, precipitation, food and water supply, this population experiences an extremely stable environment (Gonzalez-Gomez et al., 2013) (see below and Field Site description in Supporting Information). In our previous study, the rufous-collared sparrow in the Atacama Desert expressed three major LHSs in the annual cycle, but a large fraction of individuals showed overlap between breeding and moult year-round (Gonzalez-Gomez et al., 2013). The phenomenon of LHS overlap has been described elsewhere in the tropics which likely is an adaptive strategy for individuals in an environment with a constant supply of food, high predation rates and small clutch sizes (Foster, 1975a,b).

In contrast, the *Z. capensis chilensis* populations in the Fray Jorge National Park region (hereafter FJ) to the south experience a high degree of seasonality (Gutierrez et al., 2010) and environmental unpredictability such as extreme droughts and floods, associated with strong El Niño Southern Oscillation (ENSO). As a consequence of high seasonality, there is extreme variation in food availability, especially for granivorous and year-round resident animals, such as rufous-collared sparrows (Meserve, Vasquez, Kelt, Gutierrez, & Milstead, 2016) (see below and Supporting Information). It is possible for birds to raise two clutches at FJ, but this has not been consistently studied (Pyle, Engilis, & Kelt, 2015).

The endocrine system is important for coordinating physiological, behavioural and morphological changes, and its activity is linked to environmental conditions (Gonzalez-Gomez et al., 2013). Corticosterone (CORT), the main glucocorticoid in birds, at baseline levels contributes to the regulation of basic metabolic functions such as protein and lipid metabolism, water balance and glucose provisioning to cells (Landys, Ramenofsky, Guglielmo, & Wingfield, 2004). During unpredictable perturbations of the environment, CORT levels become elevated to promote rapid changes in physiology and behaviour that allow individuals to cope via activation of the emergency life-history stage (ELHS) (Wingfield et al., 1998). Elevated levels of glucocorticoids promote the rapid mobilization of energy reserves via gluconeogenesis, enhanced immune response and increase in foraging behaviour (Wingfield et al., 1998). The activation of the ELHS

can also interrupt the current LHS (i.e., abandon breeding), so that energy can be reallocated to prioritize immediate survival (Wingfield et al., 1998). We also assessed circulating testosterone (T) which strongly promotes the onset of breeding, territory acquisition and aggression, and sexual displays (Wingfield, 1984; Wingfield & Hahn, 1994). Birds found in seasonal environments, such as FJ, tend to show very distinct and high amplitude peaks in T during the breeding season (Garamszegi et al., 2008; Hau, Ricklefs, Wikelski, Lee, & Brawn, 2010), while birds in aseasonal environment show indistinct and low-amplitude T as breeding occurs nearly year-round (Goymann et al., 2004).

The cellular stress response in free-living birds and its relation to hormone levels across LHSs of the annual cycle in contrasting environments are not known. Therefore, it is unclear how the metabolic proteins related to fat accumulation may differ between sites with unique energetic challenges. To understand the interaction of the endocrine system with protein function across contrasting environments at AR and FJ, we assessed the blood proteome in both populations with emphasis on two groups of proteins: (a) chaperones, which are particularly relevant to the adrenocortical stress response, preventing alterations in cellular homoeostasis (Garbuz & Evgen'ev, 2017); and (b) metabolism and fat storage-related proteins, which could play a key role in energy demanding LHSs of the life cycle such as moult and breeding (Supporting Information Table S1).

We predicted that the degree of seasonality and predictability would influence energetic demand between the two sites, which in turn would be expressed across multiple levels of organization. It has been observed that birds inhabiting localities with low predictability of rainfall and food abundance show higher ability to adjust their organ sizes and basal metabolic rate to these changes (Williams & Tieleman, 2000). In turn, this ability is associated with energy costs of maintenance and production of plastic structures (Piersma & Drent, 2003; Pigliucci, 2001). Rufous-collared sparrows occurring in habitats with high climatic variability, such as central Chile, show higher thermal acclimation via higher adjustments in basal metabolic rates, and also seasonal changes in organ sizes (Cavieres & Sabat, 2008; Novoa, Veloso, LopezCalleja, & Bozinovic, 1996). Thus, we predicted FJ would be a more challenging environment where birds face a greater number of unpredictable perturbations over the annual cycle. At AR where birds would experience lower energetic demands due to stable biotic and abiotic conditions, we predicted much less temporal organization of LHSs as found by Gonzalez-Gomez et al. (2013). If rufous-collared sparrows are affected by seasonality in these environments, and AR represents a less energetic demanding locality, we expected to confirm previous data (Gonzalez-Gomez et al., 2013) on year-round breeding and moult (and overlap of both cycles) in the highly aseasonal AR population, and find a strong temporal division of the annual LHSs in the highly seasonal FJ. We predicted better body condition variables (i.e., body condition index, parasite load, muscle development, fat storage, haematocrit percentage), higher levels of baseline and stress-induced levels of CORT, higher T and higher degree of seasonal variation in hormone levels in FJ compared to AR. We also predicted that chaperone protein concentrations would be positively linked to baseline and stress-induced CORT levels irrespective of the locality. Additionally, metabolic protein concentrations would be positively linked to body condition and higher in the seasonal than in the aseasonal environment.

2 | METHODOLOGY

2.1 | Species and study site

Our study model was rufous-collared sparrow, *Zonotrichia capensis*, a species that inhabits a wide range of environments from Mexico to Cape Horn (Class, Wada, Lynn, & Moore, 2011). Our study in the Atacama Desert (18°20′S, 70°20′W) was conducted in two valleys near Arica, Azapa and Lluta (20 km apart), where variation in photoperiod, temperature and precipitation is low (Supporting Information). The subspecies here is *Z.c. peruviensis*. Although these desert valleys can be extremely hostile, fertile oasis exists due to riparian areas which have vegetation year-round. The water to promote high primary productivity comes from dense fog from the Pacific Ocean moving along the valleys every morning, and streams fed by snowmelt in the Andes year-round from ~6,500 m.a.s.l. These valleys also have intense agricultural activity providing high amounts of food for songbirds, especially granivorous–omnivorous rufous-collared sparrows. All of these conditions along with stable climatic variables explain high densities of birds (0.83 ± 0.06 birds/hours of mist per netting; Ralph, 1976) in comparison with other regions such as Fray Jorge.

The second site was near Fray Jorge National Park (30°30′S, 71°35′W) in a semiarid region of north-central Chile just south of the Atacama Desert, where we worked in three valleys (not more than 25 km apart), where the climatic variations are extreme. The subspecies here is *Z.c. chilensis*. This region has experienced a decline of 50% in rainfall in the past 50 years and has been subject to the three largest ENSOs of the past 100 years and have occurred since 1982 (Gutierrez et al., 2010). During ENSO, the mean annual precipitation of 133 mm (mean, 1989 and 2008) increased to three- to fourfold (1991–92, 233–229 mm; 1997, 330 mm; 2000–2002, 236–339 mm; 2004, 168 mm; 2006, 147 mm). This had large impacts on food abundance through alterations in patterns of nutrient cycling and primary productivity (López-Cortés & López, 2004). In turn, insects and seed abundance were also extremely variable (De La Maza, Lima, Meserve, Gutierrez, & Jaksic, 2009; Meserve et al., 2016). These periods are separated by intense droughts (11 to 89 mm rainfall). Temperatures vary seasonally from daily maxima of 30.8 ± 0.16°C in summer to daily minima that can reach temperatures below freezing in winter. Approximately 90% of the rainfall occurs in winter (May–September), while summers are warm and dry (Kummerow, 1966). Vegetation is characterized by sclerophyllous and evergreen shrubs strongly dependent on variable coastal fog (De La Maza et al., 2009; Meserve et al., 2016). This extreme variability probably influences the density of rufous-collared sparrows (0.30 ± 0.04 birds/hr of mist netting), which is 2.7 times lower than in our AR sampling site (see Supporting Information).

2.2 | Stage of the annual cycle and morphometric measurements

Birds were passively captured (i.e., we did not use playbacks of vocalizations or any other attractants) using mist nets over 10-day periods in each of the following months: March, July, October and December in 2015; and January, March and October in 2016. These months were chosen because they represent the four seasons, austral fall, winter, spring and summer, in the seasonal FJ. Therefore, they are appropriate to compare and contrast timing of LHSs in the annual cycle and other variables between AR and FJ. Protocols for morphometrics and scoring the stage of the annual cycle have been previously described in detail (Gonzalez-Gomez et al., 2013). At capture, all birds were banded with uniquely numbered metal bands and we measured wing, tarsus and tail length (mm), and body mass (g). Reproductive status was assigned based on the presence or absence of a brood patch or cloacal protuberance. Birds were classified as in moult if more than 20% of body feathers or more than one primary flight feather was being replaced.

Multiple metrics of body condition were measured: (a) fat (0 = "no fat" to 6 = "heavy fat") by visual inspection of subcutaneous fat in the furculum and abdomen; (b) pectoral muscle profile (0 = "prominent edge of the keel" to 4 = "fully developed muscle"; (c) parasite load (0 = "no parasites" to 4 = "heavy parasite load") according to the amount of parasites (i.e., feather mites *Amerodectes zonotrichiae*, Llanos-Soto et al., 2017) found in flight feathers by visual inspection; and (d) haematocrit percentage. Considering these measurements are qualitative, all the body condition variables in this study were assessed by three observers who received the same training. We generated an integrated estimate of body condition using principal components analysis (see Statistical analyses). The use of size-corrected mass as an index of body condition can be problematic due to all the variables that can affect these measurements (Clancey & Byers, 2014). However, we choose to use it considering our large sample size of free-living animals while controlling for variables such as season and stage in the annual life cycle. We did not attempt to use it as a proxy of survival or reproductive success in an evolutionary framework.

