



2 **Intraspecific variation in exploratory behavior and elevational affinity**  
3 **in a widely distributed songbird**

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7 **Abstract**

8 Populations of the same species can vary substantially in their behavioral and morphometric traits when they are subject to  
9 different environmental pressures, which may lead to the development of different adaptive strategies. We quantified vari-  
10 ation in exploratory behavior and morphometric traits among two rufous-collared sparrow populations that occur at low  
11 and high elevations in central Chile. Moreover, we used census and  $\delta^2\text{H}$  values of feather and blood to evaluate migration.  
12 We found that individual sparrows inhabiting high elevations were larger and showed more intense exploratory behavior in  
13 comparison with those that were captured at lower elevation. Moreover, we observed a steady decline in sparrow abundance  
14 during the winter and similar  $\delta^2\text{H}$  values for blood collected in the winter and summer at this site, which were significantly  
15 lower than blood  $\delta^2\text{H}$  values observed at low elevation. This pattern suggests that individuals do not move long distances  
16 during winter, and likely they remain at similar elevations in refuge habitats. As predicted, our results support the existent  
17 of different adaptive strategies among populations of the same species, and suggest that the combination of behavioral,  
18 morphometric, and stable isotope data is a novel and robust integrative approach to assess differences in adaptation across  
19 environmental gradients.

20 **Keywords** Exploratory behavior · Migration · *Zonotrichia capensis* ·  $\delta^2\text{H}$

21 **Introduction**

22 When species have large distributional ranges, it is likely  
23 that local populations are subject to different selective pres-  
24 sures, which can lead to the evolution of different adaptive  
25 strategies based on local environmental conditions. For  
26 birds, such ecophenotypic variation may include inter-pop-  
27 ulation differences in morphology (e.g., wing length; Chap-  
28 man 1940; Handford 1985) and physiology (testosterone lev-  
29 els; Hudson and Kimzey 1966; Broggi et al. 2004, Cavieres  
30 and Sabat 2008, Addis et al. 2011). From a behavioral per-  
31 spective, populations may also develop different strategies  
32 to address common challenges that manifest as behavioral  
33 differences in food hoarding (Quispe et al. 2009), tool use  
34 (Whiten and van Schaik 2007), social behavior (Chapman  
35 and Rothman 2009), and mating tactics (Kolluru et al. 2007).

36 One behavior that is likely to vary among populations  
37 is exploratory behavior, because it allows individuals to  
38 obtain information about food items, feeding sites, terri-  
39 tory quality, refuges, escape routes, and potential mates,  
40 among others (see Dingemanse et al. 2002; van Dongen  
41 et al. 2010; van Oers and Naguib 2013). It is unlikely that

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A2 Our research proposes a robust and integrative approach to assess  
A3 ecological and evolutionary variation across environmental  
A4 gradients, applicable to bird species difficult to monitor and  
A5 recapture.

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these environmental characteristics are consistent across the range of local habitats occupied by a species with a wide geographic distribution (e.g., Beauchamp 1999; Van Oers et al. 2004; Garamszegi et al. 2008; Minderman et al. 2009). Some studies have shown that exploration is repeatable, heritable and has consequences for fitness (Dingemanse et al. 2002; Drent et al. 2003; van Dongen et al. 2010). There are very few studies, however, that report variability in exploratory behavior among populations of the same species (e.g., van Dongen et al. 2010; Maldonado et al. 2012).

Another behavior that exhibits intraspecific variation is migration. This behavior has been associated with changes in habitat conditions, such as food availability or suitable breeding sites (Chernetsov 2012). Variation in migratory behavior results in the existence of partial migratory populations composed of residents that remain in specific areas throughout the year, groups engaged in small-scale (e.g., nomadic) movements, and/or even migratory individuals that make seasonal latitudinal or elevational movements (Newton and Dale 1996). Comparative analyses of birds have shown that migratory species are more exploratory than non-migratory species (Mettke-Hofmann et al. 2002). Moreover, several studies have shown that some morphometric measurements, such as wing size, increase with the distance covered during migration (Pennycuik 1975; Leisler and Winkler 2003; Fiedler 2005).

