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

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

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

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

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

134 Materials and Methods

135 ***Ethics statement***

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

141 ***Choice of sampling periods and sites***

142 We selected six sampling sites based on their geographical position and their aridity
143 index (AI). The AI calculation was based on Thornthwaite method (Thornthwaite, 1948;
144 see **Supplementary Material** for full formula) using rainfall and temperature data
145 collected from 2010-2021 (South African Weather Service).
146

147 We selected parapatric populations of the two species in a semi-arid region of South
148 Africa (0.2 < AI < 0.5). In this region, a hot and wet season occurs from roughly
149 December to February, and a cool dry season from May to September. We sampled
150 six sites at the onset (May 2022) of the dry season: Barberspan Bird Sanctuary,
151 Benfontein Nature Reserve, Bloemhof Dam Nature Reserve, Gariep Dam Nature
152 Reserve, Kalkfontein Dam Nature Reserve and Wolwespruit Nature Reserve (**Figures**
153 **1&3, Table 2**). We then resampled four of these sites at the end of the dry season
154 (September 2022). Two sites (Barberspan and Benfontein) were excluded from the
155 September sampling due to extreme weather conditions.

156 ***Capture and field protocol***

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

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

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

Habitat

Vegetation composition

Earlier studies have shown inter-species differences in vegetation cover and structure requirements (Dufour et al., 2015). Since habitat characteristics can vary locally, we characterized the vegetation structure (i.e. grass *versus* woody vegetation) around traps in which a *Rhabdomys* was trapped by measuring the percentage of grass, bushes, and bare soil within 2mx2m and 10x10m quadrats centered on the trap position. Each 2mx2m quadrat comprised four 1x1m subunits assessed using a 1x1m metal frame; the results obtained for the four subunits were then averaged. Each 10mx10m quadrat was assessed following a visual inspection of a 10x10m zone around each trap. We characterized 374 quadrats of each type (10x10m and 2mx2m).

Normalised Difference Vegetation Index

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

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

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

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

214

215 ***Replication statement***

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

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

219 Inferences for the physiology analyses were made at the individual scale (depending
220 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	257
(3) Successful traps	Sites	6 onset of the dry season, 4 end of the dry season

221

222 ***Data preparation and analysis***

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

224 **Habitat**

225 Vegetation composition

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

240 NDVI

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

245 **Body condition**

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

256 **Age classes**

257 Trapped *Rhabdomys* were assigned to age categories: juveniles, subadults and adults
258 based on their general appearance, size and/or breeding status. Body length of
259 trapped individuals varied between 5 and 12 cm. The body length of adult individuals
260 involved in the physiological study varied between 7.7 and 12 cm. To distinguish
261 between older and younger adults, adult mass was plotted against body length
262 (**Supplementary figure 6**), showing a curve defined by an allometric growth equation
263 with a steeper slope at the highest and lowest body length values. Since bone growth
264 can significantly alter the concentration of some physiological markers, and growth rate
265 varies with age/body length, we considered 4 length/age classes (Class A: [7.7:9 cm[;
266 Class B : [9:10 cm[; Class C : [10:11 cm[; Class D [11 cm:]).

267 **Physiology**

268

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

280 Due to covariance in variables such as blood physiological markers, multivariate
281 statistical techniques provide a unique insight into the main patterns and effects driving
282 variation in the data. Thus, we performed a multivariate analysis with 236 blood
283 samples, 12 physiological parameters as response variables, site as a random factor,
284 and session, species, body condition, age class, and NDVI as explanatory variables.
285 As with habitat, multivariate normality was violated in our physiological dataset, so
286 permutational multivariate analysis of variance (*PERMANOVA*) was performed, using
287 10 000 permutations. A backwards stepwise model selection was performed to retain
288 the most parsimonious model. The best-fitting model was determined by comparing all
289 previous models, from least to most complex, using the Corrected Akaike's Information
290 Criterion (AICc) for *PERMANOVA* (package *AICcPermanova* version 0.0.2). We then
291 sought to identify which of the 12 physiological markers drove significantly the
292 between-group differences evidenced with the *PERMANOVA*. Twelve univariate
293 *ANOVA* tests were performed, initially using the best *PERMANOVA* model followed by