2.3 | Blood sampling

We collected blood from the brachial vein using a 26-gauge needle and heparinized microhaematocrit tubes. Within 3 min of capture, we collected the first sample for baseline CORT and within 10 min of capture for the individuals in those we measured T. To assess stress-induced levels of CORT, we took a second blood sample after 30 min of standardized restraint by placing birds in a small cloth bag (hereafter "handling time") (Breuner, Wingfield, & Romero, 1999). The total amount of blood collected was less than 1% of the bird's mass. Samples were kept on ice (maximum of 4 hr) until they were centrifuged for 5 min at 2,000 × g to separate plasma and red blood cells. Haematocrit was measured in the first sample tube. Then, plasma was aspirated and stored frozen (at −20°C) until analysis.

2.4 | Endocrine analyses

Plasma concentrations of T and CORT were determined using radioimmunoassays (RIA) (Wingfield, 1984). A detailed description of RIA is presented in Supporting Information. The mean detection limits of the assay were 8.60 pg/tube. A total of four assays were run for T and eight for CORT. Intra-assay variation ranged from 1.7% to 3.2% for T, and recoveries were 78.9 ± 0.39%. For CORT, intra-assay variation ranged from 1.76% to 2.9%, and recoveries were 85.6 ± 0.38%. Interassay variation was 2.13% for T and 2.11% for CORT. The antibody was validated by checking for parallelism between diluted plasma pool that was spiked with a known amount of corticosterone and the standard curve. All samples were run in duplicate.

2.5 | Proteomics

Plasma proteome was analysed in 18 birds (AR $N = 9$, FJ $N = 9$) collected in March 2015. Plasma proteins were extracted and trypsin-digested as previously described (Kültz et al., 2015). Tryptic peptides were separated by nano-ultra-high-performance liquid chromatography (UPLC) (Waters) using a 5–35% acetonitrile gradient over a 2-hr retention time period and injected online into an Impact-HD UHR-qTOF mass spectrometer (Bruker Daltonics). Following electrospray ionization, peptides were identified using data-independent acquisition and tandem mass spectrometry as previously described (Kültz, Li, Gardell, & Sacchi, 2013). Label-free quantification was performed using PEAKSQ 8.0 for an MS1 Top3 approach and SCAFFOLD 4.8 for a spectral counting approach as previously described (Kültz et al., 2013, 2015). More details on methodology and parameters used for quantitative proteomics are provided in Supporting Information. The dataset and results of PEAKSQ and Scaffold analyses are publicly accessible at the CAMP Proteome Repository, Massive and ProteomeXchange (see the Data Accessibility section for accession numbers and links).

2.6 | Statistical analyses

As there was no effect of study site within AR ($N = 2$) or FJ ($N = 3$) localities, the data were pooled to increase statistical power. Differences in the timing of LHSs of the annual cycle, and body condition variables such as fat, muscle and parasite scores, across the year in AR and FJ were tested using an ordinal logistic model (OLM), as the response variables were qualitative. Bayesian information criterion (BIC) and maximum-likelihood (Akaike information criterion corrected for sample size, AICc) estimation methods were used to evaluate the best of three ordinal logistic models to explain timing of moulting and breeding LHSs based on the independent variables of month, locality (i.e., AR, FJ) and their interaction. Contingency analysis was used to analyse differences in the selected model. Body condition index was estimated performing

a principal components analysis (PCA) including tarsus, tail and wing lengths by locality. The first component explained 48.4% and 50.7% of the variation in AR and FJ, respectively (see Supporting Information). Then, the first principal component was regressed against body mass for each locality (Atacama Desert, $r^2 = 0.03$, $F_{1,780} = 32.07$, $p < 0.01$; Fray Jorge, $r^2 = 0.01$, $F_{1,222} = 27.82$, $p = 0.022$), and the residuals of these regressions were used as body condition index for each locality. The effects of sampling month, locality and LHS cycle on body condition index and haematocrit were assessed with a generalized linear model (GLM). The effect of handling time, sampling month and locality on CORT levels was assessed through two-way repeated-measures analysis of variance (RMANOVA). The relationship between body condition index and baseline or stress-induced CORT was assessed using linear regressions. To explore the relationships of locality, sampling month and their interaction on testosterone levels, we performed a two-way ANOVA. The association of LHS of the annual cycle on testosterone levels was assessed through one-way ANOVAs. Tukey's multiple comparisons (honest significant differences) for different sample sizes were used for post hoc tests. The relationships between hormone levels and body condition variables were assessed through ordinal logistic models. The relations between proteins and body condition index and baseline and stress-induced levels were assessed using GLM with normal and exponential distribution and identity or reciprocal links alternatively. We compared the performance of these models against a null model with no independent variable using AIC. Statistical analyses were performed with JMP®, Version 10.0 (SAS Institute Inc., Cary, NC, USA 1989–2007).

All the quantitative analyses to compare the proteome between localities have been statistically corrected for multiple testing. PEAKSQ quantification was performed via two statistical procedures: (a) the MaxQuant algorithm (Cox & Mann, 2008) and (b) ANOVA with Benjamini–Hochberg correction (Benjamini & Hochberg, 1995). Scaffold analysis was performed using *t* test with Benjamini–Hochberg correction (Benjamini & Hochberg, 1995). Out of the 18 birds with proteomics data, 12 also had CORT data to assess the relation between HSP and baseline and stress-induced levels of CORT.

3 | RESULTS

During 2015 ($N = 363$) and 2016 ($N = 239$), we captured a total of 602 individuals, in two localities: Arica valleys (AR) and the area near Fray Jorge National Park (FJ). Sample sizes in relation to season were as follows: March (AR $N = 60$, FJ $N = 24$), June/July (AR $N = 47$, FJ $N = 38$), October (AR $N = 44$, FJ $N = 14$) and December (AR $N = 67$, FJ $N = 79$) in 2015; and January (FJ $N = 39$), March (AR $N = 57$, FJ $N = 60$) and October in 2016 (AR $N = 78$, FJ $N = 5$). In the few cases of recapture, we only used data from the first capture in the analyses. We combined these data with our previous data from AR (Gonzalez-Gomez et al., 2013) to have larger sample size (Total $N = 1059$) when analysing stage of the annual cycles and hormone levels (see below).

3.1 | Timing of life-history stages in birds experiencing different degrees of environmental heterogeneity

LHSs of the annual cycle were affected by month (likelihood ratio chi-square, $G^2_{4,1001} = 225.16$, $p < 0.01$), locality (likelihood ratio chi-square, $G^2_{1,1024} = 11.75$, $p < 0.01$) and their interaction (likelihood ratio chi-square, $G^2_{1,1024} = 59.16$, $p < 0.01$). Paired contingency analysis indicated that the proportion of birds in each LHS of the annual cycle varied across all months (Figure 1).

Timing of the breeding LHS was affected by month (likelihood ratio chi-square, $G^2_{4,1024} = 297.25$, $p < 0.001$) and locality (likelihood ratio chi-square, $G^2_{4,1024} = 21.35$, $p < 0.001$). Breeding LHS in both localities peaked during October when approximately 80% of individuals were breeding in AR and 92% in FJ. In contrast, during March

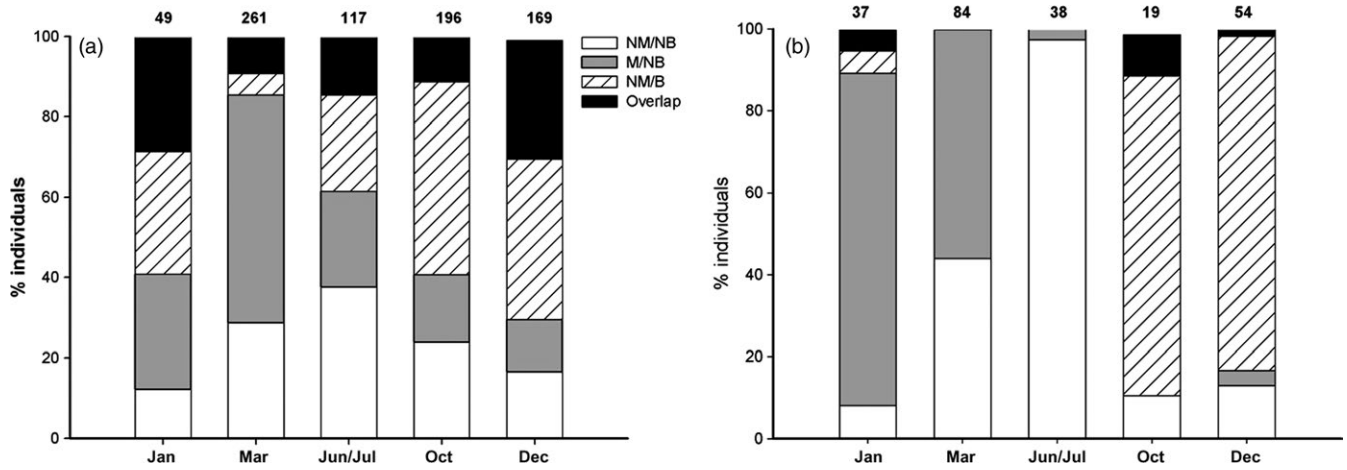

FIGURE 1 Timing of life-history stages in *Zonotrichia capensis* in the aseasonal Atacama Desert (a) and in the seasonal Fray Jorge National Park area (b). White: nonmoulting, nonbreeding (NM/NB); grey: moulting, nonbreeding (M/NB); white with black stripes: nonmoulting, breeding (NM/B); and dark grey: overlapping moult and breeding

26% of the individuals were breeding in Arica and 0% were in FJ (Figure 1).