The rufous-collared sparrow (*Zonotrichia capensis*) is an interesting species to examine inter-population variation in behavioral and morphological traits, because it is widely distributed in a broad range of habitats from southern Mexico (10°N) to the southern tip of South America (55°S), and from sea level to > 4000 m of elevation (Chapman 1940; Loughheed et al. 2013). Although there are more than 20 described subspecies based on morphological variation (Chapman 1940; Handford 1983, 1985; Lijtmaer and Tubaro 2007), recent research has identified only three distinct lineages (Loughheed et al. 2013). Genetic analysis has also found evidence of population structure among groups that inhabit different elevations (Loughheed and Handford 1992; Cheviron and Brumfield. 2009). Population-level differences in migratory behavior have also been reported. For example, individuals who inhabit higher elevations behave as migrants, while those inhabiting lower elevations are residents (Ortiz and Capllonch 2011). This pattern is expected given the adverse winter conditions of high elevation habitats, especially in the southern portion of its range in southern South America. Therefore, it is likely that individuals that inhabit high elevations show enhanced exploratory behavior, because this would allow them to obtain more information about both abiotic and biotic conditions that impact resource availability and habitat quality in these challenging and dynamic environments.

Studying migratory behavior of small birds is difficult with the traditional methods such as observation and banding, because recapture rates are very low (Hobson 2005). Hydrogen isotope ( $\delta^2\text{H}$ ) analysis of bird tissues has rapidly become an effective tool for characterizing bird migration (Hobson 2011), including elevational movement (Hobson et al. 2003; Hardesty and Fraser 2010; Newsome et al. 2015). Most studies compare tissue (typically feather)  $\delta^2\text{H}$  values to those of isoscapes depicting geographic variation in precipitation  $\delta^2\text{H}$  (Bowen et al. 2005). However, feathers are molted and regrown during short periods (e.g., weeks) and thus cannot capture seasonal movements. Comparison of feather  $\delta^2\text{H}$  data with that from metabolically active tissues (e.g., blood or liver) or continuously growing metabolically inert tissues (e.g., claws) provides a way to examine movement across seasons (Martínez Del Río et al. 2009; Dalerum and Angerbjörn 2005; Mazerolle and Hobson 2005; Newsome et al. 2015; Villegas et al. 2016). Furthermore, a multi-tissue approach also allows one to assess partial migration in populations that contain both resident and migratory individuals.

In this study, we quantified intraspecific variation in behavior, morphological traits, and habitat between two populations of *Zonotrichia capensis* that occur at different elevations in central Chile. Specifically, our novel approach coupled field experimental observations, morphological measurements, and a  $\delta^2\text{H}$ -based assessment of elevational affinity to link exploratory behavior with habitat preferences at the population level. Such detailed analyses of animal behavior may yield important insights into how animals adapt to different environmental challenges, and how such pressures produce and maintain variation between populations of the same species.

## Materials and methods

### Study sites

Rufous-collared sparrows were captured using mist nets between April and September in 2013 from two different elevation sites in central Chile: Rinconada de Maipo (33°31'S, 70°50'W, ~ 500 m) and Farellones (33°21'S, 70°17'W, 2300–2700 m) with the purpose of contrasting exploratory behavior, morphometric traits, and isotope values between populations (see below). In addition, we captured rufous-collared sparrows at two intermediate elevation sites: Yerba Loca (33°20'S, 70°18'W, ~ 1500 m) and El Arrayan (33°15'S, 70°25'W, ~ 1000 m), in an attempt to recapture individuals previously banded in Farellones and detect assess seasonal elevational movements via tissue hydrogen isotope analysis. All sites are characterized by a Mediterranean climate and dominated by Andean xeric scrubland vegetation (Thrower and Bradbury 1977), with a

143 gradient in mean annual precipitation from 300 to 400 mm  
 144 at the low elevation sites increasing to 500–900 mm at the  
 145 high elevation sites (di Castri and Hajek 1976; Dirección  
 146 meteorológica de Chile 2016).