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

Results

Variation of habitat characteristics between species and sessions

Vegetation composition

We compared the vegetation characteristics surrounding each successful trap with a *PERMANOVA*, using the first 5 Principal Component coordinates (**Supplementary Tables 7 & 9**) of each trap as response variable, and site and session as explanatory variable. For 2x2m quadrats, PC1 was most explained by variance in dry grass (39.85 %), green bushes (35.63 %) and uncovered surfaces (20.59 %), PC2 was most explained by variance in the presence of green grass (62.04 %), PC3 by presence of holes in the ground (60.13 %), PC4 by presence of dry bushes (74.2 %) and PC5 by presence of succulent plants (76.56 %). For 10x10m quadrats, PC1 was most explained by variance in dry grass (32.14 %), green bushes (26.16 %), uncovered surfaces (15.91 %) and dry bushes (10.96 %); PC2 was most explained by green grass (47.24 %), dry grass (20.85 %), holes (13.59 %), and uncovered surfaces (11.77 %); PC3 by succulent plants (53.34 %), PC4 by dry bushes (34.28 %), uncovered surfaces (28.32 %) and succulent plants (23.96 %); and PC5 by holes (43.92 %), green bushes (25.85 %) and succulent plants (18.69 %). We did not detect significant differences in overall habitat characteristics between sites (2x2m: p=0.1233; 10x10m: p=0.2998,

318 **Supplementary Tables 8 & 10**) or sessions (2x2m: p=0.9495; 10x10m: p=0.1135,
319 **Supplementary Tables 8 & 10**), suggesting that, regardless of species, mice were
320 trapped in on average similar surroundings in the different sites and at the two trapping
321 sessions.

322 **NDVI**

323 The log-transformed NDVI of sites sampled at the two sessions (2 sites per species),
324 extracted from satellite data, was also analysed between sites and sessions using
325 *ANOVA*. Site (F=115.385, df=3, p<0.001) and session (F=442.757, df=1, p<0.001),
326 and the interaction between these two variables (F=7.113, df=3, p<0.001) contributed
327 significantly to explain the NDVI variation (**Table 3**). A post-hoc Tukey multiple
328 comparisons of means test showed that all sites had a significantly lower NDVI in
329 September compared to May (**Figure 4**), indicating a poorer state of the vegetation at
330 the end of the dry season. Furthermore, while these study locations were chosen in a
331 region with similar semi-arid bioclimatic features, Gariep Dam and Kalkfontein (where
332 *R.bechuanae* was targeted) seemed to stand out as having a lower NDVI than all other
333 sites sampled in both May and in September, with Wolwespruit and Bloemhof (where
334 *R.d.dilectus* was targeted) having the highest NDVI (**Figure 4**). Therefore, to take into
335 account the impact of variations in NDVI between the two species trapping locations
336 we included site as a random factor in analyses addressing body and physiological
337 conditions.

338 ***Variation of Body condition***

339 An *ANOVA* assessed the influence of session, species, breeding status, age class and
340 NDVI on body condition, including site as a random factor. The most parsimonious
341 model included the significant effects of breeding status and age class (both p<0.001,

342 **Table 4**). Non-breeding females had lower body condition than all other categories
343 (post-hoc pairwise t-tests all $p<0.05$); body condition was also significantly higher for
344 bigger/older individuals (**Supplementary table 11**).

345

346 ***Variation in physiological responses***

347 A *PERMANOVA* was performed using the distance matrix of 12 physiological markers
348 as response variable and session, species, breeding status, body condition, age
349 class, NDVI and site (random factor) as explanatory variables (**Supplementary table**
350 **12**).

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

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

364 Six physiological markers revealed differences between the start and the end of the
365 dry season (**Figure 5**). Blood concentrations of markers of nutrition and liver function

366 (albumin, potassium, alanine aminotransferase, alkaline phosphatase, and total
367 bilirubin) were significantly lower at the end compared to the onset of the dry season
368 (**Table 4**). In contrast, globulin levels varied in the opposite direction (higher at the end
369 compared to the onset of the dry season).

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

374 Regardless of species, ALP levels were negatively correlated with body condition,
375 while the levels of phosphorus exhibited the opposite pattern. Finally, the levels of
376 glucose varied significantly with breeding statuses, being significantly lower in breeding
377 females compared with non-breeding males ($p=2*10^{-4}$) and tended to be lower than in
378 non-breeding males and breeding females (**Supplementary figure 7**).

379 Discussion

380 We asked how seasonal variation of dry conditions influenced the physiological
381 response of semi-arid parapatric populations of two sister species that evolved under
382 distinct environmental conditions (du Toit et al., 2012).
383

384 Our study took place in a relatively wet year (La Niña). During the El Niño Southern
385 Oscillation phenomenon, there is a general association between regional wetness and
386 sea surface temperatures in the neighbouring Atlantic and Indian Oceans, leading to
387 wetter episodes throughout Southern Africa during La Niña years (Nicholson & Selato,
388 2000). Vegetation growth and senescence usually follow seasonal rainfall patterns;
389 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 (NDVI).