Moult was affected by month (likelihood ratio chi-square, $G^2_{4,1000} = 129.76$, $p < 0.001$) and locality (likelihood ratio chi-square, $G^2_{4,1000} = 4.15$, $p = 0.004$). In FJ, birds moulted between January and March, while in AR, bird moulted year-round, although we found a higher proportion of birds moulting in March (Figure 1).

The proportion of birds overlapping moult and breeding was 27 times higher in AR than in FJ (likelihood ratio chi-square, $G^2_{1,843} = 37.93$, $p < 0.001$). Overlap was not significantly different across sampling months in AR, and it was coincident with the breeding season in FJ (Figure 1).

3.2 | Body condition

The BIC and AIC methods performed similarly when we analysed the effect of locality (FJ, AR), month and LHSs (i.e., moulting, breeding, nonmoulting nonbreeding, overlapping moult and breeding) on body condition.

Fat Score. The best model included month and locality (AIC = 1318.23). Fat scores were affected by locality (OLM, $\chi^2_{1,510} = 10.83$, $p < 0.001$) and sample month ($\chi^2_{4,510} = 59.51$, $p < 0.01$), but the interaction was not significant ($\chi^2_{4,510} = 0.41$, $p = 0.93$). The highest fat scores were recorded in FJ during winter when birds were not moulting and not breeding (NM/NB) (Figure 2).

For pectoralis muscle score, the model including sampling month performed significantly better than the model including stage of the annual cycle and the interaction between these variables. Pectoralis muscle score increased during winter (birds NM/NB) in both localities (OLM, $\chi^2_{4,509} = 72.79$, $p < 0.01$; Figure 2).

When we analysed parasite load score, both BIC and AIC estimation methods performed best when locality and sampling month were included. Birds had a higher parasite load in FJ than in AR (OLM, $\chi^2_{1,508} = 112.68$, $p < 0.01$) and during winter months ($\chi^2_{4,508} = 65.26$, $p < 0.01$; Figure 2).

Haematocrit levels were higher in FJ than in AR (GLM $\chi^2_{1,495} = 25.19$, $p < 0.001$; Figure 2), but followed the same seasonal pattern with higher levels during the winter (Jun/July) and lower

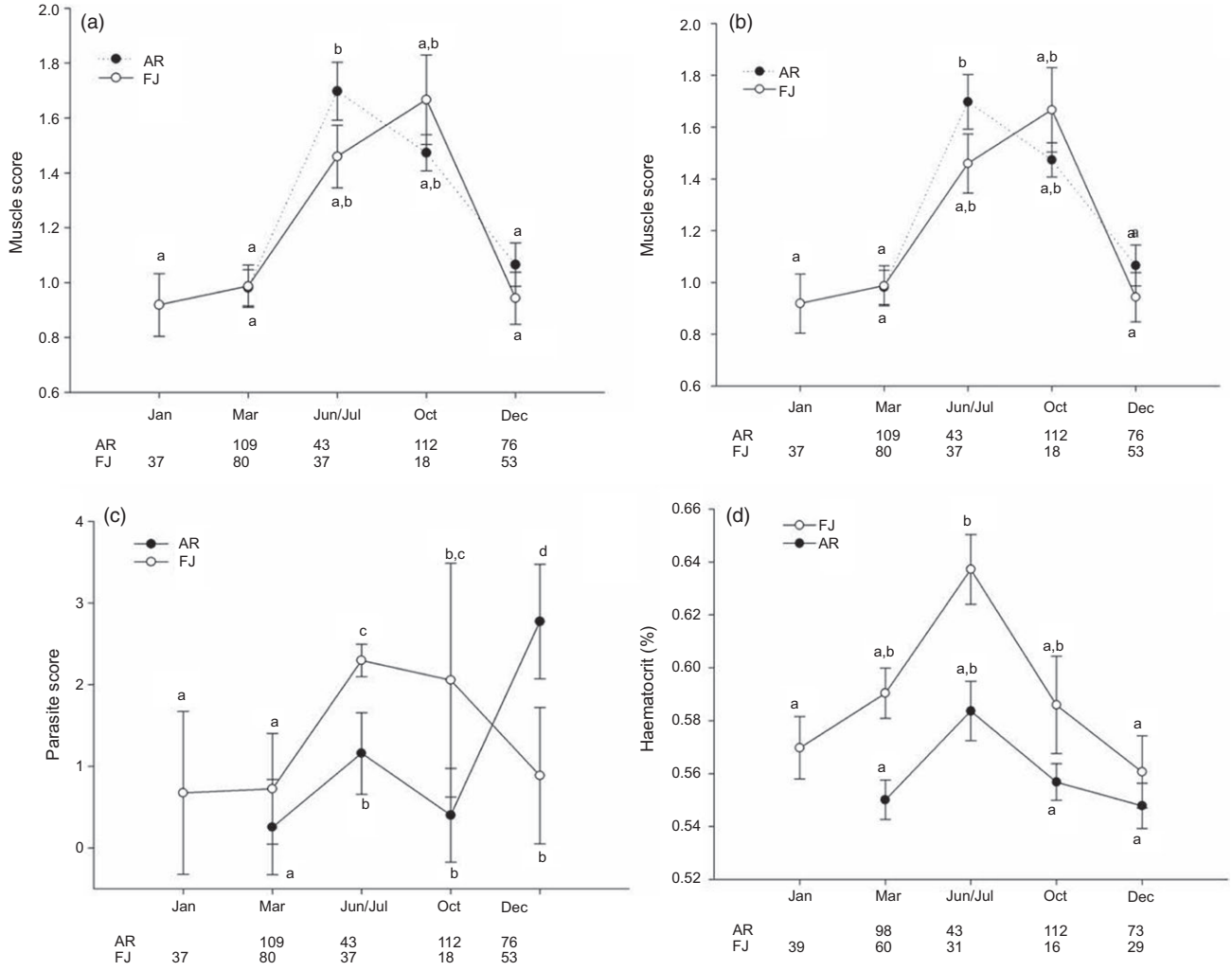

FIGURE 2 *Zonotrichia capensis* fat score (a), muscle score (b), parasite load (c) and haematocrit percentage (d) across the year in the seasonal Fray Jorge (FJ) and aseasonal Atacama Desert (AR) areas. Tukey's HSD post hoc tests, significant differences shown in *letters* ($p < 0.05$). Numbers below the axis indicate samples sizes for each group

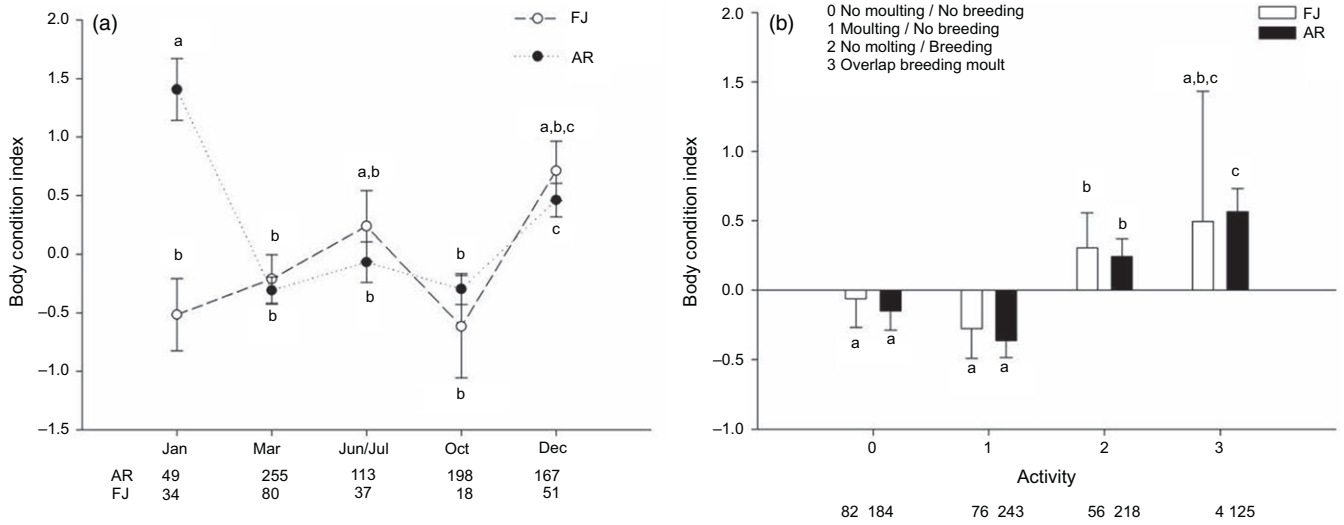

FIGURE 3 Body condition index in relation to sampling month (a) and life-history stage (b) in rufous-collared sparrows in the seasonal Fray Jorge (FJ) and the aseasonal Atacama Desert (AR). Tukey's HSD post hoc tests, significant differences shown in *letters*

levels in the austral summer in both localities (GLM $\chi^2_{4,492} = 20.49$, $p < 0.001$). We found no interaction between month and locality (GLM $\chi^2_{3,493} = 2.98$, $p = 0.39$).

