Do sister species respond similarly to dry conditions? An ecophysiological approach *in natura*

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Conflict of Interest

The authors declare they have no conflict of interest.

Author contributions

G.G. conceived the idea and designed methodology with input from H.K; G.G., H.K., P.C., collected the data; H.K. analyzed the data with input from G.G.; H.K. wrote the first draft of the manuscript with input from G.G.; N.P. and N.A. contributed critically to the manuscript, secured funding for the project with G.G. and helped with field logistics. All authors approved the final version of the manuscript.

Statement on inclusion

Our study brings together authors from France and South Africa. All authors were engaged early on with the research and study design to ensure that the diverse sets of perspectives they represent was considered from the onset. Whenever relevant, literature published by scientists from the study region was cited.

Data availability statement

Data will be available from the Dryad repository (TBA)

Abstract

1. **Understanding the proximate mechanisms of organisms' adaptations to the environment is a classical problem in evolutionary ecology. In the context of climate change, inducing increased aridity and disruption of seasonality, it is crucial to identify the potential for species responses.**
2. **Here, the physiological response to food and water limitation during seasonally dry conditions were investigated. We studied two rodent species of the genus *Rhabdomys*, one arid and one mesic, the ranges of which overlap in a semi-arid zone of South Africa. We measured blood concentrations of physiologically relevant metabolites in semi-arid populations of the two species at the onset and the end of the dry season.**
3. **We found similar shifts in blood metabolite levels in the two species, indicating malnutrition consistent with the observed degradation of habitat quality between the start and the end of the dry season. Furthermore, regardless of season, differences between the two species in blood metabolite concentrations (amylase, sodium, potassium, bilirubin, and alkaline phosphatase) were observed, that could indicate contrasting diets and water conservation abilities.**
4. **Overall, we show that, as dry conditions worsen, organisms are increasingly affected by reduced food availability, and local adaptation to arid conditions may provide the arid species with an advantage to cope with semi-arid conditions. Our study shows that even mild arid conditions could have a negative impact and questions resilience of animals to harsher arid conditions.**

Keywords

Aridity, blood metabolites, ecophysiology, liver, local adaptation, nutrition, *Rhabdomys*

Introduction

Organisms are constantly exposed to a wide range of environmental variations, including short-term changes during their lifetime and longer-term changes across generations (Lopez-Maury et al., 2008). In the coming decades, extreme and more frequent droughts are predicted by most projections, resulting from changes in precipitation patterns, increased temperature, and evaporation (Naumann et al., 2018). These changes will induce great challenges to organisms. Under such circumstances, divergent selection can lead to the evolution of local adaptations where, in a given environment, resident phenotypes outperform nonresident ones (Kawecki & Ebert, 2004).

Local ecological adaptation can be described in several different and complementary ways, such as through its genetic basis, transcriptomic, behavioral, or physiological aspects. Adaptive evolution *sensu stricto* occurs when the genetic constitution of a population changes because of natural selection (Merilä & Hendry, 2013). In contrast, local adaptation *sensu lato*, also considers concepts such as phenotypic plasticity. It is usually assessed using comparative population or species fitness estimates under novel environmental conditions (Webster & Reusch, 2017). The response to local environmental conditions is a major factor in the generation and maintenance of biodiversity, impacting population dynamics, biogeographical ranges, and species interactions (Blanquart et al., 2013; Post et al., 2009). Specifically, physiological limits can drive and be driven by evolution, shape species distributions, and define species

response capacities to future climate change, directly impacting risks of extinction (Somero, 2012). For example, adaptive variation in lethal temperature (LT_{50}) has been established in marine invertebrates inhabiting different latitudinal niches (Somero, 2010).

Studies of different physiological systems, such as the cardiovascular (Seebacher et al., 2005, Berkel & Cacan, 2022) or digestive systems (Naya et al., 2011), in response to different abiotic conditions, can improve our understanding of the causal mechanisms of responses of organisms to climate variation (Seebacher & Franklin, 2012). Biotic factors, such as sex, age, diet, strain, or breeding status also influence individual responses (McClure, 1999). With a focus on influence of air temperature, water availability and energy resources on an organism's physiological state, research in physiological ecology has addressed how variation in physiology evolves and is maintained in relation to environment conditions (Feder & Block, 1991).

As a result of climate change, organisms can be faced with warmer and drier environmental conditions (Parmesan et al., 2000), likely to impact their capacity to maintain homeostasis (Fuller et al., 2016). In dry environments, food and water can be scarce, generating strong selection on physiological attributes maximizing energy and water availability. Habitat generalist and specialist taxa may respond differently to environmental changes. Desert specialists exhibit specific physiological and/or behavioral traits, resulting from genetic adaptations to the environment, allowing them to continuously endure or evade periods of resource restriction (Rocha et al., 2021). In contrast, generalist species may seek to escape the effects of aridity through temporary avoidance (Abraham et al., 2019), plastic adjustments during unfavorable seasons (Christian et al., 1996; Kobbe et al., 2011)Compared to mesic species, arid

115 species have lower basal metabolic rates and evaporative water loss (Tieleman et al.,
116 2003; Muñoz-Garcia et al., 2022), adopt a more flexible diet (Tshikae et al., 2013), and
117 cope better with prolonged droughts (Boyers et al., 2021).

118 Analysis of blood metabolites has successfully been used to investigate kidney and
119 liver physiology of wild populations (e.g. Al-Eissa et al., 2012). The kidney and the liver
120 both contribute to homeostasis in periods of food and water scarcity; indeed the kidney
121 plays a crucial role in maintaining osmotic balance and the liver is involved in dietary
122 metabolism. Hence, dry conditions can put a strain on these organs and contribute to
123 the emergence of pathologies detrimental to survival and reproduction. Water loss can
124 also mechanically induce high concentrations of some blood markers and impact
125 organism's capacity to evacuate products of metabolic waste, such as urea, from the
126 blood (Ostrowski et al., 2006).

127 In our study, potential impact of increased aridity was addressed by investigating the
128 physiological consequences of seasonal variation in dry conditions, in two species of
129 the diurnal African striped mouse genus *Rhabdomys*. The two study species,
130 *R.bechuanae* and *R.dilectus dilectus*, are characterized by different environmental
131 niches (du Toit et al. 2012, Meynard et al. 2012). Throughout most of its distribution,
132 *R.d.dilectus* occurs in mesic habitats with vegetation cover and nests in dense grass,
133 while *R.bechuanae* is found in semi-arid and arid habitats, thriving predominantly in
134 sparsely vegetated areas and nesting in bushes (Dufour et al., 2015; Dufour et al.,
135 2019). Moreover, differences in morphology or behavior suggest adaptation to dry
136 conditions in *R.bechuanae* (Ganem et al., 2020; Dufour et al., 2019). Here we address
137 both physiological response to seasonal dry conditions and divergence in the two
138 species in the same bioclimatic region.

Indeed, at the edge of their distributions in central South Africa, the two species inhabit a semi-arid region within which they occur either as parapatric or sympatric populations. This semi-arid zone, the expansion and contraction of which depends on land use and precipitation (Kruger, 2006; Masubele et al., 2014; Department of Environmental Affairs, 2018; Lian et al., 2021), has experienced a recent trend towards desiccation (Jury, 2021). Such dry conditions could generate additional selective pressures on the semi-arid populations of the two species.

In the Succulent Karoo of South Africa, where the average daily precipitation during the dry season is 0.26 mm *versus* 0.78 mm during the wet season, the probability of survival of adult *Rhabdomys pumilio* could be predicted from their physiological response at the start of the dry season (Schoepf et al., 2017a). Indeed, Schoepf and collaborators found higher serum concentrations of albumin, glucose, potassium, and lower concentrations of globulin, in animals that survived compared to those that did not survive the dry season. At the peak of the dry season, *R.pumilio* had lower concentrations of glucose and phosphorus and higher concentrations in globulin and urea nitrogen if they survived the dry season. These results strongly suggest that such markers could be good indicators of individual fitness.

While many studies have harnessed physiology, genomics, and transcriptomics to highlight potential adaptive interspecific or interpopulational contrasts, studies under common natural environmental conditions are lacking (Rocha et al., 2021). This study took place in a natural semi-arid environment and asked how seasonal variation in dry conditions influenced the physiological responses of the two species. We compared body condition, blood concentrations of markers of kidney and liver functions and habitat characteristics of semi-arid parapatric populations of *R.bechuanae* and

164 *R.dilectus dilectus* at the start *versus* the end of the dry season. First, we expected the
165 habitat available for the mice would be drier at the end compared to the onset of the
166 dry season, inducing a reduction of food and water, impairing liver and kidney function,
167 and impacting body condition. Second, assuming local adaptive plasticity, we
168 hypothesized that both species would be able to adjust their physiological responses
169 to seasonal changes in dry conditions. Third, we expected *R.bechuanae*, having
170 evolved in arid environments, would perform better in semi-arid conditions than
171 *R.d.dilectus* (having evolved in mesic areas) and be closer to the expected local
172 optimal response to increased dry conditions, as found for *Mus musculus* (Bittner et
173 al., 2021). Alternatively, if both species evolved specific adaptations to their semi-arid
174 environment, we expected no species differences (**Table 1**).