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) was significantly elevated in non-breeders compared to breeders. Since non-breeders were mostly the smallest adults, greater ALP levels might relate to soft tissue and/or bone growth in the latter (Walker et al., 1990). Body condition was generally better in larger individuals (**Supplementary Table 11**), and ALP was as expected negatively correlated with body condition. Individuals with the lowest body condition, mostly smaller individuals, had lower phosphorus levels. High phosphorus levels can be

observed in dehydrated individuals in which osmolality is high; indeed, low phosphorus levels related to survival in *R.pumilio* at the peak of the dry season, where water balance was hardest to maintain (Schoepf et al., 2017). However, blood levels of phosphorus also depend on its consumption and hence availability in the food (Berndt & Kumar, 2009). Phosphorus reserve depletion in smaller-sized/younger adults might therefore be consistent with the role of phosphorus intake as a promoter of bone and soft tissue growth (Shapiro & Heaney, 2003). Furthermore, higher needs for phosphorus in smaller adults might be hindered by reduced access to food. Indeed, foraging activity tends to vary between individuals according to size and breeding status, possibly resulting in larger breeding individuals gaining access to higher quality food (Ebensperger & Hurtado, 2005).

Seasonal variation in physiology

Despite our study year being relatively wet, milder dry conditions impacted kidney and liver blood marker concentrations in *R.bechuanae* and *R.d.dilectus*. These changes involved 6 out of 12 tested physiological markers, and included markers expected to vary in periods of dietary restriction. Contrary to our prediction, we did not find a significant interactive effect between session and species, suggesting that the semi-arid populations of the two species were similarly affected by seasonal dry conditions. However, a follow up study during an El Niño year may be necessary before one can conclude.

Blood protein levels are generally good indicators of physiological condition (Tothova et al., 2016). They comprise two distinct and major components: albumin (ALB) and globulins (GLOB). In our study, ALB was significantly lower at the end than at the start of the dry season. ALB is synthesized in the liver and involved in the transport of

439 bilirubin and several hormones. Blood ALB levels are less prevalent in malnourished
440 individuals (Walker et al., 1990), supporting the hypothesis of reduction of quality
441 and/or availability of food at the end of the dry season. The Nubian ibex *Capra nubiana*,
442 a desert mammal, also displays low ALB during the dry season (Al-Eissa et al., 2012),
443 which could reflect poor nutritional condition, kidney dysfunction leading to poor water
444 balance and high osmolality, or liver dysfunction causing low ALB synthesis. We found
445 that GLOB was significantly higher at the end than at the start of the dry season. GLOB
446 is a family of molecules including enzymes, immunity proteins and blood proteins
447 carriers (including transferrin). An increase in GLOB levels usually indicates elevation
448 of immunoglobulins and hence could suggest heightened immune activity. Greater
449 antibody production occurs in response to a higher prevalence of respiratory infections
450 during the dry season, e.g. in the Nubian goat (Abdelatif et al., 2009), and/or reduced
451 protein nutritional status in young birds (Lochmiller et al., 1993). Higher GLOB levels,
452 indicating a strained immune system, were also reported for *R.pumilio* individuals that
453 did not survive the dry season (Schoepf et al., 2017). An increase in blood GLOB
454 concentrations may also reflect high levels of transferrin (Walker et al., 1990), a major
455 GLOB that may accumulate in the blood in cases of severe iron deficiency (Kasvosve
456 & Delanghe, 2002). Hence, elevated blood GLOB levels at the end of the dry season
457 may indicate either or both depletion in iron resource (transferrin) and higher
458 vulnerability to infection (immunoglobulin), both resulting from or being aggravated by
459 reduced access to protein. Lower levels of alkaline phosphatase (ALP), a liver enzyme,
460 at the end of the dry season in *Rhabdomys*, is also evidence of malnutrition and
461 deficiency in essential nutrients (proteins, magnesium, zinc) (Yousef et al., 2002; Saraç
462 & Saygili, 2007; Ray et al., 2017). Alanine aminotransferase (ALT), another marker of
463 liver function, also occurred at significantly lower concentrations at the end compared