When we analysed the effect of LHS of the annual cycle on body condition index, we observed birds in better body condition in FJ (2.589 ± 0.15) than in AR (−2.05 ± 0.06, mean ± *SE*, GLM $\chi^2_{1,996} = 3.99$, $p = 0.045$; Figure 3), especially during January (0.59 ± 0.2) in comparison with March and October (−0.28 ± 0.10 and −0.32 ± 0.12, respectively, GLM $\chi^2_{4,996} = 29.03$, $p < 0.001$). We also found an interaction between locality and sampling month (GLM $\chi^2_{4,996} = 23.80$, $p < 0.001$). Remarkably, birds that overlapped moult and breeding in AR had significantly better body condition index than birds in other activity categories. We observed a marked negative effect of moult on body condition in both populations ($F_{3,987} = 4.32$, $p = 0.004$).

3.3 | Hormone levels

3.3.1 | Corticosterone

Baseline CORT levels were not different across localities (RMANOVA $F_{1,352} = 0.31$, $p = 0.57$; Figure 4), months (RMANOVA $F_{1,352} = 1.43$, $p = 0.22$; Figure 4) or their interaction (RMANOVA $F_{1,352} = 0.19$, $p = 0.94$; Figure 4). Stress-induced CORT levels were higher in FJ compared to AR (RMANOVA $F_{1,352} = 33.40$, $p < 0.001$; Figure 4) and were higher in October than in the rest of the sampling months ($F_{1,352} = 22.88$, $p < 0.01$). Handling time affected stress-induced CORT levels ($F_{1,352} = 400.17$, $p < 0.001$) but interacted with sampling month ($F_{4,352} = 5.01$, $p < 0.001$) and location ($F_{1,352} = 38.075$, $p < 0.01$; Figure 4). Stress-induced levels in FJ, specifically in October, were significantly higher than stress-induced levels in AR (Figure 4).

Baseline CORT levels were similar between birds that were moulting and those that were not moulting across the year (GLM $\chi^2_{1,199} = 1.035$, $p = 0.30$), irrespective of the locality. We found a positive relationship between breeding condition and baseline CORT levels ($F_{1,197} = 24.16$, $p < 0.01$; Figure 4), but this relationship was stronger in FJ than in AR ($F_{1,199} = 39.81$, $p < 0.001$). Stress-induced CORT levels were similar between birds that were moulting and those that were not moulting across the year (GLM $\chi^2_{1,199} = 2.53$, $p = 0.11$), irrespective of the locality. As expected, we found a positive relationship of breeding LHS and stress-induced levels ($F_{1,197} = 58.71$, $p < 0.01$; Figure 4) regardless of locality ($F_{1,199} = 3.65$, $p = 0.057$) or interaction between locality and breeding LHS ($F_{1,199} = 0.41$, $p = 0.52$).

We also found a negative relationship between baseline CORT and body condition ($R^2 = 0.11$, $F_{1,358} = 4.84$, $p = 0.02$). Parasite score had a positive significant relationship to CORT levels (RMANOVA, $F_{4,180} = 3.02$, $p < 0.05$), but we found no significant relation to location ($F_{1,333} = 0.49$, $p = 0.48$) nor of the interaction between parasite score and location ($F_{1,176} = 1.01$, $p = 0.40$). Fat and muscle scores had no relationship to CORT levels (all $p > 0.05$). None of these variables had a significant association with baseline levels of CORT (all $p > 0.05$).

3.3.2 | Testosterone

T levels were not different among sampling months ($\chi^2_{1,392} = 29.15$, $p < 0.01$), and with the interaction between month and locality ($\chi^2_{1,392} = 11.88$, $p < 0.01$; Figure 5a). When we included LHS of the annual cycle, T levels were significantly higher during the reproductive season in FJ but not in AR ($\chi^2_{1,384} = 9.96$, $p < 0.01$) (Figure 5b). We did not find an association of body condition on T levels ($F_{1,356} = 0.26$, $R^2 = 0.01$, $p = 0.61$), or the interaction between condition and locality (GLM $\chi^2_{1,356} = 0.05$, $p = 0.81$). Fat and muscle scores were not related to T levels (all $p > 0.05$).

3.4 | Proteomics

Using proteomics analysis, we identified a total of 215 proteins in plasma, of which 42 proteins differed between AR and

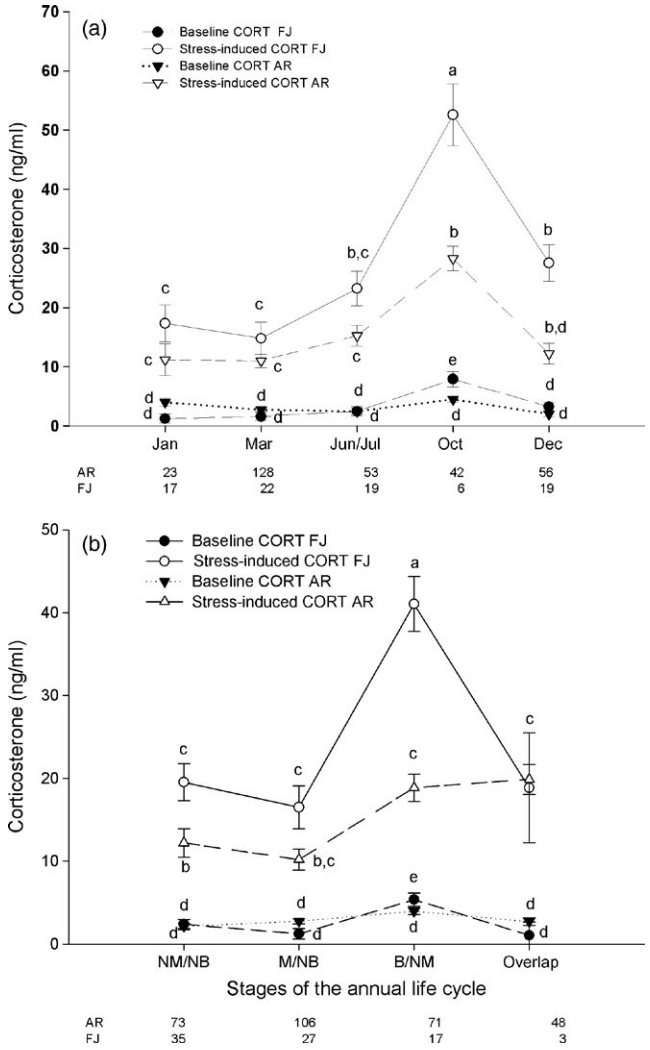

FIGURE 4 Stress-induced and baseline CORT levels of rufous-collared sparrows across the (a) year and (b) life-history stage activities (NM/NB = nonmoulting/nonbreeding; M/NB = moulting/nonbreeding; B/NM = nonmoulting/breeding; Overlap = overlapping moult and breeding) in the aseasonal Atacama Desert (AR) and the seasonal Fray Jorge (FJ). Tukey's HSD post hoc tests, significant differences shown in *letters*. Sample sizes are shown for each location

FJ. Visualization of the proteins can be found in the heat map (Supporting Information Figure S1).

For chaperone protein data, other than adenylate kinase which showed higher concentrations in FJ, we did not find any differences related to locality (Table 1). We also found no differences between localities for metabolic and endocrine-regulatory proteins (Table 1).

We found that the circulating concentrations of HSC71 and HSP90 were negatively and exponentially related to baseline levels of CORT (Table 2; Figure 6). Stress-induced levels of CORT showed a positive and linear relationship with adenylate kinase and a positive and exponential association with histone-lysine (Table 2).

Metabolic and endocrine-regulatory proteins were also related to body condition (Table 3). StAR was expressed in blood in 50% of the individuals, and it had a positive exponential and linear relationship to body condition, although the exponential fit was better. Both baseline and stress-induced CORT levels were related to StAR, although negative in the former and positive in the latter.

Interestingly, in the chaperone group of proteins such as adenylate kinase, they were marginally related to heat-shock proteins (Table 3). In the group of metabolic and endocrine-regulatory HSC71 and HSP90, they were positively both linearly and exponentially related to creatine kinase (Table 3).

4 | DISCUSSION

In this study, we assessed the association of seasonality (i.e., environmental heterogeneity) on annual LHSs, body condition, hormone levels and the proteome. We expected strong seasonal patterns in all of these aspects in the variable environment (FJ), and an aseasonal pattern in the Atacama Desert (AR) where the environmental variation across the year is extremely low. We observed a strong relationship between seasonality and the expression of LHSs and hormone levels, only. The proteome and morphological body condition variables were linked to individual physiological variables rather than degree of seasonality.