175 Materials and Methods

176 ***Ethics statement***

177 Permits to work and handle animals in the field were obtained from the DESTEA of the
178 Free State, the North West DEDECT and Northern Cape DENC (respectively,
179 n°202110000007994, NW 38956/04/2022, FAUNA 0389/2022). Animal handling was
180 performed under ethical clearance from the Languedoc Roussillon ethical committee
181 for animal experimentation (n°2022041512256467 v5).

182 ***Choice of sampling periods and sites***

183 We selected six sampling sites based on their geographical position and their aridity
184 index (AI). The AI calculation was based on Thornthwaite method (Thornthwaite, 1948;
185 see **Supplementary Material** for full formula) using rainfall and temperature data
186 collected from 2010-2021 (South African Weather Service).
187

188 We selected parapatric populations of the two species in a semi-arid region of South
189 Africa (0.2 < AI < 0.5). In this region, a hot and wet season occurs from roughly
190 December to February, and a cool dry season from May to September. We sampled
191 six sites at the onset (May 2022) of the dry season: Barberspan Bird Sanctuary,
192 Benfontein Nature Reserve, Bloemhof Dam Nature Reserve, Gariep Dam Nature
193 Reserve, Kalkfontein Dam Nature Reserve and Wolwespruit Nature Reserve (**Figures**
194 **1&3, Table 2**). We then resampled four of these sites at the end of the dry season
195 (September 2022). Two sites (Barberspan and Benfontein) were excluded from the
196 September sampling due to extreme weather conditions.

197 ***Capture and field protocol***

198 We used small mammal PVC traps (LxHxW= 29.6x7.5x7.5 cm), baited with a mixture
199 of oats, peanut butter, salt and sunflower seeds, and added a piece of cotton wool.
200 They were placed approximately every 15 m along roughly 150 to 300 m transects.
201 Since *Rhabdomys* is mostly diurnal, the traps were checked twice a day (8 AM and 3
202 PM). On the field, all trapped striped mice were measured (body length and mass),
203 their sex and breeding status assessed based on external morphological metrics, and
204 a 0.5 mm piece of tail was collected and kept in 98 % ethanol for species identification.
205 One of four possible breeding statuses was recorded: breeding males; breeding
206 females; non-breeding males; and non-breeding females. All striped mice were marked
207 with a unique ear-tag before their release at the trapping location unless they were
208 kept for the physiological study (i.e. kept in their trap with cotton and food).

209 Overall, trapping effort was 9688 trap nights (**Supplementary Table 1**). Out of 903
210 trapped small mammals, 694 were *Rhabdomys*. For the physiological study, we
211 selected adults, avoiding related individuals as much as possible, by selecting pairs of

212 mice (a male and a female) trapped at least 100 m apart. This distance was based on
213 data obtained for *R. pumilio* indicating minimal relatedness (R=0 between males and
214 R=0.06 between females) at this distance (Solmsen et al. 2012). Trapping took place
215 between 3 and 6 days per site, until we reached our target of 20 individuals.

216 ***Habitat***

217 **Vegetation composition**

218 Earlier studies have shown inter-species differences in vegetation cover and structure
219 requirements (Dufour et al., 2015). Since habitat characteristics can vary locally, we
220 characterized the vegetation structure (i.e. grass *versus* woody vegetation) around
221 traps in which a *Rhabdomys* was trapped by measuring the percentage of grass,
222 bushes, and bare soil within 2mx2m and 10x10m quadrats centered on the trap
223 position. Each 2mx2m quadrat comprised four 1x1m subunits assessed using a 1x1m
224 metal frame; the results obtained for the four subunits were then averaged. Each
225 10mx10m quadrat was assessed following a visual inspection of a 10x10m zone
226 around each trap. We characterized 236 quadrats of each type (10x10m and 2mx2m).

227 **Normalised Difference Vegetation Index**

228 NDVI, a satellite imagery-based index informative of ground vegetation greenness,
229 was retrieved from the Copernicus Open Access Data Hub (Copernicus Sentinel-2
230 data, 2023). For each site and sampling session, we used NDVI data available for the
231 closest day to the beginning of a sampling session, which was expected to represent
232 the conditions experienced by the mice at the time of capture **(Supplementary Table**
233 **3).**
234

235 ***Blood extraction and assessment of blood metabolites concentrations***

236 A total of 273 animals were euthanized by means of cervical dislocation on their day
237 of capture, and a blood volume of at least 100 µL was collected in lithium-heparinized
238 tubes by cardiac puncture. The breeding status was confirmed for all individuals during
239 dissection. The whole liver, the left kidney, and the skull of every individual were also
240 collected for a complementary study. Because the two species could not be
241 distinguished visually, the spleen was also harvested from each mouse and
242 subsequently analyzed for post-hoc species identification using Cytochrome Oxidase
243 I genotyping (as described in Ganem et al. 2012).

244 Levels of albumin, alkaline phosphatase, alanine aminotransferase, amylase, total
245 bilirubin, blood urea nitrogen, total protein, globulin, glucose, calcium, phosphorus,
246 potassium, and sodium were successfully measured in the whole blood of 257
247 individuals immediately after blood collection (Abaxis VETSCAN V2 technology with
248 the Comprehensive diagnostic profile cartridge), following the manufacturer's
249 instructions. The coefficient of variation (CV%) of measured levels of these markers
250 was determined from data obtained by analysing a given blood sample twice. Samples
251 from six adult striped mice (5 *R.bechuanae*, 1 *R.d.dilectus*) were used for these
252 calculations. For these six samples, the calculated CV equaled 5.6 ± 1.9 %, which is
253 consistent with the intra-test CV% provided in the manufacturer's instruction manual
254 for other mammal species (**Supplementary Table 4**).

255

256 ***Replication statement***

257 Inferences for the habitat analyses were made at the scale of successful traps (**3**).

258 Inferences for the body condition analysis were made at the individual scale
259 (depending on the factor of interest, **1** or **2**).

260 Inferences for the physiology analyses were made at the individual scale (depending
261 on the factor of interest, **1** or **2**).

Scale of inference	**Scale at which the factor of interest is applied**	**Number of replicates at the appropriate scale**
(1) Individuals	Populations	3 *R.bechuanae* onset of the dry season, 3 *R.d.dilectus* onset of the dry season, 2 *R.bechuanae* end of the dry season, 2 *R.d.dilectus* end of the dry season
(2) Individuals	Individuals	236
(3) Successful traps	Sites	6 onset of the dry season, 4 end of the dry season

262

263 ***Data preparation and analysis***

264 All statistical tests were carried out using the R software (version 4.2.1).

265 **Habitat**

266 Vegetation composition

267 Principal Component Analysis (package FactoMineR version 2.9) was performed,
268 including the 7 variables measured within the 4m^2 quadrats and within the 100m^2
269 quadrats for each successful trap (traps in which *Rhabdomys* was captured). In each
270 case, over 80 % of the variance was explained by the first five Principal Components
271 (**Supplementary Tables 7 & 9**). As Multivariate normality was violated in our dataset,
272 the coordinates of all traps on the first 5 PCs were used as response variables of a
273 *PERMANOVA* (package *vegan*, version 2.6-4). *PERMANOVA* is a multivariate
274 statistical inference tool using permutational algorithms (Anderson, 2001). This
275 equivalent to MANOVA operates in a distribution-free setting and is robust to non-
276 normality of residuals as well as dispersion heterogeneity, even in slightly unbalanced
277 designs (Anderson, 2013). We tested differences between sites nested within sessions
278 (start and end of dry season), with 10 000 permutations, to assess whether the sites
279 had similar enough habitat characteristics to be considered as suitable replicates in
280 other analyses.

281 NDVI

282 NDVI was averaged over a 60 m radius circular buffer for each trap and then compared
283 across sessions and sites using a GLM Analysis of Variance, with the site nested within
284 session. To meet *ANOVA* prerequisites, the response variable was log-transformed;
285 the analysis was followed by Tukey multiple comparisons of means post-hoc tests.

286 **Body condition**

We calculated an index of body condition (mass/length), to assess the state of each individual's fat reserves (Schulte-Hostedde et al., 2005). To test whether breeding status, age, habitat quality, increased dryness, and/or interspecific differences influence body condition of individuals trapped in semi-arid locations, an *ANOVA* was used with body condition as a response variable, site as a random factor and session, species, session*species, breeding status, age class and NDVI as explanatory variables. Assumptions of normality and homoscedasticity of residuals were checked using diagnostic plots (*graphics* package v.3.6.2.). A Tukey multiple comparisons of means post-hoc test was applied to determine which inter-level differences were driving the significance of each factorial effect having more than two modalities.

Age classes

Trapped *Rhabdomys* were assigned to age categories: juveniles, subadults and adults based on their general appearance, size and/or breeding status. Body length of trapped individuals varied between 5 and 12 cm. The body length of adult individuals involved in the physiological study varied between 7.7 and 12 cm. To distinguish between older and younger adults, adult mass was plotted against body length (**Supplementary figure 8**), showing a curve defined by an allometric growth equation with a steeper slope at the highest and lowest body length values. Since bone growth can significantly alter the concentration of some physiological markers, and growth rate varies with age/body length, we considered 4 length/age classes (Class A: [7.7:9 cm[; Class B : [9:10 cm[; Class C : [10:11 cm[; Class D [11 cm:]).