464 to the start of the dry season, indicating a reduction in liver activity (Walker et al., 1990).
465 During malnutrition, ALT levels could be low (Le Couteur et al., 2010). Indeed, as
466 environmental resources become scarce, essential nutrients, such as pyridoxine (or
467 vitamin B6), that constitute some ALT coenzymes, may be less available, leading to a
468 reduction of ALT blood levels (Vespasiani-Gentilucci et al., 2018).
469
470 Acute starvation typically causes bilirubin (TBIL) to accumulate in the blood, as the
471 enzymatic process for conversion of heme into TBIL is stimulated (Thaler et al., 1972).
472 Yet, TBIL was lower at the end compared to the onset of the dry season, rejecting the
473 starvation hypothesis.
474 Lower TBIL concentrations at the end of the dry season in *Rhabdomys* could stem
475 from low hemoglobin counts because the body produces fewer red blood cells than
476 usual; low hemoglobin can be induced by several factors, among which dietary factors
477 such as iron deficiencies (Clark, 2008). Iron deficient anemia is also supported by
478 higher GLOB concentrations observed at the end of the dry season. Infection can
479 likewise be a cause of both decreased TBIL levels (Zhao et al., 2019) and higher GLOB
480 concentrations at the end of the dry season. Finally, malnutrition and/or lack of
481 potassium intake are among the main potential causes of the reduction of its levels in
482 the blood (Their, 1986), which could explain why they were the lowest at the end of the
483 dry season, when the available resources were lowest, in our study.
484
485 Overall, our results indicate that seasonal variation of physiological markers could
486 reflect reduced primary productivity throughout the dry season, which is coherent with
487 our observation of habitat degradation (lower NDVI) at the end of the dry season. Blood
488 concentrations of various markers seem to indicate difficulties in maintaining nutritional

489 functions and the necessity of a higher immune output, caused or exacerbated by food
490 restriction. However, since glucose levels were maintained during the dry season,
491 shifts in six physiological markers related to nutrition and immunity during the milder
492 than usual dry season suggest a reduction of liver function due to malnutrition, rather
493 than starvation.

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

500 We did not observe seasonal variation of sodium or blood urea nitrogen, blood markers
501 of kidney function or osmoregulation. Even if such effects may have been mitigated by
502 the relatively wet conditions, reduced environmental moisture at the end of the dry
503 season could have caused an overall increase in blood osmolality, increasing blood
504 concentrations of most biomarkers. In contrast, most markers exhibited a significant
505 reduction in blood concentrations. This tends to indicate that water availability does not
506 worsen as strongly as food availability for striped mice throughout the dry season in
507 this semi-arid environment.

508 The two species responded similarly to increased dryness, while maintaining stable
509 body conditions and levels of energy (glucose), a proxy of fat reserves. *R.pumilio*
510 individuals tended to reduce their energy expenditure and physical activity during
511 periods of limited food availability (Rimbach, Blanc et al., 2018; Rimbach, Jäger et al.,
512 2018). The semi-arid populations of *R.bechuanae* and *R.d.dilectus* could display a

513 similar behavioral strategy, which could explain their relatively stable levels of glucose
514 during the dry season.

515 ***Interspecific differences***

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

522 Among the markers exhibiting a significant difference between *R.bechuanae* and
523 *R.d.dilectus*, all but amylase showed higher blood concentrations in *R.d.dilectus*.
524 Amylase is positively correlated with digestive activity and reflects differences in
525 feeding status or diet composition between the species (Hidalgo et al., 1999). Given
526 the role of alpha-amylase in digestion, lower blood amylase levels in *R.d.dilectus* than
527 *R.bechuanae* may indicate lower digestive activity due to reduced access to food, or
528 lower starch content in the diet. Pajic et al. (2019) found a correlation between dietary
529 starch content and the number of amylase gene copies in several mammalian
530 genomes, even in different habitats and with different diets. Since amylase activity and
531 amylase gene expression are directly correlated with amylase gene copy number
532 (Arendt et al., 2014), further studies could elucidate the potential genetic basis of the
533 different levels of amylase in our two study species.

534 Sodium (NA) and potassium (K) concentrations, which depend on both dietary and
535 water intake, were higher in *R.d.dilectus* than in *R.bechuanae*. These differences,

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

551 We found relatively little evidence that the two species suffer differentially from lack of
552 food in their semi-arid environment during a La Niña phenomenon. Overall, energy
553 levels, as indicated by blood glucose, and body condition, did not vary between the
554 species. Instead, it seems that the overall differences between the two species can be
555 accounted for by lower blood concentrations in *R.bechuanae*, and on average higher
556 blood concentrations in key metabolites in *R.d.dilectus,* possibly due to overall poorer
557 water regulation. High average blood amylase levels in *R.bechuanae* in the dry season
558 might indicate an interspecific difference in diet composition or food intake, but not of
559 protein-energy malnutrition. Little is known about the *R.bechuanae* and *R.d.dilectus*
560 diets, although the genus was characterised as a generalist feeding on seeds and