4.1 | Timing of life-history stages

Across the distribution of rufous-collared sparrows, there are varying degrees of seasonality, and as a result, different patterns of timing and duration of their stages of the annual cycle (Class et al., 2011). In the aseasonal environment (AR), we found breeding and moult LHSs occurred year–round, and for some individuals, these stages were coexpressed. Despite a lack of strong seasonal signals, we observed individuals in different LHSs of the annual cycle across the year but with a higher percentage of birds breeding in October and moulting in March, indicating a weak seasonal pattern. We also found heavy overlap between moult and breeding (Figure 1). In contrast, we observed a strong division of LHSs of the annual cycle in the highly seasonal environment at FJ, when most of the birds were breeding between October and December, moulted in January and started wintering towards March, presumably avoiding the high costs of overlapping LHSs of the annual cycle (Johnson, Stouffer, & Bierregaard, 2012). Similar to our observations in FJ, most of the studied populations of *Z. capensis* show breeding and moulting stages of the annual cycle in separate seasons once a year (Davis, 1971; King, 1973; Miller, 1959; Moore, Bonier, & Wingfield, 2005; Wolf, 1969), although high-altitude populations in Colombia can moult and breed twice a year, but overlap was not observed (Miller, 1962). Challenging this idea, five individuals at the seasonal environment (FJ) overlapped breeding and low rate moult, while no other instances of overlap of stages of the annual cycle were observed. However, overlapping has never been described outside of the extremely

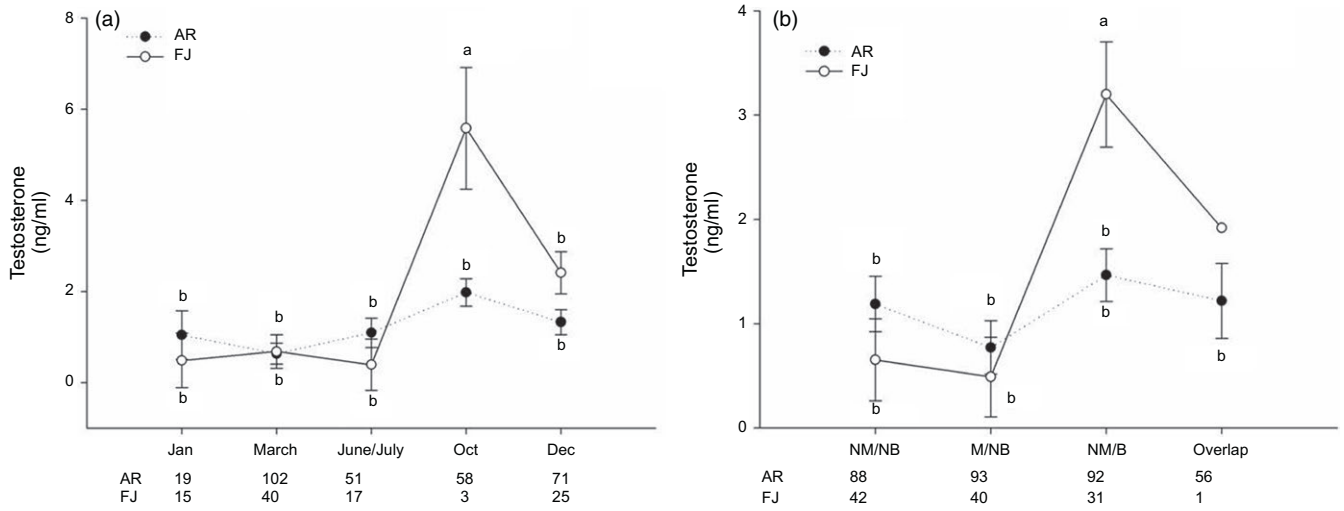

FIGURE 5 (a) Testosterone levels across sampling months in the aseasonal Atacama Desert (AR) and the seasonal Fray Jorge (FJ). (b) Testosterone levels across life-history stage (NM/NB = nonmoulting/nonbreeding; M/NB = moulting/nonbreeding; M/B = nonmoulting/breeding; Overlap = overlapping moult and breeding). Tukey's HSD post hoc tests, significant differences shown in *letters*

aseasonal AR sites (Gonzalez-Gomez et al., 2013). In other species, moult and breeding overlap has been described in numerous tropical species (Foster, 1975b), and it seems to be adaptive under certain conditions, such as small clutch sizes, high and constant food supply, long breeding seasons and probably high nest predation rates (Foster, 1975a). Although our results support the idea that energy restrictions are an important modulating factor in the timing and duration of stages of the annual cycles in these populations, mores studies assessing clutch size, and nest success rate in individual birds, are needed to determine the net effect of both strategies (overlap versus nonoverlap) on fitness.

4.2 | Body condition

In general, we found a stronger link between body condition and the time of year as opposed to the degree of seasonality. The degree to which seasonality affects this is open to interpretation at AR. Fat scores peaked in the austral winter at both localities, although greater fat scores were measured in birds from the seasonal environment (FJ) than in the aseasonal environment (AR). Many bird species follow this pattern, depositing fat reserves during nonbreeding LHSs, and depleting them during breeding season (Williams, 2012), although more so in environments with greater seasonality (Rogers & Smith, 1993). Remarkably, despite the extremely low seasonality that birds in AR experience, they followed the same pattern, although attenuated. It is possible that minimal changes in temperature or changes in the annual photoperiod trigger hyperphagia and increase fat storages, but more studies are necessary to clarify this.

Pectoralis muscle score also followed a seasonal (time of year) pattern, with marked peaks during the reproductive season, although higher in the seasonal (FJ) than in the aseasonal environment (AR). At least in FJ, this could be a result of the increases in circulating androgens during the reproductive season (Ramenofsky & Nemeth, 2014). However, birds in AR showed an increase in pectoralis muscle despite undetectable changes in T levels (see below), which suggests that other mechanisms could be acting, such as seasonal expression of androgen receptors and metabolizing enzymes in relation to circulating androgens (see Refs).

Parasite score had higher peaks during winter and spring in FJ, and lower values in summer. Coincidently, several studies have shown a peak in ectoparasite abundance during breeding season, most likely because feather mites in birds are commonly transmitted from parents to nestlings (Harbison, Bush, Malenke, & Clayton, 2008). Lower parasite loads during the summer at FJ can be explained because mite infections are restricted by feather lost during prebasic moult (Moyer, Gardiner, & Clayton, 2002), and lower humidity (Moyer, Drown, & Clayton, 2002). In contrast, these factors do not explain seasonality in feather mite abundance in the arid and aseasonal environment in the AR. Further studies are needed to investigate parasite load variations in AR birds.

Haematocrit levels followed a marked seasonal pattern in both localities, with higher peaks in birds wintering, although absolute levels were higher in FJ than in AR (Figure 2). Previous studies have found higher haematocrit levels during the winter LHS in seasonal environments, most likely associated with enhanced oxygen uptake due to increased thermogenesis (Fair, Whitaker, & Pearson, 2007; Krause et al., 2016). In our study where both subspecies are resident, we found higher values in general and variations of ~12% in the seasonal FJ, similar to variations in migratory white-crowned sparrows (Krause et al., 2016), which could be explained by the strong variations in temperature that these birds experience in FJ. It is remarkable that birds in the AR population follow an apparent seasonal pattern, even when the variations in temperature are minimal. Body condition index was strongly associated with LHS of the annual cycle with moult and wintering birds in lower body condition coincident with higher values of haematocrit percentage

TABLE 1 Metabolic and chaperone proteins in two populations of rufous-collared sparrows

	Protein	Function	Locality *df* = 11, *N* = 12
Metabolic proteins	Apolipoprotein	Lipid transport	AR = 14,961.7 ± 5,136.2 FJ = 25,905.7 ± 7,581.2 (mean ± *SE*) *t* = 1.19, *p* = 0.12
	Steroidogenic acute regulatory protein (StAR)	Role in the production of steroid hormones	AR = 2,443.3 ± 1,986 FJ = 21,285.7 ± 13,686 (mean ± *SE*) *t* = 1.36, *p* = 0.11
	Creatine kinase	Energy reserves	AR = 826.66 ± 2,024.91 FJ = 934.857 ± 410.05 (mean ± *SE*) *t* = 0.11, *p* = 0.45
	Albumin	Fatty acid transport	AR = 7,078,333.0 ± 1,127,810.3 FJ = 5,088,571 ± 720,691.41 (mean ± *SE*) *t* = 7.78, *p* = 0.08
	Fatty acyl-CoA hydrolase	Fatty acid metabolism	AR = 1,795.17 ± 621.775 FJ = 730.86 ± 276.24 (mean ± *SE*) *t* = −1.51, *p* = 0.08
	L-Lactate dehydrogenase	Glycolysis	AR = 643.33 ± 416.24 FJ = 762.42 ± 295.53 (mean ± *SE*) *t* = 0.23, *p* = 0.41
	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	Glycolysis	AR = 2,605 ± 1,589.81 FJ = 1,643.57 ± 1,439.7 (mean ± *SE*) *t* = −1.13, *p* = 0.85
	HSC71	Folding protein stability	AR = 2,603.15 ± 1,233.70 FJ = 1,825.02 ± 574.00 (mean ± *SE*) *t* = −0.57, *p* = 0.70
Chaperone proteins	HSP90	Folding protein stability	AR = 2,492.66 ± 1,589.81 FJ = 1,514.25 ± 575.2 (mean ± *SE*) *t* = −0.49, *p* = 0.68
	Ankyrin repeat domain	Folding protein stability	AR = 548,200 ± 403,886 FJ = 181,857 ± 403,886 (mean ± *SE*) *t* = −0.87, *p* = 0.78
	Adenylate kinase	**Cellular energy homoeostasis**	**AR = 563.67 ± 371.21** **FJ = 5,108.57 ± 1,838.30 (mean ± *SE*)** **t_{11} = 2.02, *N* = 12, *p* = 0.043**
	Histone-lysine N-methyltransferase	Epigenetic gene regulation	AR = 83,828 ± 63,240 FJ = 151,264 ± 88,909 (mean ± *SE*) *t* = 0.61, *p* = 0.27
	DNA mismatch repair protein Msh6	DNA repair	AR = 201,780 ± 67,803 FJ = 98,200 ± 28,102 (mean ± *SE*) *t* = −1.41, *p* = 0.89

Significant results are shown in bold.

during the wintering (LHS). Interestingly, we found that birds overlapping moult and breeding, especially in the aseasonal AR site, had higher body condition than birds just breeding, suggesting that this phenomenon is associated with the lack of energy constraints.