Physiology

310 We performed a Principal Component Analysis to identify outliers in the data set that
311 could be potential measure errors. We identified twelve outliers that we removed from
312 further analyses. These outliers were animals from which we collected smaller volume
313 of blood samples than recommended for the Vetscan or were heavily hemolyzed and
314 flagged as such in the Vetscan analysis output. In addition, as total protein values were
315 used by the Vetscan analyzer to infer the Globulin (GLOB) fraction of blood protein
316 (with GLOB=TP-ALB), we considered this marker as not informative and removed this
317 variable from our analyses. Finally, for 199 out of 257 samples, blood concentrations
318 of creatinine (CRE) were flagged as being below the instrument's sensitivity threshold;
319 we hence excluded CRE from subsequent analyses, as this imprecision would have
320 hindered analytical performance.

321 Due to covariance in variables such as blood physiological markers, multivariate
322 statistical techniques provide a unique insight into the main patterns and effects driving
323 variation in the data. Thus, we performed a multivariate analysis with 236 blood
324 samples, 12 physiological parameters as response variables, site as a random factor,
325 and session, species, body condition, age class, and NDVI as explanatory variables.
326 As with habitat, multivariate normality was violated in our physiological dataset, so
327 permutational multivariate analysis of variance (*PERMANOVA*) was performed, using
328 10 000 permutations. A backwards stepwise model selection was performed to retain
329 the most parsimonious model. The best-fitting model was determined by comparing all
330 previous models, from least to most complex, using the Corrected Akaike's Information
331 Criterion (AICc) for *PERMANOVA* (package *AICcPermanova* version 0.0.2). We then
332 sought to identify which of the 12 physiological markers drove significantly the
333 between-group differences evidenced with the *PERMANOVA*. Twelve univariate
334 *ANOVA* tests were performed, initially using the best *PERMANOVA* model followed by

335 backwards stepwise model selection. Assumptions of normality and homoscedasticity
336 of residuals were checked using diagnostic plots. In the case of total bilirubin, a
337 permutation test had to be performed instead of the *ANOVA*. Through a backwards
338 stepwise model selection process, the final model retained was based on the Akaike's
339 Information Criterion (AIC). For all tests, the significance level (α) was set at 0.05.
340

341 ## Results

342 ***Variation in habitat characteristics between species and sessions***

343 **Vegetation composition**

344 We compared the vegetation characteristics surrounding each successful trap with a
345 *PERMANOVA*, using the first 5 Principal Component coordinates (**Supplementary**
346 **Tables 7 & 9**) of each trap as response variable, and site and session as explanatory
347 variable. For 2x2m quadrats, PC1 was most explained by variance in dry grass (39.85
348 %), green bushes (35.63 %) and uncovered surfaces (20.59 %), PC2 was most
349 explained by variance in the presence of green grass (62.04 %), PC3 by presence of
350 holes in the ground (60.13 %), PC4 by presence of dry bushes (74.2 %) and PC5 by
351 presence of succulent plants (76.56 %). For 10x10m quadrats, PC1 was most
352 explained by variance in dry grass (32.14 %), green bushes (26.16 %), uncovered
353 surfaces (15.91 %) and dry bushes (10.96 %); PC2 was most explained by green grass
354 (47.24 %), dry grass (20.85 %), holes (13.59 %), and uncovered surfaces (11.77 %);
355 PC3 by succulent plants (53.34 %), PC4 by dry bushes (34.28 %), uncovered surfaces
356 (28.32 %) and succulent plants (23.96 %); and PC5 by holes (43.92 %), green bushes
357 (25.85 %) and succulent plants (18.69 %). We detected significant differences in
358 overall habitat characteristics between sessions (2x2m and 10x10m: $p<0.001$), and

359 sites nested within sessions (2x2m and 10x10m: $p<0.001$), indicating clear site
360 differences and seasonal effects on habitat structure (**Supplementary Figures 6 & 7**).
361 Although a permutational dispersion test showed these patterns could be attributable
362 in part to differences in dispersion between sessions or sites (see **Supplementary**
363 **Material**), some general trends could be inferred from the data. Indeed, *R.d.dilectus*
364 quadrats tended to feature higher proportions of grass (2x2 : 83.99 %, 10x10 : 71.46
365 %) than *R.bechuanae* quadrats (2x2 : 54.30 %, 10x10 : 53.16 %). Conversely,
366 *R.bechuanae* quadrats tended to feature higher proportions of bushes (2x2 : 31.07 %,
367 10x10 : 27.35 %) than *R.d.dilectus* quadrats (2x2 : 7.33 %, 10x10 : 10.49 %).
368 Furthermore, quadrats surveyed at the end of the dry season featured more dry grass
369 (2x2 : 74.65 %, 10x10 : 50.15 %) than at its onset (2x2 : 50.75 %, 10x10 : 40.92 %).

370 **NDVI**

371 The log-transformed NDVI of sites sampled at the two sessions (2 sites per species),
372 extracted from satellite data, was also analysed between sites and sessions using
373 *ANOVA*. Site ($F=115.385$, $df=3$, $p<0.001$) and session ($F=442.757$, $df=1$, $p<0.001$),
374 and the interaction between these two variables ($F=7.113$, $df=3$, $p<0.001$) contributed
375 significantly to explain the NDVI variation (**Table 3**). A post-hoc Tukey multiple
376 comparisons of means test showed that all sites had a significantly lower NDVI in
377 September compared to May (**Figure 4**), indicating a poorer state of the vegetation at
378 the end of the dry season. Furthermore, while these study locations were chosen in a
379 region with similar semi-arid bioclimatic features, Gariep Dam and Kalkfontein (where
380 *R.bechuanae* was targeted) seemed to stand out as having a lower NDVI than all other
381 sites sampled in both May and in September, with Wolwespruit and Bloemhof (where
382 *R.d.dilectus* was targeted) having the highest NDVI (**Figure 4**). Therefore, to take into
383 account the impact of variations in NDVI between the two species trapping locations

384 we included site as a random factor in analyses addressing body and physiological
385 conditions.

386 ***Variation of Body condition***

387 An *ANOVA* assessed the influence of session, species, breeding status, age class and
388 NDVI on body condition, including site as a random factor. The most parsimonious
389 model included the significant effects of breeding status and age class (both $p<0.001$,
390 **Table 4**). Non-breeding females had lower body condition than all other categories
391 (post-hoc pairwise t-tests all $p<0.05$); body condition was also significantly higher for
392 bigger/older individuals (**Supplementary table 11**).

393

394 ***Variation in physiological responses***

395 A *PERMANOVA* was performed using the distance matrix of 12 physiological markers
396 as response variable and session, species, breeding status, body condition, age
397 class, NDVI and site (random factor) as explanatory variables (**Supplementary table**
398 **12**).

399 The most parsimonious model **(Table 5)** indicated an overall difference in physiological
400 parameter concentrations between *R.bechuanae* and *R.d.dilectus* ($p=0.001$), as well
401 as an influence of breeding status ($p=0.008$), that differed between the two species
402 (interaction effect, $p=0.005$). Session ($p=0.062$), and body condition ($p=0.074$) verged
403 on overall significance and were also included in the model, indicating that
404 physiological markers overall tend to shift between the start and end of the dry season,
405 and to vary with body condition. Importantly, both species showed the same pattern of
406 variation between sessions (no significant species*session interaction effect,
407 **Supplementary Table 12**).

408 Univariate *ANOVAs* performed for each of the 12 physiological markers, with the initial
409 model being the one retained in the *PERMANOVA* analysis, showed that maximum
410 parsimony was achieved for 9 of the physiological markers without the site random
411 effect, suggesting little impact of inter-site variations on these markers.

412 Six physiological markers revealed differences between the start and the end of the
413 dry season (**Figure 5**). Blood concentrations of markers of nutrition and liver function
414 (albumin, potassium, alanine aminotransferase, alkaline phosphatase, and total
415 bilirubin) were significantly lower at the end compared to the onset of the dry season
416 (**Table 4**). In contrast, globulin levels varied in the opposite direction (higher at the end
417 compared to the onset of the dry season).

418 The levels of five markers were significantly lower in *R.bechuanae* and *R.d.dilectus*
419 (R^2=0.12, $p<0.001$), including total bilirubin, alkaline phosphatase (ALP), sodium and
420 potassium (**Figure 6**). However, amylase showed the opposite pattern between the
421 species for all breeding statuses except breeding males (**Figure 7**).

422 Regardless of species, ALP levels were negatively correlated with body condition,
423 while the levels of phosphorus exhibited the opposite pattern. Finally, the levels of
424 glucose varied significantly with breeding statuses, being significantly lower in breeding
425 females compared with non-breeding males ($p=2*10^{-4}$) and tended to be lower than in
426 non-breeding males and breeding females (**Supplementary figure 9**).

427 ## Discussion

428 We asked how seasonal variation of dry conditions influenced the physiological
429 response of semi-arid parapatric populations of two sister species that evolved under
430 distinct environmental conditions (du Toit et al., 2012).

431

Our study took place in a relatively wet year (La Niña). During the El Niño Southern Oscillation phenomenon, there is a general association between regional wetness and sea surface temperatures in the neighbouring Atlantic and Indian Oceans, leading to wetter episodes throughout Southern Africa during La Niña years (Nicholson & Selato, 2000). Vegetation growth and senescence usually follow seasonal rainfall patterns; above-average rainfall widens the temporal window for vegetation growth, improving habitat conditions at the start of the typical dry season (Anyamba et al., 2002). Despite this, and consistent with our predictions, milder dry conditions did alter habitat quality of our study sites (as attested by shifts in NDVI and habitat structure).