561 other plant material, and insects (Curtis & Perrin, 1979). Some studies show variation
562 in diet composition within *Rhabdomys* genus associated with variation in local
563 resources (Taylor & Green, 1976). The two species could have adjusted their diet to
564 the respective local availabilities of different food resources within their home ranges.
565 For example, in the semi-arid zone, arthropod biomass and diversity is affected by
566 seasonal patterns in rainfall. Dalerum et al. (2017) found lower arthropod trapping
567 success and diversity during the dry season, in shrub habitats compared to any other
568 biomes, in Benfontein Game Reserve, one of our study sites. Thus, compared to
569 seeds, the reliability of arthropods as a food source during dry conditions could be
570 lower in shrublands, inhabited by *R.bechuanae* and by both species in parapatry, than
571 in grasslands inhabited by *R.d.dilectus* in allopatry. Variation in seed composition could
572 also explain this interspecific difference in amylase concentrations. Indeed, granivores
573 prefer seeds that have higher protein content and lower secondary metabolites but
574 may be forced to take a wider range of seeds in dry areas than in mesic ones (Wilmer
575 et al., 2009). If the hypothesis of a relatively ancient adaptation of *R.bechuanae* to dry
576 habitats is true, it could be expected that it would also have adjusted its diet to the
577 specific conditions encountered in dry habitats. Within this ecosystem, *R.bechuanae*
578 could forage more efficiently compared with *R.d.dilectus*, which evolved in a more
579 mesic environment; its diet could also be richer in starch (such as containing more
580 seeds/grains than leaves or fibre). Higher starch intake could help maintaining water
581 balance; starch is the best substrate for metabolic water production in a dry
582 environment (Adolph, 1964). This is consistent with the other interspecific differences
583 revealed in this study, as diet can also significantly affect osmoregulation (Sabat et al.,
584 2009).

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

592 Conclusions

593

594 We found that two closely related species with different ecological characteristics
595 displayed similar negative shifts in blood concentrations of markers of nutrition and
596 liver function in spite of a relatively mild seasonal drying of their habitats. We hence
597 expect the physiological impact of harsher dry conditions, to affect the individuals'
598 fitness even more dramatically. The fact that the habitat of *R.bechuanae* studied
599 populations was the driest at both time points, combined with our findings concerning
600 their overall better water balance and nutrition than *R.d.dilectus*, point towards
601 *R.bechuanae* coping better in the semi-arid environment. If, as projected, the
602 frequency and length of drought episodes increase in this region, we predict that
603 *R.bechuanae* will expand its range at the expense of *R.d.dilectus*. Though adaptation
604 *per se* cannot be inferred from our study, this comparative study considering
605 populations of the two species in the same biogeographic zone adds to the growing
606 evidence that these cryptic species of striped mice have diverged phenotypically.
607 These findings support the hypothesis of adaptation to distinct ecological conditions
608 associated with their divergence. Further studies might seek to identify signatures of
609 selection in genes linked to crucial physiological functions in dry conditions to

610 understand the underlying mechanisms of divergence. Moreover, investigation of
611 seasonal variation in gene expression in the liver and kidney should help identify the
612 physiological pathways, key to survival, affected by seasonal variation in dry
613 conditions.
614