4.3 | Hormone levels

We observed no differences between baseline levels of CORT across the year or between our sites. In contrast, we found lower plasma stress-induced levels of CORT in the aseasonal (AR) than in the seasonal environment (FJ). This could be related to the stable conditions at AR, implying lower energy challenges than for birds inhabiting more heterogeneous environments such as FJ (Cavieres & Sabat, 2008). In the Northern Hemisphere, studies have found that individuals at sites with more benign conditions often have lower corticosterone levels than those at sites exposed to harsher condition (Addis, Davis, Miner, & Wingfield, 2011; Boelman et al., 2015; Krause, McGuigan, Bishop, Wingfield, & Meddle, 2015; Krause, Meddle, & Wingfield, 2015; Walker et al., 2015; Wingfield, Kubokawa, Ishida, Ishii, & Wada, 1995; Wingfield et al., 2015). However, in some species inhabiting harsh environments such as the arctic where the breeding season is short, CORT levels are low during the parental care, most likely to preserve the only possible reproductive attempt (O'Reilly &

TABLE 2 Associations between body condition, baseline and stress-induced CORT levels and metabolic and chaperone plasma proteins

Protein	Body condition (*df* = 1,12)		Baseline CORT (*df* = 1,11)		Stress-induced CORT (*df* = 1,12)	
	GLM normal distribution, link: identity	GLM exponential distribution, link: reciprocal	GLM normal distribution, link: identity	GLM exponential distribution, link: reciprocal	GLM normal distribution, link: identity	GLM exponential distribution, link: reciprocal
Metabolic proteins						
Apolipoprotein	X^2 = 1.11 *p* = 0.29 AICc = 274.01 Null AICc = 271.45	X^2 = 0.89, *p* = 0.34 AICc = 264.78 Null AICc = 262.74	X^2 = 1.30 *p* = 0.25 AICc = 274.68 Null AICc = 272.32	X^2 = 0.68 *p* = 0.40 AICc = 265.51 Null AICc = 263.27	X^2 = 0.52 *p* = 0.46 AICc = 297.71 Null AICc = 294.77	X^2 = 0.28 *p* = 0.59 AICc = 289.49 Null AICc = 286.94
Steroidogenic acute regulatory protein (StAR)	**X^2 = 4.78** **Coef = −2841.6** ***p* = 0.028** **AICc = 260.31** Null AICc = 306.90	**X^2 = 22.17** **Coef = 0.002** ***p* = 0.0001** **AICc = 213.88** Null AICc = 273.81	X^2 = 0.61 *p* = 0.43 AICc = 287.65 Null AICc = 284.59	**X^2 = 5.86** **Coef = 0.001** ***p* = 0.015** **AICc = 251.81** Null AICc = 254.74	**X^2 = 7.12** **Coef = 2,451.23** ***p* = 0.007** **AICc = 303.24** Null AICc = 306.90	**X^2 = 12.03** **Coef = −7.07** ***p* = 0.005** **AICc = 264.35** Null AICc = 273.81
Creatine kinase	X^2 = 0.92 *p* = 0.33 AICc = 216.25 Null AICc = 213.50	**X^2 = 4.32** **Coef = 0.001** ***p* = 0.04** **AICc = 183.36** Null AICc = 184.75	X^2 = 1.37 *p* = 0.24 AICc = 217.09 Null AICc = 214.79	**X^2 = 9.75** **Coef = 0.01** ***p* = 0.002** **AICc = 184.35** **Null AICc = 191.17**	X^2 = 0.79 *p* = 0.37 AICc = 234.15 Null AICc = 231.48	X^2 = 1.52 *p* = 0.21 AICc = 206.10 Null AICc = 204.78
Albumin	X^2 = 0.001 Coef = 8,242.81 *p* = 0.97 AICc = 393.67 Null AICc = 390.01	X^2 = 0.00 Coef = −2.12 *p* = 0.99 AICc = 404.81 Null AICc = 401.88	X^2 = 0.15 *p* = 0.68 AICc = 393.17 Null AICc = 389.67	X^2 = 0.02 *p* = 0.88 AICc = 404.88 Null AICc = 401.96	X^2 = 2.15 *p* = 0.14 AICc = 423.83 Null AICc = 422.52	X^2 = 0.31 *p* = 0.57 AICc = 436.70 Null AICc = 434.18
Fatty acyl-CoA hydrolase	X^2 = 1.64 Coef = 200.1 *p* = 0.19 AICc = 211.04 Null AICc = 209.03	X^2 = 0.90 Coef = −0.001 *p* = 0.34 AICc = 201.32 Null AICc = 199.30	X^2 = 0.15 *p* = 0.69 AICc = 213.08 Null AICc = 209.56	X^2 = 0.00 *p* = 0.98 AICc = 293.23 Null AICc = 290.29	X^2 = 1.11 *p* = 0.29 AICc = 228.43 Null AICc = 226.08	X^2 = 1.17 *p* = 0.27 AICc = 215.25 Null AICc = 213.59
L-lactate dehydrogenase	X^2 = 2.94 *p* = 0.086 AICc = 201.39 Null AICc = 200.67	X^2 = 2.81 *p* = 0.09 AICc = 181.67 Null AICc = 181.55	X^2 = 1.66 Coef = 466.35 *p* = 0.19 AICc = 199.37 Null AICc = 197.37	X^2 = 1.66 Coef = −0.001 *p* = 0.19 AICc = 180.26 Null AICc = 178.99	X^2 = 0.01 *p* = 0.89 AICc = 220.23 Null AICc = 216.78	X^2 = 0.02 *p* = 0.87 AICc = 201.77 Null AICc = 198.96

(Continues)

TABLE 2 (Continues)

Protein	Body condition (*df* = 1,12)		Baseline CORT (*df* = 1,11)		Stress-induced CORT (*df* = 1,12)	
	GLM normal distribution, link: identity	GLM exponential distribution, link: reciprocal	GLM normal distribution, link: identity	GLM exponential distribution, link: reciprocal	GLM normal distribution, link: identity	GLM exponential distribution, link: reciprocal
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	X^2 = 0.67 *p* = 0.41 AICc = 216.47 Null AICc = 213.47	X^2 = 0.40 *p* = 0.52 AICc = 210.43 Null AICc = 207.90	X^2 = 0.23 *p* = 0.63 AICc = 214.57 Null AICc = 211.14	X^2 = 0.09 *p* = 0.75 AICc = 209.86 Null AICc = 207.03	X^2 = 1.28 *p* = 0.25 AICc = 233.87 Null AICc = 231.69	X^2 = 0.50 *p* = 0.50 AICc = 229.42 Null AICc = 227.09
Chaperone proteins						
HSC71	X^2 = 0.60 *p* = 0.437 AICc = 227.75 Null AICc = 224.68	X^2 = 0.73 *p* = 0.39 AICc = 214.86 Null AICc = 210.34	X^2 = 3.23 *p* = 0.07 AICc = 224.50 Null AICc = 224.07	**X^2 = 6.27** **Coef = 0.01** ***p* = 0.01** **AICc = 209.17** **Null AICc = 212.52**	X^2 = 0.02 *p* = 0.88 AICc = 245.33 Null AICc = 241.88	X^2 = 0.02 *p* = 0.88 AICc = 231.09 Null AICc = 228.27
HSP90	X^2 = 0.54 *p* = 0.45 AICc = 236.42 Null AICc = 233.30	X^2 = 1.77 *p* = 0.18 AICc = 209.78 Null AICc = 208.62	X^2 = 1.03 *p* = 0.31 AICc = 235.74 Null AICc = 233.11	**X^2 = 5.49** **Coef = 0.001** ***p* = 0.02** **AICc = 207.21** **Null AICc = 209.77**	X^2 = 0.05 *p* = 0.82 AICc = 254.54 Null AICc = 251.13	X^2 = 0.11 *p* = 0.73 AICc = 228.25 Null AICc = 225.53
Ankyrin repeat domain	**X^2 = 10.86** **Coef = 2,125,223.73** ***p* = 0.001** **AICc = 350.94** **Null AICc = 358.14**	**X^2 = 11.29** **Coef = −9.039e-7** ***p* = 0.001** **AICc = 323.33** **Null AICc = 331.68**	X^2 = 0.60 *p* = 0.43 AICc = 361.21 Null AICc = 358.14	**X^2 = 3.59** **Coef = 6.3862e-6** ***p* = 0.057** **AICc = 331.02** **Null AICc = 331.68**	X^2 = 1.12 *p* = 0.28 AICc = 389.03 Null AICc = 386.69	X^2 = 4.94 Coef = 3.6e-7 *p* = 0.03 AICc = 354.90 Null AICc = 357.01
Adenylate kinase 2 mitochondrial	X^2 = 0.01 *p* = 0.89 AICc = 246.03 Null AICc = 242.38	X^2 = 0.03 *p* = 0.85 AICc = 223.46 Null AICc = 220.56	X^2 = 1.91 *p* = 0.17 AICc = 244.13 Null AICc = 242.38	X^2 = 2.36 *p* = 0.12 AICc = 221.12 Null AICc = 220.56	X^2 = 0.33 Coef = 73.56 *p* = 0.56 **AICc = 255.34** **Null AICc = 261.43**	X^2 = 0.16 *p* = 0.68 AICc =239.29 Null AICc = 236.62
Histone-lysine N-methyltransferase	X^2 = 2.22 *p* = 0.13 AICc = 325.28 Null AICc = 323.84	**X^2 = 9.06** **Coef = 6.7403e-6** ***p* = 0.003** **AICc = 290.53** Null AICc = 296.66	X^2 = 0.66 *p* = 0.41 AICc = 333.88 Null AICc = 330.88	X^2 = 2.76 *p* = 0.09 AICc = 307.88 Null AICc = 307.71	X^2 = 2.95 Coef = 11,798.23 *p* = 0.08 AICc = 357.76 Null AICc = 357.25	**X^2 = 4.20** **Coef = 329.68** ***p* = 0.04** **AICc = 329.68** **Null AICc = 331.05**
DNA mismatch repair protein Msh6	X^2 = 0.23 *p* = 0.63 AICc = 322.66 Null AICc = 319.23	X^2 = 0.13 *p* = 0.71 AICc = 316.74 Null AICc = 313.94	X^2 = 1.31 *p* = 0.25 AICc = 321.23 Null AICc = 318.87	X^2 = 1.20 *p* = 0.27 AICc = 312.74 Null AICc = 311.01	X^2 = 0.47 *p* = 0.49 AICc = 348.05 Null AICc = 345.06	X^2 = 0.58 *p* = 0.58 AICc = 341.00 Null AICc = 338.47