Consistent with our prediction, we observed seasonal variation of the physiological response in both species related to energetic resource depletion at the end of the dry season. Additionally, as predicted, irrespective of season, we found differences in the physiological responses between species.

Water and nutritional stress are expected to impact the body condition of individuals (Fuller et al., 2021). However, the body condition of the species did not vary between the two study sessions, which may relate to the wetter conditions the sampling year. As expected, body condition varied with breeding status, and several physiological markers varied significantly with body condition or breeding status, indicating their association with physiological processes involved in reproduction and body mass regulation. Reproduction in mammals has direct physiological costs, associated with increased energy, protein and calcium demands and indirectly through compensatory effects such as reduction in thermogenesis, immune function and physical activity (Speakman, 2008; Rintoul & Bringham, 2014; Stawski & Rojas, 2016; Schoepf et al., 2017b). In support, our results indicate lower levels of blood glucose in breeding females compared with all other individuals. Moreover, alkaline phosphatase (ALP)

457 was significantly elevated in non-breeders compared to breeders. Since non-breeders
458 were mostly the smallest adults, greater ALP levels might relate to soft tissue and/or
459 bone growth in the latter (Walker et al., 1990). Body condition was generally better in
460 larger individuals (**Supplementary Table 11**), and ALP was as expected negatively
461 correlated with body condition. Individuals with the lowest body condition, mostly
462 smaller individuals, had lower phosphorus levels. High phosphorus levels can be
463 observed in dehydrated individuals in which osmolality is high; indeed, low phosphorus
464 levels related to survival in *R.pumilio* at the peak of the dry season, where water
465 balance was hardest to maintain (Schoepf et al., 2017). However, blood levels of
466 phosphorus also depend on its consumption and hence availability in the food (Berndt
467 & Kumar, 2009). Phosphorus reserve depletion in smaller-sized/younger adults might
468 therefore be consistent with the role of phosphorus intake as a promoter of bone and
469 soft tissue growth (Shapiro & Heaney, 2003). Furthermore, higher needs for
470 phosphorus in smaller adults might be hindered by reduced access to food. Indeed,
471 foraging activity tends to vary between individuals according to size and breeding
472 status, possibly resulting in larger breeding individuals gaining access to higher quality
473 food (Ebensperger & Hurtado, 2005).

474 ***Seasonal variation in physiology***

475 Despite our study year being relatively wet, milder dry conditions impacted kidney and
476 liver blood marker concentrations in *R.bechuanae* and *R.d.dilectus*. These changes
477 involved 6 out of 12 tested physiological markers, and included markers expected to
478 vary in periods of dietary restriction. Contrary to our prediction, we did not find a
479 significant interactive effect between session and species, suggesting that the semi-
480 arid populations of the two species were similarly affected by seasonal dry conditions.

481 However, a follow up study during an El Niño year may be necessary before one can
482 conclude.

483 Blood protein levels are generally good indicators of physiological condition (Tothova
484 et al., 2016). They comprise two distinct and major components: albumin (ALB) and
485 globulins (GLOB). In our study, ALB was significantly lower at the end than at the start
486 of the dry season. ALB is synthesized in the liver and involved in the transport of
487 bilirubin and several hormones. Blood ALB levels are less prevalent in malnourished
488 individuals (Walker et al., 1990), supporting the hypothesis of reduction of quality
489 and/or availability of food at the end of the dry season. The Nubian ibex *Capra nubiana*,
490 a desert mammal, also displays low ALB during the dry season (Al-Eissa et al., 2012),
491 which could reflect poor nutritional condition, kidney dysfunction leading to poor water
492 balance and high osmolality, or liver dysfunction causing low ALB synthesis. We found
493 that GLOB was significantly higher at the end than at the start of the dry season. GLOB
494 is a family of molecules including enzymes, immunity proteins and blood proteins
495 carriers (including transferrin). An increase in GLOB levels usually indicates elevation
496 of immunoglobulins and hence could suggest heightened immune activity. Greater
497 antibody production occurs in response to a higher prevalence of respiratory infections
498 during the dry season, e.g. in the Nubian goat (Abdelatif et al., 2009), and/or reduced
499 protein nutritional status in young birds (Lochmiller et al., 1993). Higher GLOB levels,
500 indicating a strained immune system, were also reported for *R.pumilio* individuals that
501 did not survive the dry season (Schoepf et al., 2017). An increase in blood GLOB
502 concentrations may also reflect high levels of transferrin (Walker et al., 1990), a major
503 GLOB that may accumulate in the blood in cases of severe iron deficiency (Kasvosve
504 & Delanghe, 2002). Hence, elevated blood GLOB levels at the end of the dry season
505 may indicate either or both depletion in iron resource (transferrin) and higher

506 vulnerability to infection (immunoglobulin), both resulting from or being aggravated by
507 reduced access to protein. Lower levels of alkaline phosphatase (ALP), a liver enzyme,
508 at the end of the dry season in *Rhabdomys*, is also evidence of malnutrition and
509 deficiency in essential nutrients (proteins, magnesium, zinc) (Yousef et al., 2002; Saraç
510 & Saygili, 2007; Ray et al., 2017). Alanine aminotransferase (ALT), another marker of
511 liver function, also occurred at significantly lower concentrations at the end compared
512 to the start of the dry season, indicating a reduction in liver activity (Walker et al., 1990).
513 During malnutrition, ALT levels could be low (Le Couteur et al., 2010). Indeed, as
514 environmental resources become scarce, essential nutrients, such as pyridoxine (or
515 vitamin B6), that constitute some ALT coenzymes, may be less available, leading to a
516 reduction of ALT blood levels (Vespasiani-Gentilucci et al., 2018).
517
518 Acute starvation typically causes bilirubin (TBIL) to accumulate in the blood, as the
519 enzymatic process for conversion of heme into TBIL is stimulated (Thaler et al., 1972).
520 Yet, TBIL was lower at the end compared to the onset of the dry season, rejecting the
521 starvation hypothesis.
522 Lower TBIL concentrations at the end of the dry season in *Rhabdomys* could stem
523 from low hemoglobin counts because the body produces fewer red blood cells than
524 usual; low hemoglobin can be induced by several factors, among which dietary factors
525 such as iron deficiencies (Clark, 2008). Iron deficient anemia is also supported by
526 higher GLOB concentrations observed at the end of the dry season. Infection can
527 likewise be a cause of both decreased TBIL levels (Zhao et al., 2019) and higher GLOB
528 concentrations at the end of the dry season. Finally, malnutrition and/or lack of
529 potassium intake are among the main potential causes of the reduction of its levels in

530 the blood (Their, 1986), which could explain why they were the lowest at the end of the
531 dry season, when the available resources were lowest, in our study.
532
533 Overall, our results indicate that seasonal variation of physiological markers could
534 reflect reduced primary productivity throughout the dry season, which is coherent with
535 our observation of habitat degradation (lower NDVI) at the end of the dry season. Blood
536 concentrations of various markers seem to indicate difficulties in maintaining nutritional
537 functions and the necessity of a higher immune output, caused or exacerbated by food
538 restriction. However, since glucose levels were maintained during the dry season,
539 shifts in six physiological markers related to nutrition and immunity during the milder
540 than usual dry season suggest a reduction of liver function due to malnutrition, rather
541 than starvation.

542 Individuals undergoing malnutrition during dry conditions are expected to show low
543 blood glucose concentrations, and deficiencies in essential nutrients, leading to
544 pathologies such as anemia (Gordon et al., 1988). Indeed, animals foraging on soils
545 potentially poor in nutrients such as iron, zinc, and magnesium are more prone to
546 malnutrition (Graham, 1991; Gupta et al., 2008). Higher GLOB, lower TBIL and lower
547 ALP blood concentrations in our study might relate to such deficiencies.

548 We did not observe seasonal variation of sodium or blood urea nitrogen, blood markers
549 of kidney function or osmoregulation. Even if such effects may have been mitigated by
550 the relatively wet conditions, reduced environmental moisture at the end of the dry
551 season could have caused an overall increase in blood osmolality, increasing blood
552 concentrations of most biomarkers. In contrast, most markers exhibited a significant
553 reduction in blood concentrations. This tends to indicate that water availability does not

554 worsen as strongly as food availability for striped mice throughout the dry season in
555 this semi-arid environment.

556 The two species responded similarly to increased dryness, while maintaining stable
557 body conditions and levels of energy (glucose), a proxy of fat reserves. *R.pumilio*
558 individuals tended to reduce their energy expenditure and physical activity during
559 periods of limited food availability (Rimbach, Blanc et al., 2018; Rimbach, Jäger et al.,
560 2018). The semi-arid populations of *R.bechuanae* and *R.d.dilectus* could display a
561 similar behavioral strategy, which could explain their relatively stable levels of glucose
562 during the dry season.

563 ***Interspecific differences***

564 *R.bechuanae* is thought to have evolved in drier environments than *R.d.dilectus*.
565 Moreover, in the semi-arid region where they co-occur, *R.bechuanae* populations
566 occupy drier habitats than *R.d.dilectus*. While body condition did not differ significantly
567 between species, some interspecific contrasts support our hypothesis that
568 *R.bechuanae* may have developed a better physiological capacity to cope with semi-
569 arid conditions than *R.d.dilectus*.