Main tables & figures

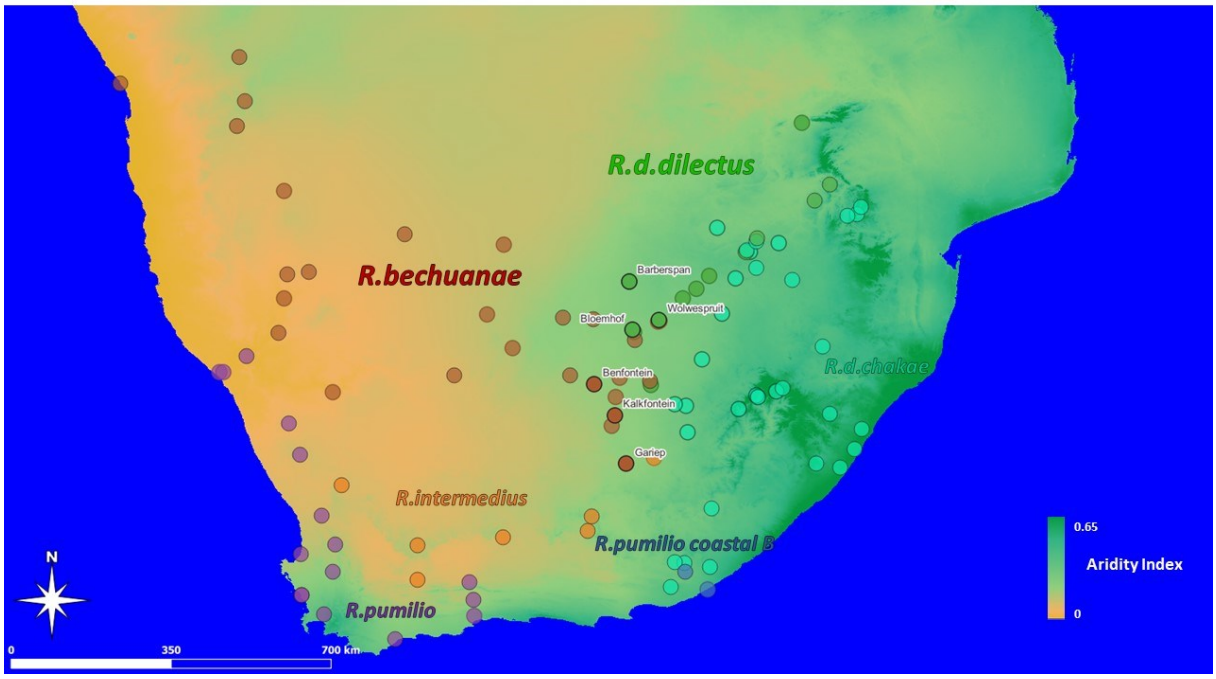


Figure 1: Map of known occurrences of 6 main clades of Rhabdomys (translucent dots) and locations sampled in this study (full colour dots) in southern Africa

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).

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

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

625 ***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	-	+

626

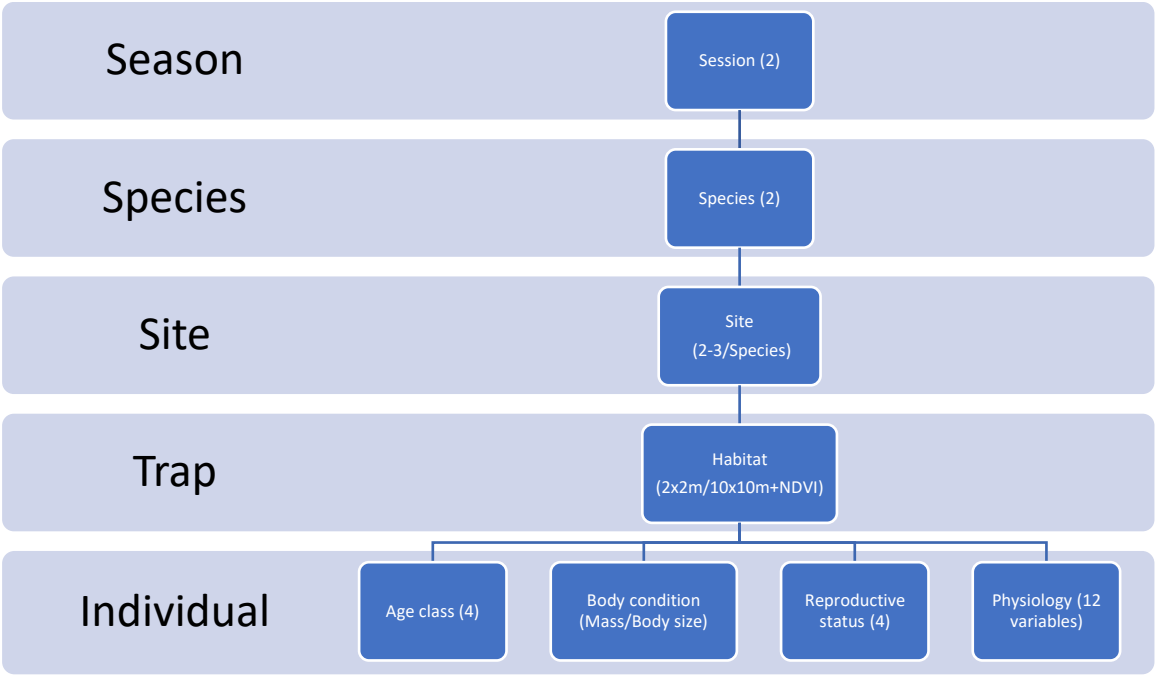
627 *Adaptation to harsher arid conditions could have enabled *R.bechuanae* to withstand

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

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

630 developed local adaptation.

631



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

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

635 of categories included in the factor.