Significant results are shown in bold.

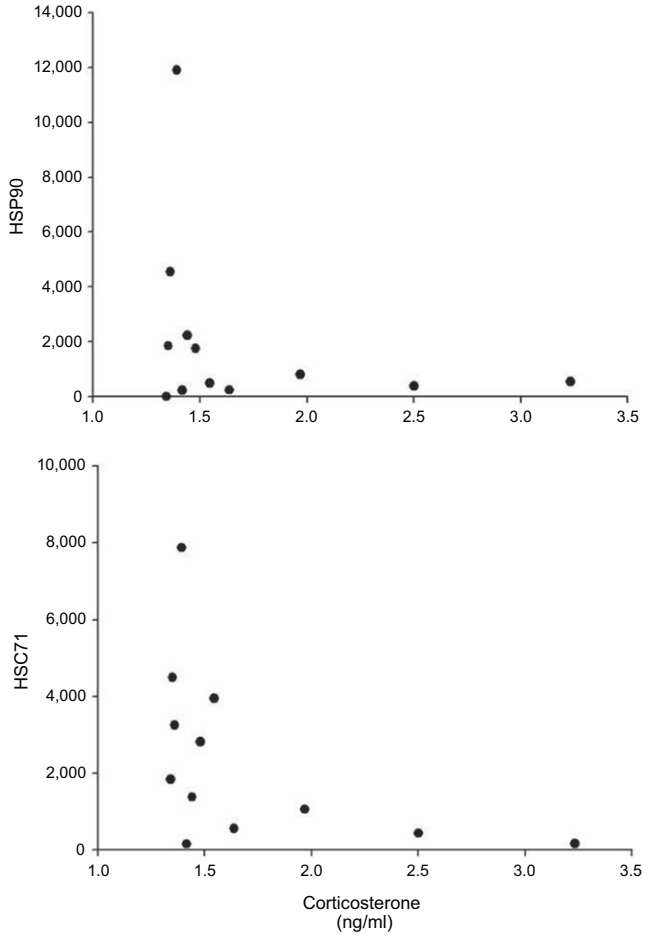

FIGURE 6 The relationship between heat-shock proteins 70 and 90 and baseline corticosterone levels in *Zonotrichia capensis*

Wingfield, 2001). This example highlights the necessity to measure CORT levels at different times during the reproductive season, and, in future studies, follow individuals through the year. We also found seasonal variations in stress-induced levels of CORT in AR as well as in FJ, with higher levels during the reproductive season. However, birds in the seasonal environment had greater variation in plasma levels of CORT through the year compared to the aseasonal environment. Variations in CORT levels across the year were expected (Ramenofsky et al., 2017), allowing birds to modulate their breeding season, and improve their reproductive success depending on local conditions (Goutte, Antoine, & Chastel, 2011; Schoech, Rensel, Bridge, Boughton, & Wilcoxen, 2009). In particular, our findings in the seasonal FJ site are coincident with higher levels of CORT during breeding season in comparison with other life cycles in nonmigratory species (Lattin, Breuner, & Romero, 2016). In the aseasonal AR site, we also observed a peak of CORT in October which aligned with the high proportion of birds breeding at this time of the year. However, when individuals were grouped per LHS and stress-induced CORT levels compared, we observed no significant differences, implying that in October birds have higher levels of CORT regardless of their breeding status, although more studies are needed to elucidate this finding.

Although in both localities we found that CORT levels significantly decreased during moult in comparison with other cycles, birds did not completely suppress the stress response (i.e., stress-induced levels of CORT were still significantly higher than baseline levels). In seasonal environments, the suppression of the stress response during moult has been commonly observed (Astheimer, Buttemer, & Wingfield, 1994; Cornelius, Perfito, Zann, Breuner, & Hahn, 2011; Romero, Strochlic, & Wingfield, 2005), most likely because higher levels of CORT could have negative impacts on feather quality (Cornelius et al., 2011). Equatorial populations of *Z. capensis* with long moult periods have similar patterns to birds at the AR and FJ sites where they exhibited lower levels of CORT during moult in comparison with other LHSs of the annual cycle, although they did not suppress the stress response (Wada, Moore, Breuner, & Wingfield, 2006). In our study, we did not monitor individual birds, but we observed moult year-round in AR and from January through March in FJ, suggesting that moult could be spread over three months. Therefore, the cost of moult could be lower than in other localities, and presumably the cost of suppressing stress response for a long period of time could be high. Interestingly in the seasonal FJ site, birds moulted during the driest and warmest period of the year, forcing birds to move longer distances to unfamiliar sites to find water (P. L. Gonzalez-Gomez, personal observation). In this context, birds could face numerous unpredictable events ranging from drought to novel predators, to competitors, and therefore, the complete suppression of the stress response could be detrimental. Further research is needed to determine whether there are variations in receptors or carriers such as corticosteroid-binding proteins across the year, and/or local variations in sensitivity of feather follicles to prevent the detrimental effects of CORT on feather quality.

The testosterone data were consistent with our finding of a defined breeding season in FJ, where we found a peak in plasma T levels during the reproductive season. This pattern of seasonal modulation of T has been described in many species inhabiting seasonal environments (Goymann & Landys, 2011; Wingfield, 1984), where higher levels of T allow males to acquire resources to attract mates and develop secondary sexual traits (Wingfield, Hegner, Dufty, & Ball, 1990). In contrast, in AR we observed lower and not variable levels of T year-round, which is consistent with birds breeding across the year, a pattern that is most likely driven by environmental homogeneity rather than the annual photoperiod as shown in more temperate species (Hau et al., 2010).

4.4 | Proteomics

Overall, roughly 20% of the blood proteome showed differences between localities. With the exception of adenylate kinase, we did not find differences between localities in the concentration of proteins related to metabolic and regulatory functions or chaperone proteins. Adenylate kinase is an enzyme that plays a key role in regulation of cellular energy state under different metabolic stresses by monitoring

TABLE 3 Associations between heat-shock proteins and metabolic and chaperone plasma proteins