## 147 Field study

148 We quantified exploratory behavior via novel environ-  
 149 ment experiments in a large field-portable cage [270 cm  
 150 (length) × 150 cm (width) × 150 cm (height)] made of PVC  
 151 poles and semitransparent black shading cloth (Fig. 1a).  
 152 Four wooden perches (80 cm long, 2 cm in diameter) were  
 153 hung throughout the cage at varying heights between 50  
 154 and 110 cm off the ground. One perch was placed diagon-  
 155 ally at the opposite corner at the far end of the cage, and  
 156 the remaining three were spaced at regular intervals (70 cm  
 157 apart) along the long axis of the cage. The floor was divided  
 158 into nine similar quadrants that when added to the four  
 159 perches, four walls, the top and the central pole division  
 160 yielded 19 possible perching locations (see van Dongen  
 161 et al. 2010). Thirty-eight non-reproductive adult birds were  
 162 captured and introduced into the small acclimatization cage  
 163 (30 cm length, 25 cm width, and 39 cm height) in a corner  
 164 of the experimental cage and covered with a cloth during a  
 165 5-min acclimatization period. At the beginning of the experi-  
 166 mental period, the cloth was removed, then, the door of the  
 167 holding cage was opened, and finally, an observer dictated  
 168 the number and destination of all movements made by each  
 169 subject onto a digital voice recorder over a period of 10 min.

170 We then measured to the nearest 0.1 cm the bill and tarsus  
 171 length with digital calipers, the wings, and tail length with  
 172 both wing and tail rules, and mass in grams ( $\pm 0.1$  g) with  
 173 a 60 g Pesola ( $\pm 0.1$  g). A blood sample was obtained from  
 174 the brachial vein, and several drops were dried on two glass  
 175 microscope slides. The remaining blood was stored on FTA

176 cards for later genetic sexing. Finally, a primary feather (p9)  
 177 was collected from the left wing and the bird was released.

## 178 Counts

179 We conducted weekly census counts from January 2013  
 180 to December 2013. We designated a ~ 10-km monitoring  
 181 area that spanned from 2000- to 3000-m elevation. This  
 182 area included five ~ 1 km<sup>2</sup> plots, which contained 5 points  
 183 separated by ~ 250 m, where all sparrows that occurred  
 184 within 50 m around each point were counted for 5 min from  
 185 6:30 AM to 12:00 AM (Ralph et al. 1996).

## 186 Genetic sexing

187 Sex could not be determined from morphological measure-  
 188 ments during the pre-breeding stage. We extracted DNA  
 189 from the collected blood that was stored on FTA cards using  
 190 a QIAGEN DNA extraction kit (Valencia, CA, USA). The  
 191 gene fragments CHD-W (unique to females) and CHD-Z  
 192 (found in both males and females) were amplified using  
 193 polymerase chain reactions (PCR) with primers P2 and P8.  
 194 The PCR product was run on a 3% agarose gel to separate  
 195 from the gene fragments. Sex was determined by visualiza-  
 196 tion of the gel, with males exhibiting bands only of CHD-Z  
 197 and females having bands of both CHD-W and CHD-Z. This  
 198 protocol was adapted from Griffiths et al. (1998).

## 199 Exploratory behavior

200 Audio recordings were analyzed using JWatcher 1.0 to  
 201 obtain the duration and frequency of behaviors and location  
 202 of the sparrows in the experimental cage. With this informa-  
 203 tion, we calculated richness and diversity metrics of explora-  
 204 tory behavior using a richness index and Shannon's diver-  
 205 sity index. The richness index is equal to  $S = n/N$ , where n