570 Among the markers exhibiting a significant difference between *R.bechuanae* and
571 *R.d.dilectus*, all but amylase showed higher blood concentrations in *R.d.dilectus*.
572 Amylase is positively correlated with digestive activity and reflects differences in
573 feeding status or diet composition between the species (Hidalgo et al., 1999). Given
574 the role of alpha-amylase in digestion, lower blood amylase levels in *R.d.dilectus* than
575 *R.bechuanae* may indicate lower digestive activity due to reduced access to food, or
576 lower starch content in the diet. Pajic et al. (2019) found a correlation between dietary
577 starch content and the number of amylase gene copies in several mammalian

genomes, even in different habitats and with different diets. Since amylase activity and amylase gene expression are directly correlated with amylase gene copy number (Arendt et al., 2014), further studies could elucidate the potential genetic basis of the different levels of amylase in our two study species.

Sodium (NA) and potassium (K) concentrations, which depend on both dietary and water intake, were higher in *R.d.dilectus* than in *R.bechuanae*. These differences, coupled with higher ALP and TBIL concentrations in *R.d.dilectus* compared to *R.bechuanae*, could have been induced by nutritional deficiencies, which would suggest a better nutrition state for *R.d.dilectus* than *R.bechuanae*. However, higher levels of amylase in *R.bechuanae* cast doubt on this hypothesis. Instead, lower levels of blood NA, K, ALP and TBIL, would be more parsimoniously attributed to lower osmolality due to better water balance in *R.bechuanae*. Indeed, detection of water balance shifts may be muddled by the opposing effects of malnutrition and water constraints, as both influence osmolality (Zhang et al., 2022). A better overall ability of *R.bechuanae* to conserve body moisture could rely on physiological mechanisms such as more efficient excretion or osmoregulation, or through feeding on a diet optimizing water intake. We hypothesize that during the evolutionary history of *R.bechuanae*, selection pressure may have favored specific strategies allowing an overall more efficient water regulation compared to *R.d.dilectus*. These results, combined with the reduction in the levels of K, ALP and TBIL at the end of the dry season suggest the species are exposed to both water and nutrition stress.

We found relatively little evidence that the two species suffer differentially from lack of food in their semi-arid environment during a La Niña phenomenon. Overall, energy levels, as indicated by blood glucose, and body condition, did not vary between the

species. Instead, it seems that the overall differences between the two species can be accounted for by lower blood concentrations in *R.bechuanae*, and on average higher blood concentrations in key metabolites in *R.d.dilectus,* possibly due to overall poorer water regulation. High average blood amylase levels in *R.bechuanae* in the dry season might indicate an interspecific difference in diet composition or food intake, but not of protein-energy malnutrition. Little is known about the *R.bechuanae* and *R.d.dilectus* diets, although the genus was characterised as a generalist feeding on seeds and other plant material, and insects (Curtis & Perrin, 1979). Some studies show variation in diet composition within *Rhabdomys* genus associated with variation in local resources (Taylor & Green, 1976). The two species could have adjusted their diet to the respective local availabilities of different food resources within their home ranges. For example, in the semi-arid zone, arthropod biomass and diversity is affected by seasonal patterns in rainfall. Dalerum et al. (2017) found lower arthropod trapping success and diversity during the dry season, in shrub habitats compared to any other biomes, in Benfontein Game Reserve, one of our study sites. Thus, compared to seeds, the reliability of arthropods as a food source during dry conditions could be lower in shrublands, inhabited by *R.bechuanae* and by both species in parapatry, than in grasslands inhabited by *R.d.dilectus* in allopatry. Variation in seed composition could also explain this interspecific difference in amylase concentrations. Indeed, granivores prefer seeds that have higher protein content and lower secondary metabolites but may be forced to take a wider range of seeds in dry areas than in mesic ones (Wilmer et al., 2009). If the hypothesis of a relatively ancient adaptation of *R.bechuanae* to dry habitats is true, it could be expected that it would also have adjusted its diet to the specific conditions encountered in dry habitats. Within this ecosystem, *R.bechuanae* could forage more efficiently compared with *R.d.dilectus*, which evolved in a more

627 mesic environment; its diet could also be richer in starch (such as containing more
628 seeds/grains than leaves or fibre). Higher starch intake could help maintaining water
629 balance; starch is the best substrate for metabolic water production in a dry
630 environment (Adolph, 1964). This is consistent with the other interspecific differences
631 revealed in this study, as diet can also significantly affect osmoregulation (Sabat et al.,
632 2009).

633 Differences in blood amylase concentrations between the two species were observed
634 in all breeding statuses, except for breeding males. This is consistent with findings in
635 *R.pumilio* (Scantlebury et al., 2006), a species with similar levels of sociality as
636 *R.bechuanae*, where daily energy expenditure is reduced in mice living in groups. In
637 the solitary *R.d.dilectus*, higher thermoregulatory energy expenditure might reduce
638 foraging time, allowing only the largest individuals (breeding males) to meet their
639 foraging needs as efficiently as their *R.bechuanae* counterparts.

640 Conclusions

641

642 We found that two closely related species with different ecological characteristics
643 displayed similar negative shifts in blood concentrations of markers of nutrition and
644 liver function in spite of a relatively mild seasonal drying of their habitats. We hence
645 expect the physiological impact of harsher dry conditions, to affect the individuals'
646 fitness even more dramatically. The fact that the habitat of *R.bechuanae* studied
647 populations was the driest at both time points, combined with our findings concerning
648 their overall better water balance and nutrition than *R.d.dilectus*, point towards
649 *R.bechuanae* coping better in the semi-arid environment. If, as projected, the
650 frequency and length of drought episodes increase in this region, we predict that

651 *R.bechuanae* will expand its range at the expense of *R.d.dilectus*. Though adaptation
652 *per se* cannot be inferred from our study, this comparative study considering
653 populations of the two species in the same biogeographic zone adds to the growing
654 evidence that these cryptic species of striped mice have diverged phenotypically.
655 These findings support the hypothesis of adaptation to distinct ecological conditions
656 associated with their divergence. Further studies might seek to identify signatures of
657 selection in genes linked to crucial physiological functions in dry conditions to
658 understand the underlying mechanisms of divergence. Moreover, investigation of
659 seasonal variation in gene expression in the liver and kidney should help identify the
660 physiological pathways, key to survival, affected by seasonal variation in dry
661 conditions.
662

Main tables & figures

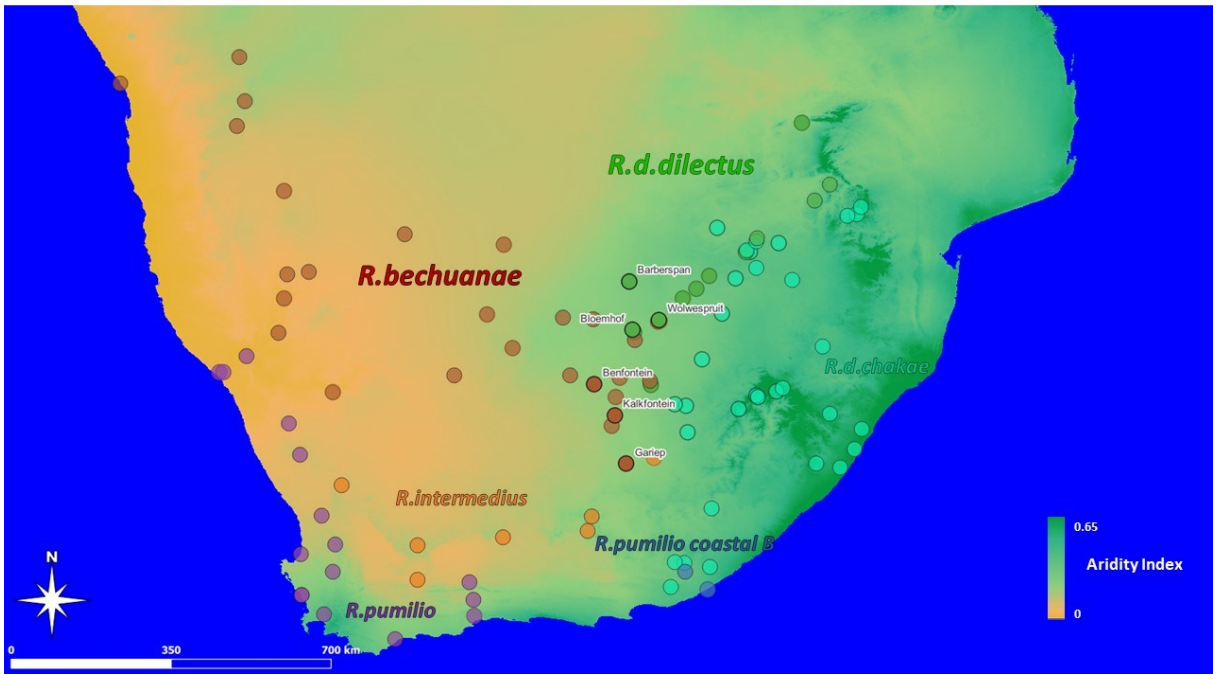


Figure 1: Map of known occurrences of 6 main clades of Rhabdomys (translucent dots) and locations sampled in this study (full colour dots) in southern Africa

Based on published data and unpublished data (origin details are available in doi: 0d1b3414-7e2a-11ea-a38d-00163e26bfb0). Base map: World Topographic Map Esri Standard, Aridity Index layer was computed from a 0.5° global grid, using data from the Version 3 of the Global Aridity Index and Potential Evapotranspiration Database (Zomer et al.,2022).