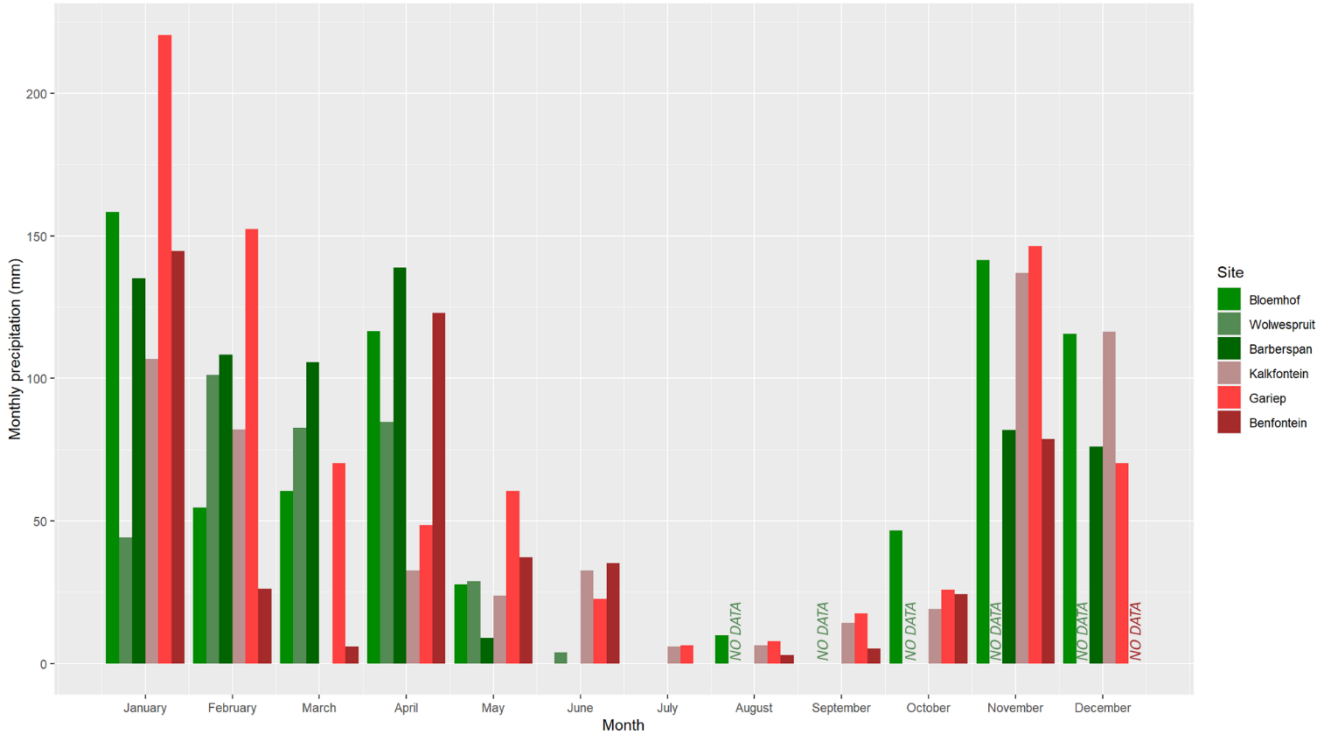
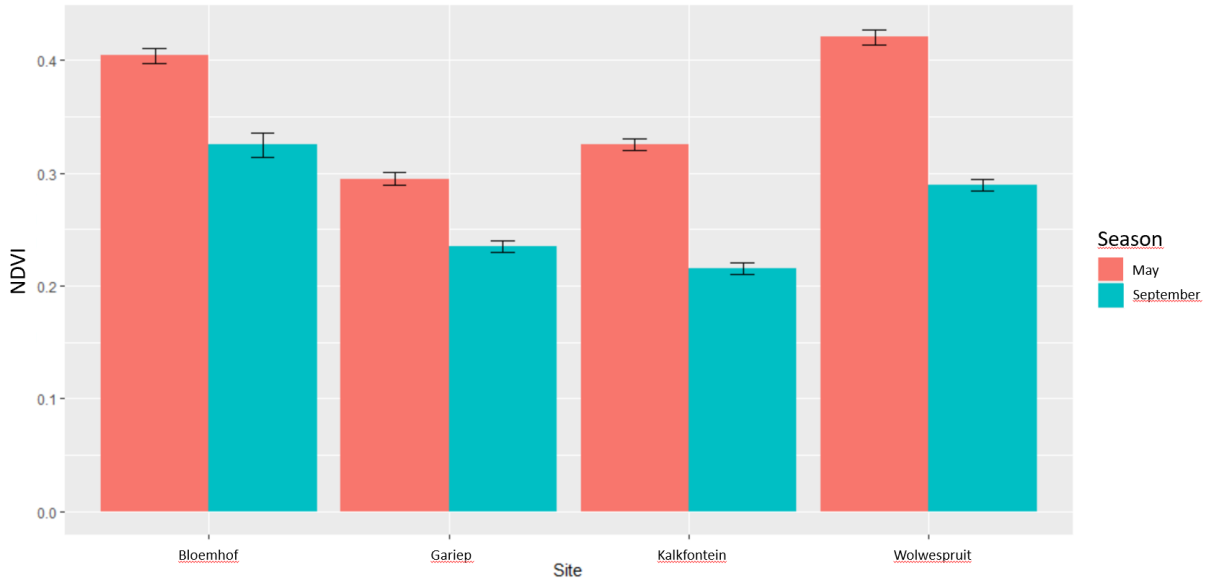