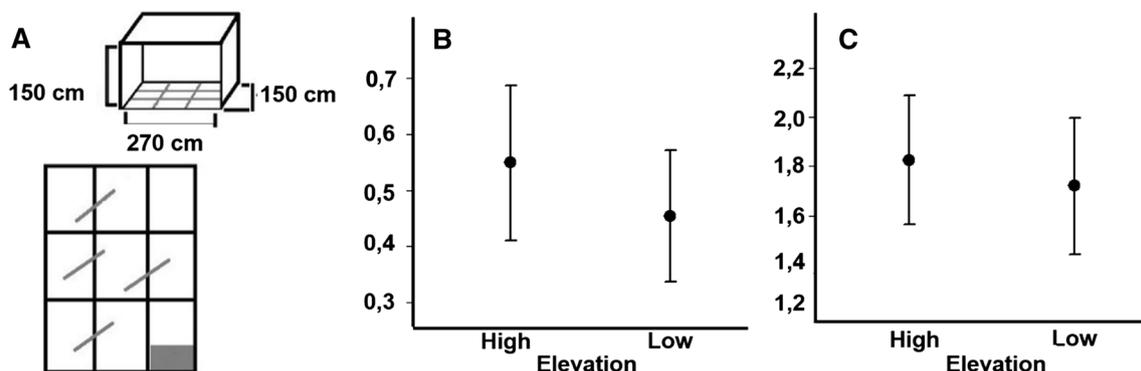


Fig. 1 Experimental aviary to describe the exploratory behavior by novel environment. The figure shows the measurements and divisions inside of the aviary (a). *Z. capensis* exploratory behavior

(mean  $\pm$  SE) as estimated with richness (b) and diversity (c) index for birds from the Farellones (~ 2300 m,  $n = 16$ ) and Rinconada de Maipú (~ 450 m,  $n = 20$ )

206 represents the total number of locations occupied during the  
 207 experiment and  $N$  represents the total number of locations in  
 208 the experimental cage. Diversity in exploratory behavior was  
 209 quantified via Shannon's diversity index using the equation  
 210  $H = -\sum p_i \ln p_i$ , where  $p$  represents the total number of times  
 211 a perch  $i$  was visited, expressed as a proportion of the total  
 212 number of perch visits throughout the trial (see van Dongen  
 213 et al. 2010 for further details).

## 214 Stable isotope analysis

215 Primary feathers were treated with a 2:1  
 216 chloroform:methanol solvent mixture to remove surface  
 217 contaminants and barbs were cut into small ( $< 1$  mm) pieces  
 218 with scissors and air-dried. Dried whole blood samples were  
 219 scraped off from the glass microscope slides into micro-  
 220 centrifuge tubes and homogenized by mixing.  $\delta^2\text{H}$  values  
 221 were measured with a Thermo Scientific high-temperature  
 222 conversion elemental analyzer (TCEA) coupled to a Thermo  
 223 Scientific Delta V isotope ratio mass spectrometer at the  
 224 Center for Stable Isotopes at the University of New Mexico  
 225 (Albuquerque, N.M., USA). Isotopic results are expressed  
 226 as  $\delta$  values,  $\delta^2\text{H} = 1000 \times [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}]$   
 227 ], where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are  $^2\text{H}/^1\text{H}$  of the sample and  
 228 standard, respectively. Vienna Standard Mean Ocean Water  
 229 (V-SMOW) is the internationally accepted standard for  
 230  $\delta^2\text{H}$  analysis and the units are parts per thousand or per mil  
 231 (‰) (IAEA/WMO 2011). Precision for ( $\delta^2\text{H}$ ) was deter-  
 232 mined by analysis of the three internal reference materials  
 233 for each tissue (blood or feather); within-run variation (SD)  
 234 in  $\delta^2\text{H}$  values of these reference materials was 3–4‰.