671 ***Table 1: Summary of main ecological characteristics of R.bechuanae and***

672 ***R.d.dilectus and our predictions on their physiological response during the***

673 ***dry season***

ECOLOGICAL CHARACTERISTICS	*R.BECHUANAE*	*R.D.DILECTUS*
ENVIRONMENTAL NICHE	Arid to semi-arid (Ganem et al., 2020)	Mesic to semi-arid (Ganem et al., 2020)
PREFERRED HABITAT	Bushy patches (Dufour et al., 2015)	Continuous cover / grassland (Dufour et al., 2015)
SOCIAL STRUCTURE	Group-living (Dufour et al., 2018)	Solitary / opportunistic group-living (Dufour et al., 2018)
SPECIES-SPECIFIC RESPONSE TO SEASONAL VARIATIONS OF DRY CONDITIONS: PREDICTIONS		
BODY CONDITION	↘↘ or ↘*	↘↘
NUTRITIONAL METABOLISM	↘↘ or ↘*	↘↘
LIVER ACTIVITY	↘↘ or ↘*	↘↘
WATER BALANCE	↘↘ or ↘*	↘↘
SENSITIVITY TO INFECTION	↗↗ or ↗*	↗↗
ASEASONAL DIFFERENCES: PREDICTIONS		
BODY CONDITION	+	-
LIVER ACTIVITY	+	-
WATER BALANCE	+	-
SENSITIVITY TO INFECTION	-	+

674

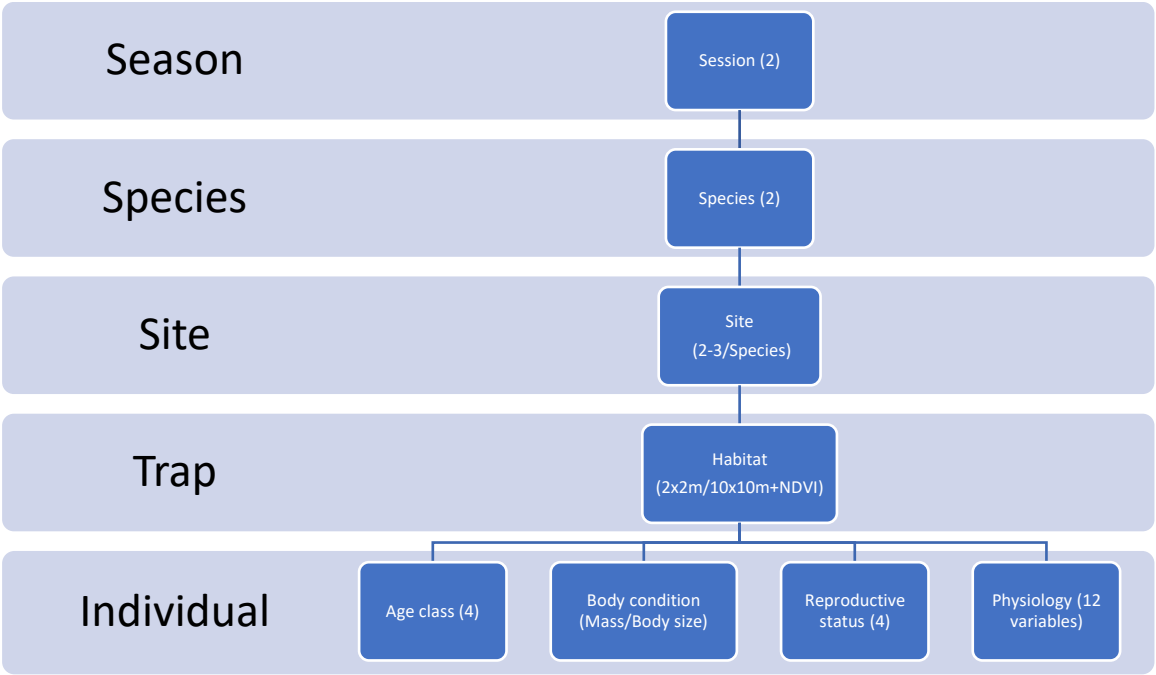
675 *Adaptation to harsher arid conditions could have enabled *R.bechuanae* to withstand

676 growing aridity without suffering as many adverse effects as *R.d.dilectus* in the semi-

677 arid zone. Alternatively, semi-arid populations of both species could both have

678 developed local adaptation.

679



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681 ***Figure 2: Graphical summary of organization and composition of data used for***

682 ***analysis in this study.*** Figures between brackets indicate number of modalities or types

683 of categories included in the factor.

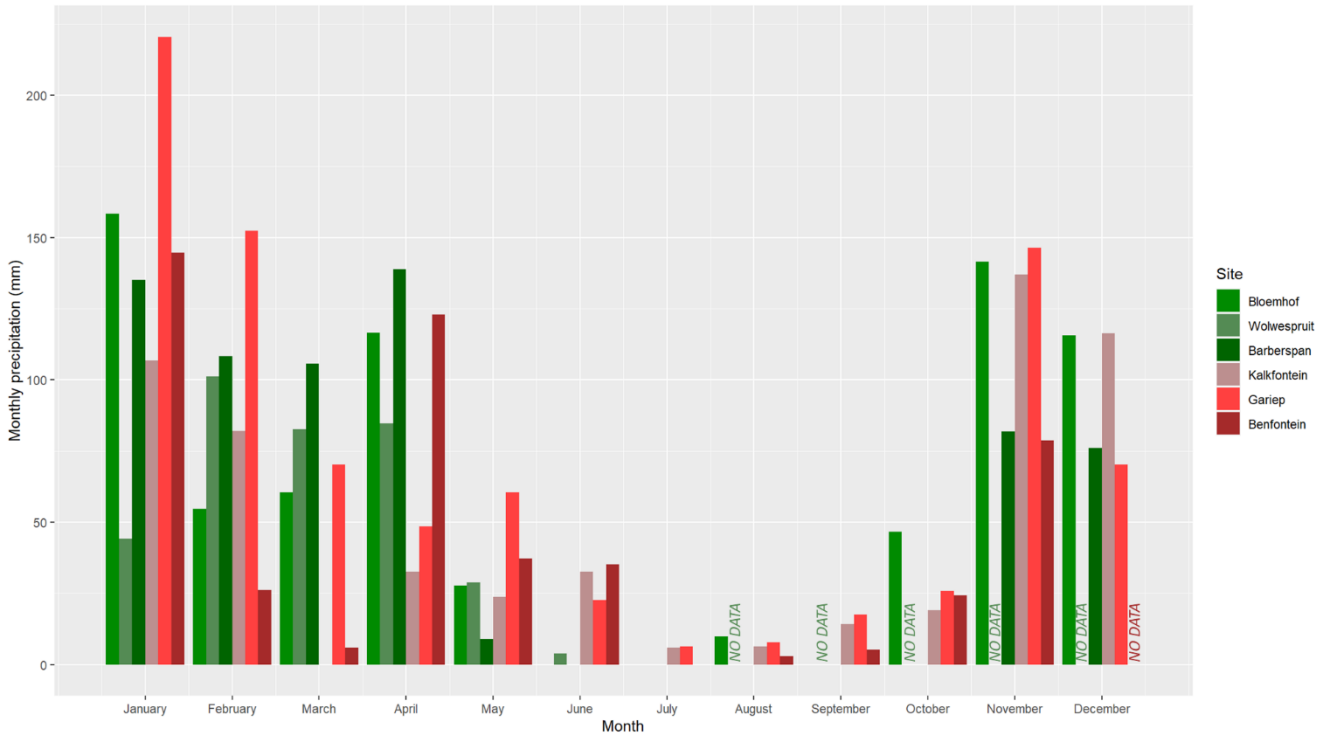

Figure 3: Total monthly precipitation for all sampled sites as recorded in 2022.

Table 2: Geographical position and Aridity Index of sampled sites for each species

SPECIES	SAMPLED SITES	GEOGRAPHICAL COORDINATES	ARIDITY INDEX*
R.bechuanae	Gariep Dam Nature Reserve	E25.55537; S30.57589	0.35-0.49
	Kalkfontein Dam Nature Reserve	E25.28031; S29.52058	0.47-0.61
	Benfontein Nature Reserve**	E24.81824; S28.82747	0.32-0.46
R.d.dilectus	Bloemhof Dam Nature Reserve	E25.65144; S27.66063	0.29-0.41
	Barberspan Bird Sanctuary**	E25.58354; S26.56538	0.34-0.44
	Wolwespruit Nature Reserve	E26.26326; S27.41418	0.43-0.63

690 *Average Aridity index (see **Supplementary material** for formula) ± Standard
691 Error of the Mean, based on climate data collected from 2010 to 2021 (South
692 African Weather Service)
693 ** Sampled only in May 2022 (dry season onset).
694