Group	Protein	HSC71 (*df* = 1,11)		HSP90 (*df* = 1,11)	
		GLM normal distribution, link: identity	**GLM exponential distribution, link: reciprocal**	**GLM normal distribution, link: identity**	**GLM exponential distribution, link: reciprocal**
Metabolic and endocrine-regulatory proteins	Apolipoprotein	X^2 = 0.01 *p* = 0.92 AICc = 298.23 Null AICc = 294.77	X^2 = 0.00 *p* = 0.94 AICc = 289.77 Null AICc = 286.94	X^2 = 0.007 *p* = 0.93 AICc = 298.23 Null AICc = 294.77	X^2 = 0.00 *p* = 0.94 AICc = 289.77 Null AICc = 286.94
	Steroidogenic acute regulatory protein (StAR)	X^2 = 0.30 *p* = 0.58 AICc = 310.06 Null AICc = 306.90	X^2 = 1.04 *p* = 0.30 AICc = 275.61 Null AICc = 273.81	X^2 = 0.01 *p* = 0.89 AICc = 310.35 Null AICc = 306.90	X^2 = 0.06 *p* = 0.79 AICc = 276.59 Null AICc = 273.81
	Creatine kinase	**X^2 = 14.30** **Coef = 0.54** ***p* = 0.0002** **AICc = 220.64** **Null AICc = 231.48**	**X^2 = 12.67** **Coef = −3.482e-7** ***p* = 0.0008** **AICc = 194.94** **Null AICc = 204.78**	**X^2 = 14.56** **Coef = 0.385** ***p* = 0.0001** **AICc = 220.38** **Null AICc = 231.48**	**X^2 = 9.41** **Coef = −1.64e-7** ***p* = 0.002** **AICc = 198.20** **Null AICc = 204.78**
	Albumin	X^2 = 0.14 *p* = 0.70 AICc = 425.84 Null AICc = 422.52	X^2 = 0.01 *p* = 0.88 AICc = 436.99 Null AICc = 434.18	X^2 = 0.001 *p* = 0.93 AICc = 425.98 Null AICc = 422.52	X^2 = 0.001 *p* = 0.97 AICc = 437.181 Null AICc = 434.18
	Fatty acyl-CoA hydrolase	X^2 = 0.87 *p* = 0.35 AICc = 228.67 Null AICc = 226.08	X^2 = 0.95 *p* = 0.32 AICc = 215.47 Null AICc = 213.59	X^2 = 1.54 *p* = 0.21 AICc = 227.99 Null AICc = 226.08	X^2 = 2.90 *p* = 0.08 AICc = 213.51 Null AICc = 213.59
	L-lactate dehydrogenase	X^2 = 0.01 *p* = 0.89 AICc = 220.23 Null AICc = 216.78	X^2 = 2.53 *p* = 0.11 AICc = 199.26 Null AICc = 198.96	X^2 = 0.69 *p* = 0.40 AICc = 219.56 Null AICc = 216.78	X^2 = 1.69 *p* = 0.19 AICc = 200.11 Null AICc = 198.96
	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	X^2 = 0.11 *p* = 0.73 AICc = 235.04 Null AICc = 231.69	X^2 = 0.050 *p* = 0.81 AICc = 229.87 Null AICc = 227.09	X^2 = 0.08 *p* = 0.76 AICc = 235.07 Null AICc = 231.69	X^2 = 0.3 *p* = 0.84 AICc = 229.89 Null AICc = 227.09
Chaperone proteins	Ankyrin repeat domain	X^2 = 0.19 *p* = 0.66 AICc = 389.96 Null AICc = 386.69	X^2 = 0.79 *p* = 0.37 AICc = 359.05 Null AICc = 228.27	X^2 = 0.70 *p* = 0.40 AICc = 389.45 α Null AICc = 241.88	X^2 = 7.90 Coef = 3.0102e-9 *p* = 0.005 AICc = 351.94 Null AICc = 357.01

(Continues)

TABLE 3 (Continued)

Group	Protein	HSC71 (*df* = 1,11)		HSP90 (*df* = 1,11)	
		GLM normal distribution, link: identity	GLM exponential distribution, link: reciprocal	GLM normal distribution, link: identity	GLM exponential distribution, link: reciprocal
	Adenylate kinase 2 mitochondrial (1)	X^2 = 1.08 *p* = 0.29 AICc = 263.81 Null AICc = 261.43	**X^2 = 3.77** **Coef = 1.2996e-7** ***p* = 0.051** **AICc = 235.68** **Null AICc = 236.62**	X^2 = 0.19 *p* = 0.65 AICc = 264.70 Null AICc = 261.43	X^2 = 0.67 *p* = 0.41 AICc =238.78 Null AICc = 236.62
	Histone-lysine N-methyltransferase	X^2 = 0.18 *p* = 0.66 AICc = 360.53 Null AICc = 357.25	X^2 = 0.41 *p* = 0.52 AICc = 333.47 Null AICc = 331.05	X^2 = 0.19 *p* = 0.66 AICc = 360.52 Null AICc = 357.25	X^2 = 0.38 *p* = 0.53 AICc = 333.50 Null AICc = 331.05
	DNA mismatch repair protein Msh6	X^2 = 0.06 *p* = 0.79 AICc = 348.45 Null AICc = 345.06	X^2 = 0.03 *p* = 0.84 AICc = 341.26 Null AICc = 338.47	X^2 = 0.51 *p* = 0.47 AICc = 348.01 Null AICc = 345.06	X^2 = 0.39 *p* = 0.53 AICc = 340.91 Null AICc = 338.47

Significant results are shown in bold.

and altering the levels of ATP, ADP and AMP, and triggering cascade effects, such as the stimulus of AMP-dependent receptors linked to glycolytic pathways (Dzeja & Terzic, 2009). Consistent with our hypothesis, we found higher adenylate kinase in individuals in FJ than in AR. Adenylate kinase was also positively related to stress-induced levels of CORT. Thus, the upregulation of this protein in the more challenging seasonal environment of FJ is consistent with the idea that this protein could act as primary stress-response pathway (Kong, Binas, Moon, Kang, & Kim, 2013) by receiving direct cues from the cellular environment (i.e., phosphate nucleotide levels) and indirect cues from increased CORT levels avoiding eventual ATP depletion and promoting energy balance under stressful unpredictable events.

We identified that heat-shock protein 70 (HSP70) and heat-shock protein 90 (HSP90), which are involved in functions such as protein folding and signal transduction, were negatively correlated with plasma glucocorticoids (Garbuz & Evgen'ev, 2017). HSP70 is expressed constitutively and under normal conditions acts as an ATP-dependent molecular chaperone assisting multiprotein complex assemblages and transport of proteins across cellular membranes (Jego, Hazoume, Seigneuric, & Garrido, 2013; Shi & Thomas, 1992). HSP90 is inducible and it binds prefolded or fully folded proteins and helps them to achieve or maintain tertiary structures (Grad & Picard, 2007). In mammals, the relation between HSPs and plasma levels of CORT is mediated by the low-affinity glucocorticoid receptors (GR) and the high-affinity mineralocorticoid receptors (MR) (Breuner & Orchinik, 2001; Landys, Ramenofsky, & Wingfield, 2006; Landys et al., 2004). In the absence of hormone, GR and MR are thought to be primarily located in the cytoplasm as part of hetero-oligomeric complexes that contain HSP90 and HSP70, which are pivotal in the maturation processes and biological actions of these receptors (Grad & Picard, 2007). It has been argued that MR and GR have differential roles in the regulation of CORT signalling, where MR is activated at low CORT levels and GR is activated only after CORT levels rise (Joels, Karst, DeRijk, & de Kloet, 2008). Although in humans stress-induced levels of HSP90 were correlated with GR (Matic et al., 2014), we did not find stress-induced levels of CORT related to HSP90. This could be a result of our small sample size, more complex interactions with cochaperones (Bimston et al., 1998), or differential regulation of receptors and hormone levels, which has been shown in birds (Breuner & Orchinik, 2001; Landys et al., 2006). Our finding that HSP90 and HSC70 were negatively related to baseline levels of CORT is counterintuitive and likely related to the interaction between MR, HSP and cochaperones, but further studies are necessary.

We also found HSP70 and HSP90 positively related to levels of creatine kinase, which was expected considering that both HSP70 and HSP90 are ATP-dependent proteins (Mayer & Bukau, 2005), and creatine kinase regulates cellular energy reservoirs (Arakawa et al., 2016). In our study, this protein was also related to baseline levels of CORT and body condition, which was also consistent with its role in cellular energy supply.

Our findings of nonsignificant differences in the expression of certain proteins between AR and FJ could suggest less flexibility

in these traits; however, our results need to be interpreted with caution. On the one hand, our sample size was small, we used one tissue, and we did not assess changes in the proteome across the year. It could be that differences are higher during the reproductive season when CORT and T levels were higher in FJ than in AR.

4.5 | Integration of proteome, behavioural and endocrine traits under different environmental conditions

Our results suggest that environmental heterogeneity (i.e., seasonality as well as unpredictable climatic events) could be a strong modulator of endocrine and behavioural traits such as timing and duration of LHSs over annual cycles, through correlated levels of CORT and T. In contrast, morphological traits such as body condition variables and protein expression showed very little or no relationships to seasonality but revealed stronger correlations with physiological traits or other protein levels. It is possible that organisms use labile responses, such as behaviour and endocrine responses, to inhabit harsh unpredictable environments, and might be more limited by less flexible traits such as morphology. In this regard, we could expect a new scenario of selective pressures, where more flexible phenotypes would be possibly selected.

In the context of increasing unpredictable events in frequency and magnitude linked to global change, coping strategies will be likely shaped by the trade-off between each level of organization (LHSs of the annual cycle) and flexibility in timing and duration (overlap) of LHSs. The results of this comprehensive study showed the complexity of responses at organism level, and it is a preliminary approach to integrative studies in free-living animals.

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AUTHORS' CONTRIBUTIONS

P.L.G.-G. designed the experiments, collected field data, carried out laboratory analysis and statistical analysis and wrote the manuscript. V.E. collected field data. C.F.E. provided field support and discussed the results. P.S. provided laboratory support in Chile and discussed the results. J.S.K. wrote the manuscript and discussed the results. J.H.P. carried out laboratory analysis and wrote the manuscript. J.L. and D.K. carried out protein analysis. J.C.W. designed the experiments, discussed the results and wrote the manuscript.

DATA ACCESSIBILITY

The proteomics dataset are publicly accessible at Massive and ProteomeXchange, with the following accession numbers:

Massive: MSV000082313.

ProteomeXchange: PXD009616.

The data are also accessible at the CAMP Proteome: https://kueltzlab.ucdavis.edu/camp_dda_profiles.cfm?campid=101&species=zonca.

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