## 235 Data analysis

236 To compare the elevation of capture with  $\delta^2\text{H}$  based esti-  
 237 mates of elevation, we applied an elevational lapse rate  
 238 of 8–10‰ per 500 m (see Poage and Chamberlain 2001;  
 239 Hardesty and Fraser 2010) to precipitation  $\delta^2\text{H}$  data col-  
 240 lected from Rinconada de Maipu, where sparrows are year-  
 241 round residents. All statistical analyses were performed in  
 242 STATISTICA 7.0 (StatSoft, 2004). All data were tested for  
 243 normality and homoscedasticity via the Shapiro–Wilk test  
 244 and Levene's test, respectively. We used ANOVA and Kol-  
 245 mogorov–Smirnov tests to examine the effects of location

and sex on exploratory behavior and body measurements. 246  
 We used a repeated measures ANOVA to test for differences 247  
 between feather and blood  $\delta^2\text{H}$  values among populations. 248

## Results

### Exploratory behavior and morphology

250 We found significant differences in richness and diversity 251  
 of exploratory behavior among populations, as both indi- 252  
 ces were higher for birds from Farellones than those from 253  
 Rinconada de Maipu (richness index:  $F = 9.58$ ,  $P = 0.003$ ; 254  
 diversity index:  $F = 7.45$ ,  $P = 0.009$ ; see Fig. 1b and c). 255  
 Furthermore, we found that sparrows from the high elevation 256  
 site (Farellones) had significantly greater mass, tarsus, wing, 257  
 tail, and total length (Table 1; mass:  $F = 20.72$ ,  $P = 0.001$ ; 258  
 tarsus length:  $F = 19.23$ ,  $P = 0.001$ ; tail length:  $F = 7.26$ , 259  
 $P = 0.011$ ; wing length:  $F = 25.40$ ,  $P = 0.001$ ; and total 260  
 length:  $F = 7.90$ ,  $P = 0.008$ ). We did not find significant 261  
 differences in behavior and morphometric traits between 262  
 males and females in either population (diversity index: 263  
 $F = 1.60$ ,  $P = 0.21$ ; richness index:  $F = 0.78$ ,  $P = 0.38$ ; 264  
 mass:  $F = 0.49$ ,  $P = 0.48$ ; bill length:  $F = 0.20$ ,  $P = 0.65$ ; 265  
 tarsus length:  $F = 1.33$ ,  $P = 0.25$ ; tail length:  $F = 7.24$ , 266  
 $P = 0.65$ ; wing length:  $F = 0.28$ ,  $P = 0.10$ ; and total length: 267  
 $F = 0.70$ ,  $P = 0.40$ ). 268

### Counts

269 Rufous-collared sparrows were observed during the entire 270  
 year at all three low elevation sites, while at Farellones we 271  
 observed a decline in abundance from May to July (Fig. 2) 272  
 with few birds present during the winter (June and July). 273  
 With the beginning of spring in late August, we observed an 274  
 increase in the abundance of sparrows at Farellones, which 275  
 remained high during the summer months (Fig. 2). More- 276  
 over, despite observing sparrows at the low elevation site at 277  
 Rinconada de Maipu throughout the year, we did not capture 278  
 sparrows during the winter at the intermediate elevation sites 279  
 at Yerba Loca and El Arrayan. 280

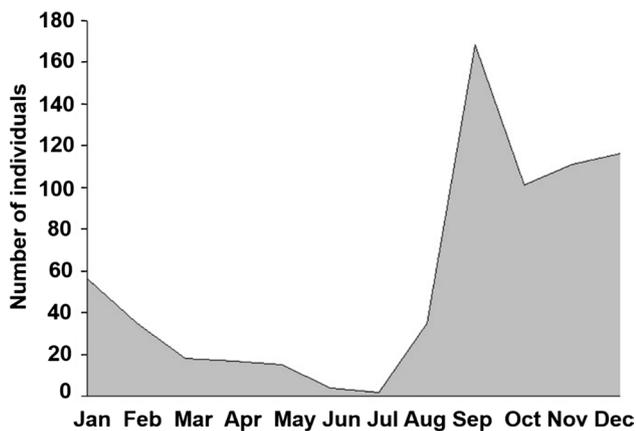
### Feather and blood $\delta^2\text{H}$