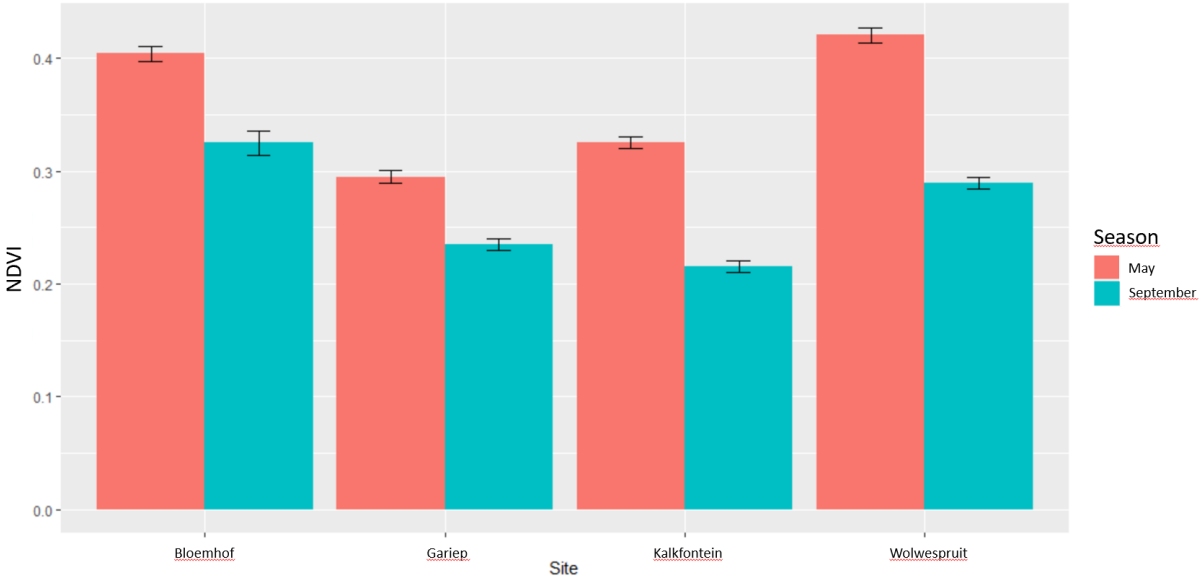

Figure 4: Average Normalized Differential Vegetation Index calculated within a buffer circle of 60m radius around each successful trap per site and session.

NDVI was retrieved from the Copernicus Open Access Data Hub (Copernicus Sentinel-2 data [2023]). For each site and session, we retained NDVI data available for the closest day to the beginning of a sampling session, expected to represent the conditions experienced by the mice at the time of capture.

***Table 3:* Results of the Analysis of Variance (response variable: NDVI)**

Variable	*Df*	*R²*	*F*	*P*
Session	1	0.445	442.757	**$< 2.2*10^{-16}$**
Site	3	0.348	115.385	**$< 2.2*10^{-16}$**
*Session*Site*	3	0.021	7.113	**$1.512*10^{-4}$**
Residual	229	0.185		

704 ***Table 4:*** **Results of the Analysis of Variance (response variable : body**
705 **condition)**

Variable	*Df*	*R²*	*F*	*P*
Age class	3	0.151	14.665	**$9.081*10^{-9}$**
Breeding status	3	0.065	6.309	**$3.952*10^{-4}$**
Residual	229	0.784		

706

707 ***Table 5: Summary of the results of the best fitting Permutational multivariate***

708 ***analysis of variance model, addressing variation in physiological response***

709 ***(site= random factor)***

710

Variable	*Df*	*R²*	*Pseudo-F*	*P*
Session	1	0.011	3.000	0.062
Species	1	0.042	11.108	**0.001**
Breeding status	3	0.044	3.874	**0.008**
Body condition	1	0.010	2.710	0.074
*Species * Breeding status*	3	0.047	4.202	**0.005**
Residual	226	0.846		

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719 ***Table 6: summary of the results of univariate analyses of variance addressing***

720 ***variation in responses of each of the 12 studied physiological variables (best***

721 ***fitting models).***

722

Physiological marker	*Variable(s) kept in Best Model and their significance*	*adjusted R^2*	*F*	*df Model*	*df Residual*	*P (model)*
Albumin	**Session*******,** Reproductive status, body condition	0.097	6.048	5	230	$2.786*10^{-5}$
Alkaline phosphatase	**Session*******, Species*****, Reproductive status*******, Body condition*****	0.321	19.390	6	228	$< 2.2*10^{-16}$
Alanine aminotransferase	**Session***, Species, Reproductive status, Species*Reproductive Status	0.060	2.884	8	227	0.005
Amylase	**Species*****, Reproductive status, body condition, **Species*Reproductive Status***	0.196	8.164	8	227	$1.079*10^{-9}$
Calcium	Species	0.009	3.095	1	234	0.08
Globulin	**Session*****, Body condition	0.300	51.450	2	233	$< 2.2*10^{-16}$
Glucose	Session$^{.}$, **Reproductive status****	0.085	6.481	4	231	$5.849*10^{-5}$
Potassium	**Session*****, Species***	0.068	9.569	2	233	0.0001
Sodium	Session, **Species*******,** Reproductive status, Body condition	0.125	6.614	6	229	$1.83*10^{-6}$
Blood Urea Nitrogen	*Null Random effect model*	0.072				/
Phosphorus	Session, Species, Reproductive status, **Body condition***** + random	0.294				(χ^2 test vs. null model) $9.838*10^{-9}$

723

*Note: The full models, before backwards elimination, included session, species, body condition, reproductive status and reproductive status*species as variables, and site as a partially-crossed random factor nested within species.*

Bold: *significant effects ($p < 0.05$) following Bonferroni correction with:*

***: $p < 0.001$; **: $0.001 < p < 0.01$; *: $0.01 < p < 0.05$

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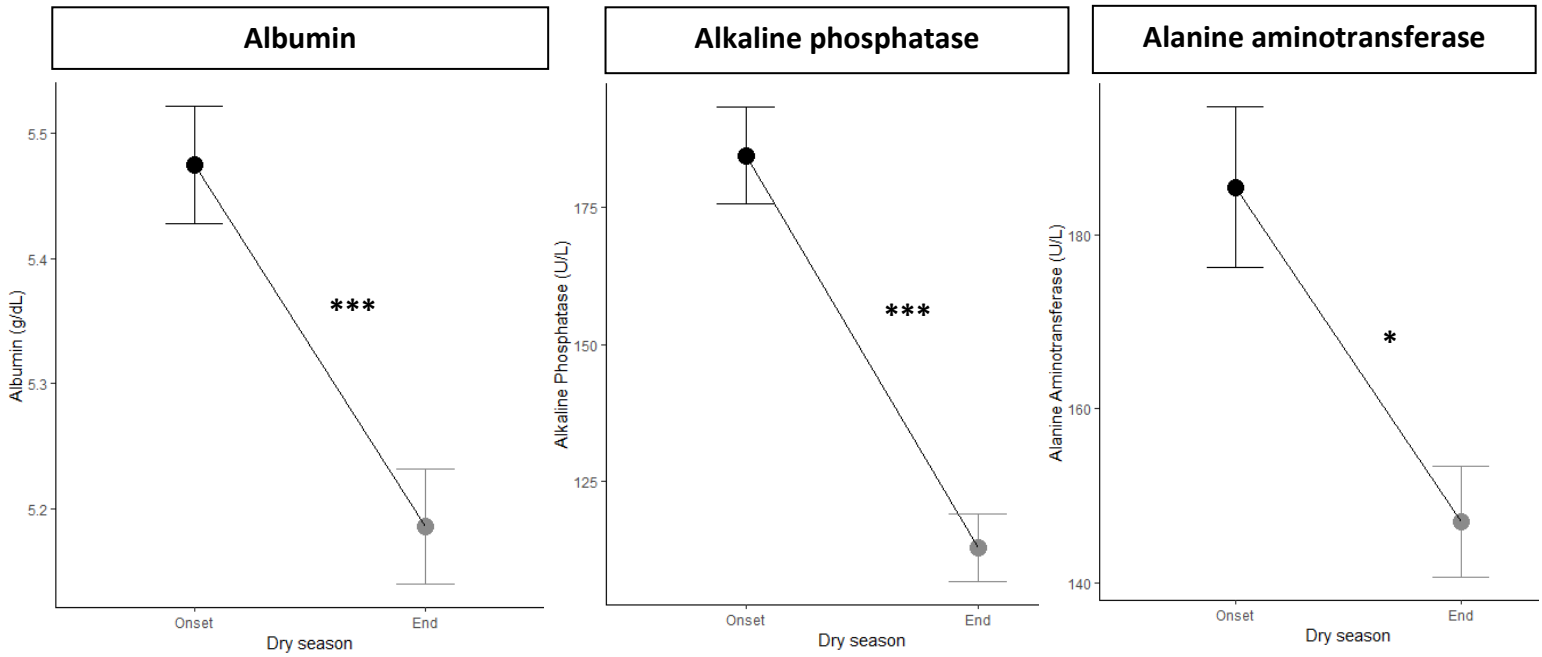