281 Feather  $\delta^2\text{H}$  values across all sites were negatively and sig- 282  
 nificantly associated with elevation ( $\beta = -0.48$ ,  $R^2 = 0.55$ , 283

**Table 1** Morphological traits (mean  $\pm$  SE) in *Z. capensis*, from Farellones ( $n = 15$ ) and Rinconada de Maipu ( $n = 19$ )

	Body mass	Tarsus length	Wing length	Tail length	Total length
Rinconada	20.6 $\pm$ 0.2	20.7 $\pm$ 0.1	73.3 $\pm$ 0.6	63.2 $\pm$ 0.6	143.0 $\pm$ 1.3
Farellones	22.0 $\pm$ 0.2	21.7 $\pm$ 0.2	78.0 $\pm$ 0.8	66.1 $\pm$ 0.8	144.7 $\pm$ 3.3
<i>P</i>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>

Significance is denoted by *P* values



**Fig. 2** Rufous-collared sparrow abundance at Farellones in 2013. The dates show the total number of individuals observed and listened in each month (see “Materials and Methods” for details)

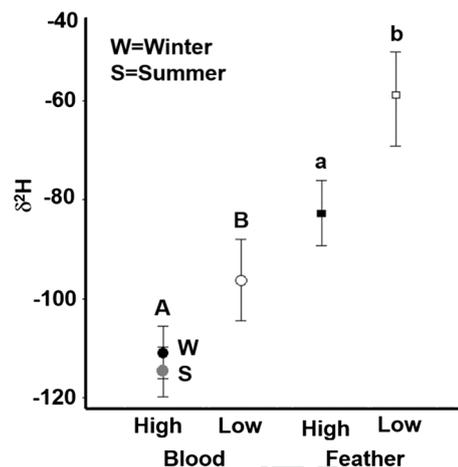
**Table 2** Values feather and blood  $\delta^2\text{H}$  (mean  $\pm$  SE) of *Z. capensis* among high and low elevation sites; sample sizes are in parentheses

Sites (m)	Blood $\delta^2\text{H}$	Feather $\delta^2\text{H}$
High elevation (> 1500)	- 111 $\pm$ 2 (5)	- 83 $\pm$ 2 (10)
Low elevation (< 1000)	- 95 $\pm$ 3 (12)	- 57 $\pm$ 3 (12)
<i>P</i>	< 0.001	< 0.001

Significance is denoted by *P* values

284 *P* < 0.001). The Farellones population had mean ( $\pm$  SD)  
 285 ( $- 86 \pm 3\%$ ) feather  $\delta^2\text{H}$  values that were not significantly  
 286 different from the Yerba Loca population ( $- 79 \pm 2\%$ )  
 287 ( $F = 4.08, P = 0.08$ ). However,  $\delta^2\text{H}$  values of feathers  
 288 collected from sparrows at Farellones were significantly lower  
 289 than feathers collected at the lower elevation sites of El  
 290 Arrayan ( $- 56 \pm 4\%$ ) ( $F = 40.7, P < 0.001$ ) and Rinconada  
 291 de Maipu ( $- 58 \pm 4\%$ ) ( $F = 15.5, P < 0.001$ ). Feathers  
 292 collected from Yerba Loca also had significantly lower  $\delta^2\text{H}$   
 293 values than those from El Arrayan ( $F = 31.09, P < 0.001$ )  
 294 and Rinconada de Maipu ( $F = 9.12, P = 0.01$ ). Finally, we did  
 295 not find significant differences in feather  $\delta^2\text{H}$  values between  
 296 El Arrayan and Rinconada de Maipu populations ( $F = 0.08,$   
 297  $P = 0.77$ ). Based on these results, we segregated these sites  
 298 into two groups: low elevation (Rinconada de Maipu and El  
 299 Arrayan) and high elevation (Yerba Loca and Farellones)  
 300 sites.

301 The high elevation population had mean ( $- 83 \pm 2\%$ )  
 302 feather  $\delta^2\text{H}$  value that were significantly lower than that  
 303 of the low elevation site ( $- 57 \pm 3\%$ ) (Table 2;  $F = 35.4,$   
 304  $P = 0.001$ ). Likewise, the mean  $\delta^2\text{H}$  value of blood repre-  
 305 senting winter collected from Farellones ( $- 111 \pm 2.1\%$ )  
 306 was significantly lower than that collected from the low  
 307 elevation site ( $- 95 \pm 3\%$ ) (Fig. 3;  $F = 9.01, P = 0.01$ ). In



**Fig. 3** Whole blood and feather  $\delta^2\text{H}$  (mean  $\pm$  SE) values in *Z. capensis* in populations that occur over 1500 m and under 1000 m. The dates show the absence of significant differences between blood samples among seasons in high elevation population, and significant differences between both, blood and feather samples among populations

**Table 3** Elevation of capture (m a.s.l.) and estimated elevation in *Z. capensis*

Sites	High elevation (> 1500)	Low elevation (< 1000)
Capture elevation	2300	450
Estimated elevation	2115	421
<i>P</i>	> 0.05	> 0.05

Significance is denoted by *P* values

308 addition, the offset in  $\delta^2\text{H}$  values between metabolically inert  
 309 feathers representing summer and metabolically active blood  
 310 representing winter ( $\Delta^2\text{H}_{\text{feather-blood}}$ ) was similar at high  
 311 and low elevation populations (Fig. 3;  $F = 0.74, P = 0.4$ ).  
 312 There were no significant differences in the  $\Delta^2\text{H}$  values of  
 313 blood or feathers collected in the summer or winter at the  
 314 high elevation site Farellones (Fig. 3  $\delta^2\text{H}_{\text{feather}}$ :  $F = 4.57,$   
 315  $P = 0.07$ ;  $\delta^2\text{H}_{\text{blood}}$ :  $F = 0.61, P = 0.45$ ). Finally, we did not  
 316 find significant differences between capture elevation and  
 317 estimated elevation among populations based on elevational  
 318 lapse rates in precipitation  $\delta^2\text{H}$  values; see “Materials and  
 319 Methods” (Table 3).

### Discussion

320 Our understanding of behavioral variation has been sub-  
 321 ject to remarkable shifts in recent years. For decades,  
 322 it was assumed that individual behavior was very plas-  
 323 tic or even noise, that could rapidly change in accord-  
 324 ance with the behavior of other conspecifics, and/or to  
 325

326 changing environmental conditions (see Sih et al. 2004;  
 327 Réale et al. 2007; Dingemanse et al. 2010). Despite the  
 328 presumed advantages of behavioral plasticity, it has been  
 329 found that specific behavioral patterns exist and can be  
 330 different among and/or within populations (Sih et al. 2004;  
 331 Laland and Janik 2006; Bell 2007). Our study found dif-  
 332 ferences in exploratory behavior among populations; spe-  
 333 cifically, exploration was higher in birds inhabiting higher  
 334 elevations (> 1500 m) relative to those occurring at lower  
 335 elevations (< 1000 m). This result was predicted because  
 336 exploratory behavior is an important mechanism to obtain  
 337 information about environmental conditions and quality  
 338 (see Dingemanse et al. 2002; Careau et al. 2009), particu-  
 339 larly in extremely seasonal environments such as those  
 340 that occur at high elevations. For example, in the Farellones  
 341 site (~ 2300 m), environmental conditions are harsh,  
 342 particularly in winter, with limited primary production  
 343 and decreased food availability for rufous-collared sparrows  
 344 relative to populations that occur at lower elevations  
 345 (Körner 2007). Therefore, the ability to rapidly obtain  
 346 environmental information via exploration is likely impor-  
 347 tant for finding food or shelter under these conditions  
 348 (see Mettke-Hofmann 2006). In addition to differences  
 349 in exploratory behavior, we also found population-level  
 350 differences in morphological traits, as all body measure-  
 351 ments were significantly larger in birds inhabiting higher  
 352 elevation sites year-round. This result was also expected  
 353 because body size should increase in response to colder  
 354 conditions in accordance with Bergmann's rule (Meiri and  
 355 Dayan 2003). Overall, these behavioral and morphologi-  
 356 cal patterns suggest that different environmental pressures  
 357 lead to the development of different adaptive strategies in  
 358 this species. Cheviron and Brumfield (2009) found sig-  
 359 nificantly reduced mitochondrial gene flow along eleva-  
 360 tional transects in *Z. capensis* in the Peruvian Andes and  
 361 concluded that divergent selection pressures along envi-  
 362 ronmental gradients can drive differentiation among popu-  
 363 lations. The highly seasonal climatic conditions present  
 364 at higher elevations, however, likely promotes elevational  
 365 migration (Newton and Dale 1996) such that morphomet-  
 366 ric adaptations may be related to the distance that birds  
 367 migrate (Leisler and Winkler 2003). In such scenario,  
 368 exploratory behavior could be important for deciding when  
 369 its best to migrate.