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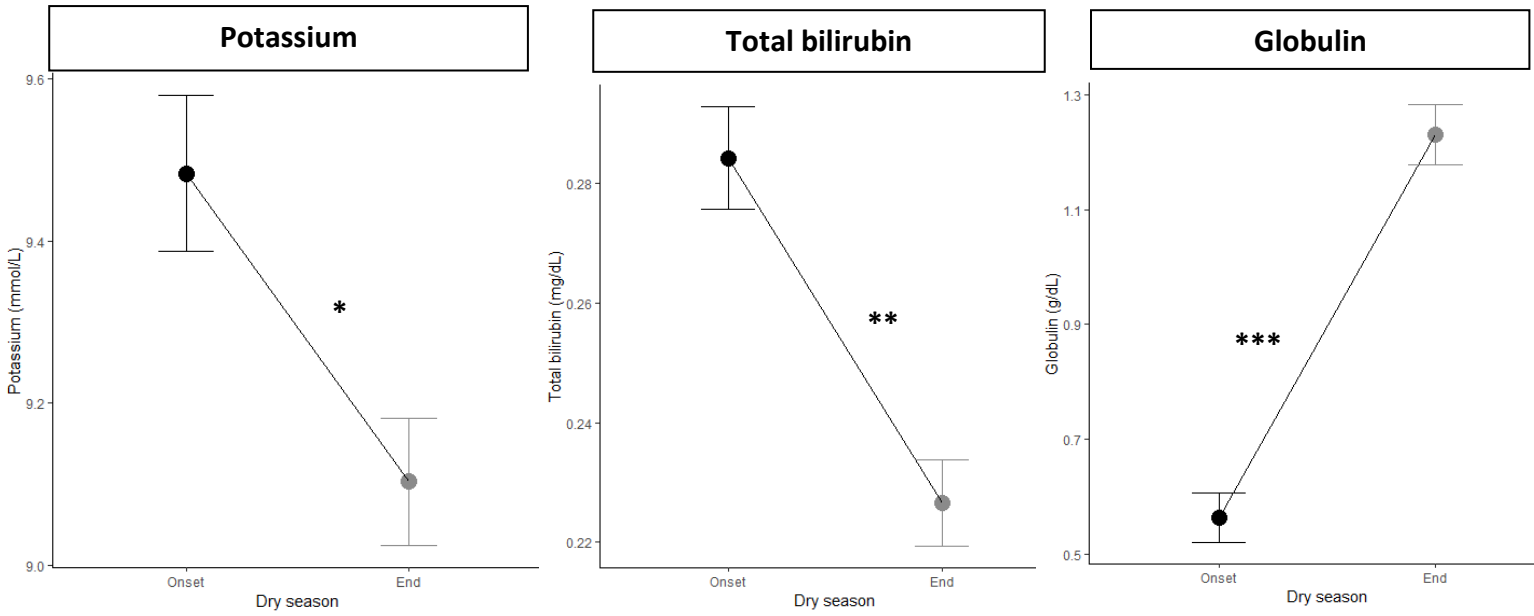

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734 ***<u>Figure 5: Significant shifts in blood physiological marker concentrations</u>***

735 ***<u>between the onset and the end of the dry season (mean ± SE)</u>***

736

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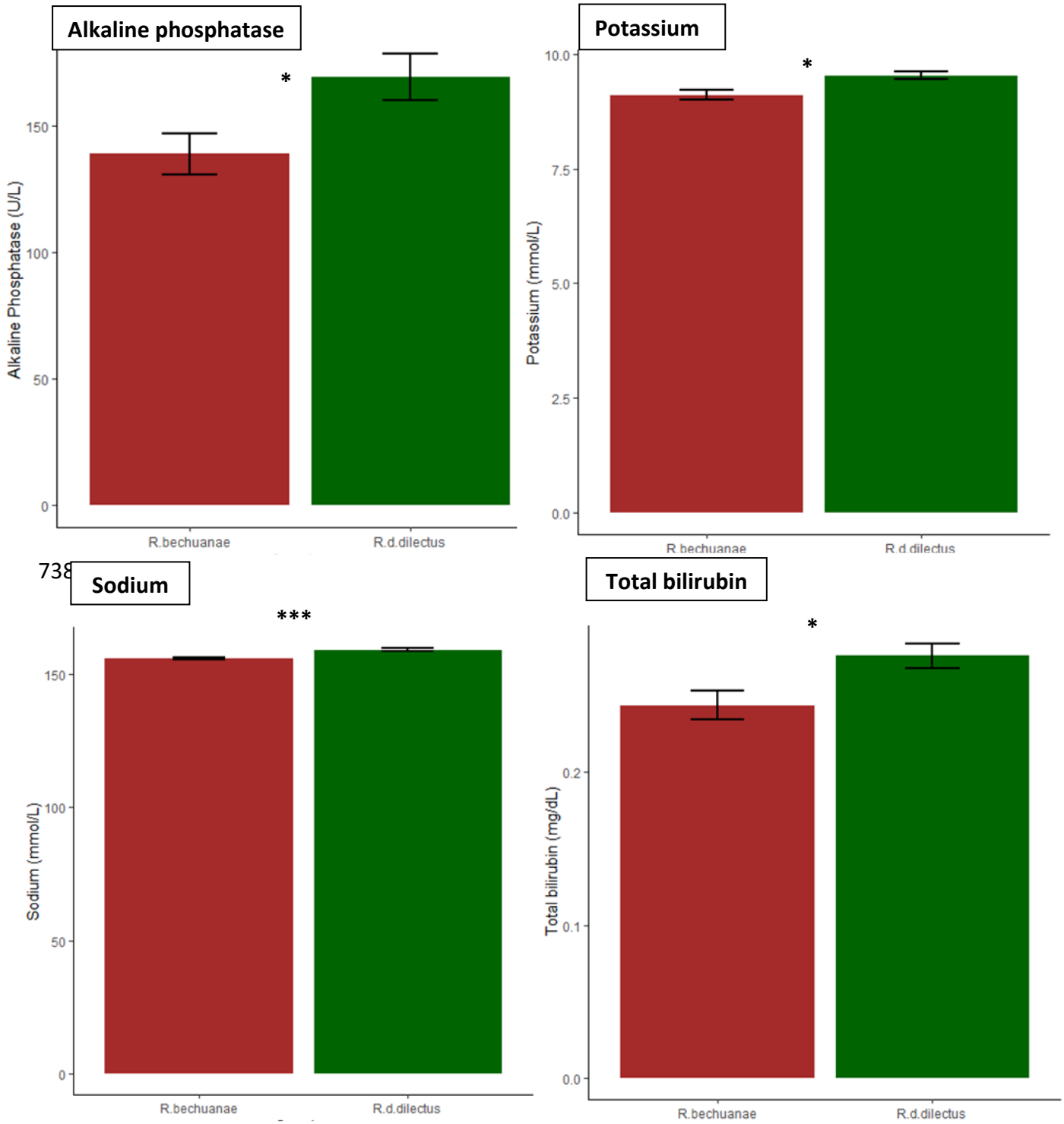

Figure 6: Significant interspecific differences involving four blood physiological marker concentrations (mean ± SE)

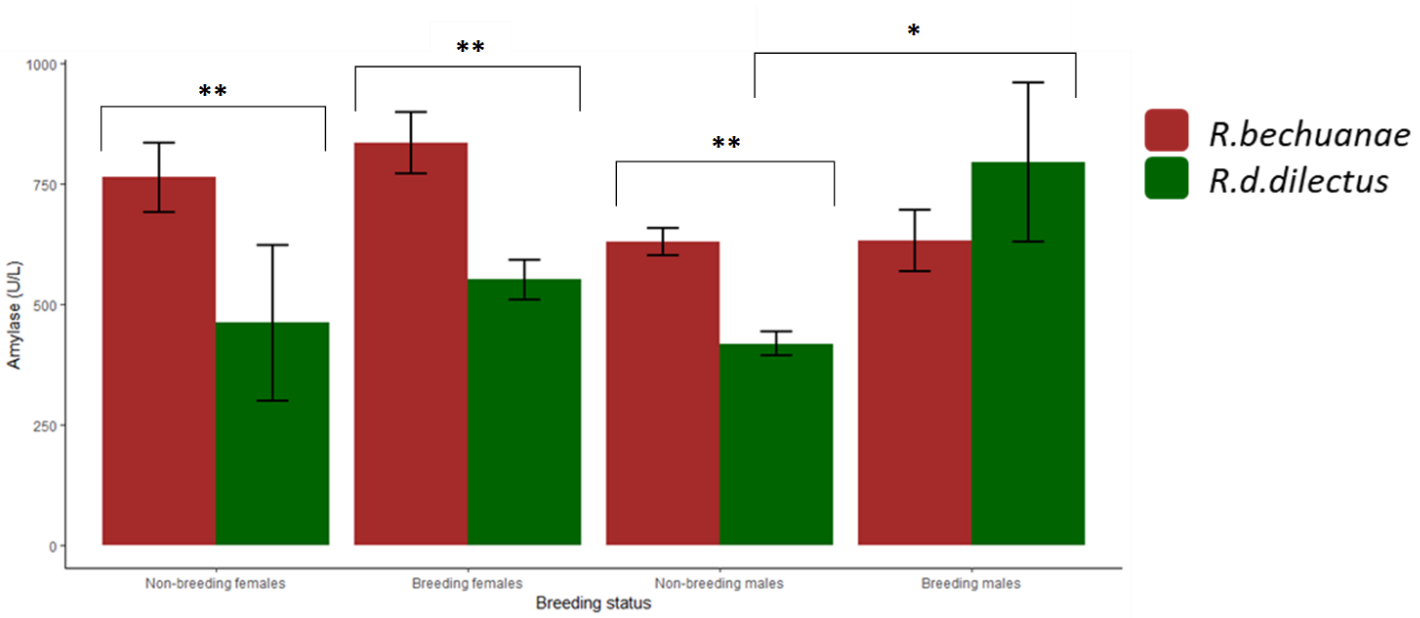

Figure 7: Variation of blood amylase concentration between species and breeding status (mean ± SE)

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