370 Descriptive studies show that some populations of *Z.*  
 371 *capensis* in Chile and Argentina undertake latitudinal  
 372 and elevational migrations (Chapman 1926; Olrog 1979;  
 373 Narosky and Di Giacomo 1993; Ortiz and Capllonch  
 374 2011). These patterns have been gleaned from seasonal  
 375 fluctuations in abundance of birds at different elevations  
 376 throughout the year and occasionally from recapture of  
 377 banded individuals (see Stiles 1985; Loiselle and Blake  
 378 1991; Johnson and Maclean 1994; Winker et al. 1997;

Burgess and Mlingwa 2000; Wright 2005; Brown 2006).  
 Most studies that have recaptured marked individuals,  
 however, have not directly assessed seasonal migration,  
 since they only note presence or absence during a single  
 period of the annual life cycle. To our knowledge, there  
 are no data that show the Farellones population of rufous-  
 collared sparrow are elevational migrants. Even though we  
 observed birds at Farellones (2300 m) all year-round, our  
 data showed that they diminished strongly during winter  
 (Fig. 2). Furthermore, we did not recapture individuals  
 banded at our highest elevation site at other localities.

Fortunately, isotope analysis revealed that blood repre-  
 senting the winter months collected from high elevation  
 had lower mean  $\delta^2\text{H}$  values than blood collected at the  
 same time from sparrows at low elevation (Fig. 3). The  
 magnitude of the  $\delta^2\text{H}$  offset in blood among high and low  
 elevation sites is similar to that in feathers representing  
 the summer months (Fig. 3), suggesting that these popu-  
 lations are residents and remain at their respective eleva-  
 tions year-round. Furthermore, the  $\delta^2\text{H}$  values of blood  
 collected from high elevation sites during the winter and  
 summer months were similar (Fig. 3). These patterns show  
 that  $\delta^2\text{H}$  analysis is a faithful recorder of elevation of ori-  
 gin in *Z. capensis chilensis* and that the high elevation  
 population does not migrate downslope during the winter.  
 Finally, the difference in observed  $\delta^2\text{H}$  values for either  
 blood or feathers between sparrows that occur in low and  
 high elevation sites conforms to expectations based on the  
 application of published elevational lapse rates in precipi-  
 tation  $\delta^2\text{H}$  for the Andes (Table 3).

The differences found in exploratory behavior, morpho-  
 logical traits, and lack of elevational movement show the  
 existence of ecological differentiation between high and  
 low elevation *Z. capensis chilensis* populations. Those  
 results support the idea that different adaptive strate-  
 gies could evolve in response to the steep gradient in the  
 environmental conditions present across elevation in the  
 Andes. Finally, we show that our combination of behavio-  
 ral, morphometric, and isotope data is an informative and  
 novel approach to assess ecological and evolutionary dif-  
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## 437 Compliance with ethical standards

438 **Conflict of interest** The authors have no conflict of interest to declare.

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