Figure 3: Total monthly precipitation for all sampled sites as recorded in 2022.

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

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



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

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

652

653 ***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		

654 ***Table 4:*** **Results of the Analysis of Variance (response variable : body**
655 **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		

656

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

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

659 ***(site= random factor)***

660

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		

661

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

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

664 ***fitting models).***

665

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$^{\cdot}$, **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) $9.838*10^{-9}$

666

667 *Note: The full models, before backwards elimination, included session, species, body condition,*

668 *reproductive status and reproductive status*species as variables, and site as a partially-crossed*

669 *random factor nested within species.*

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

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

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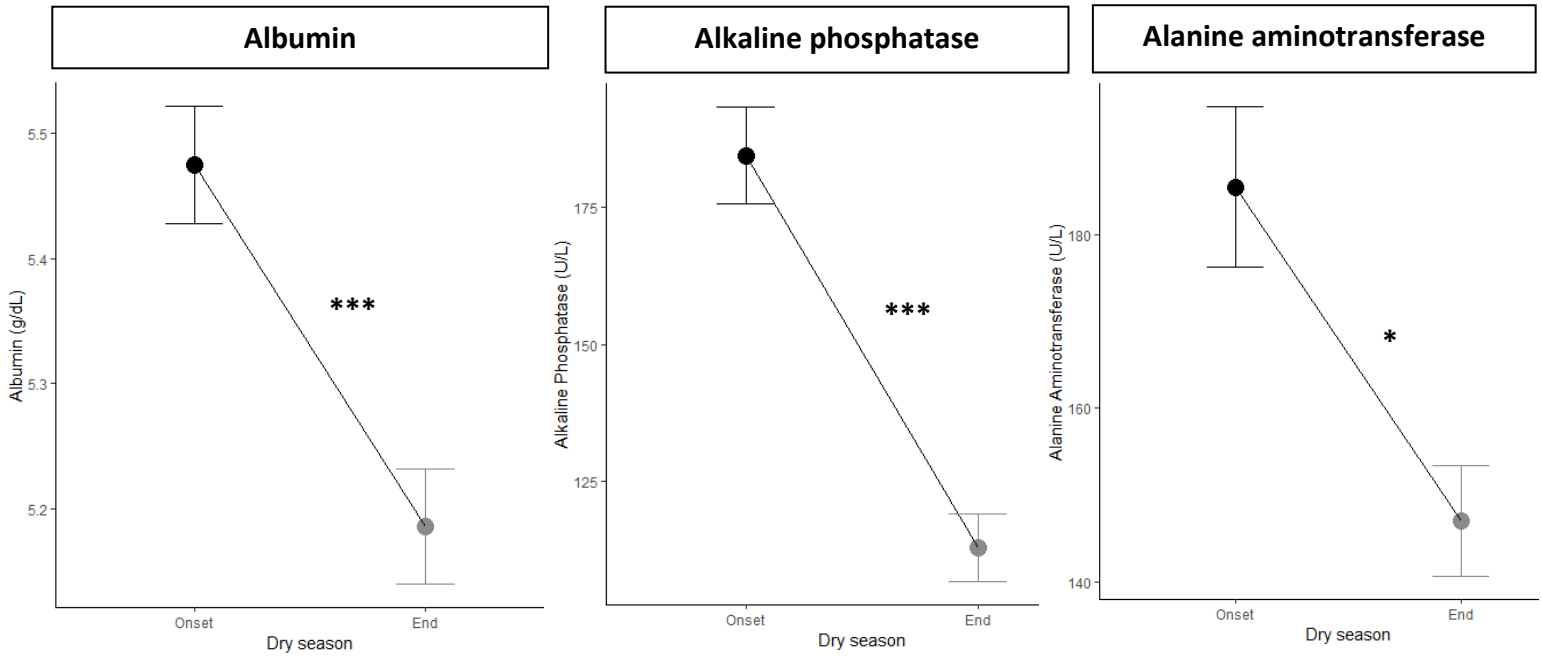

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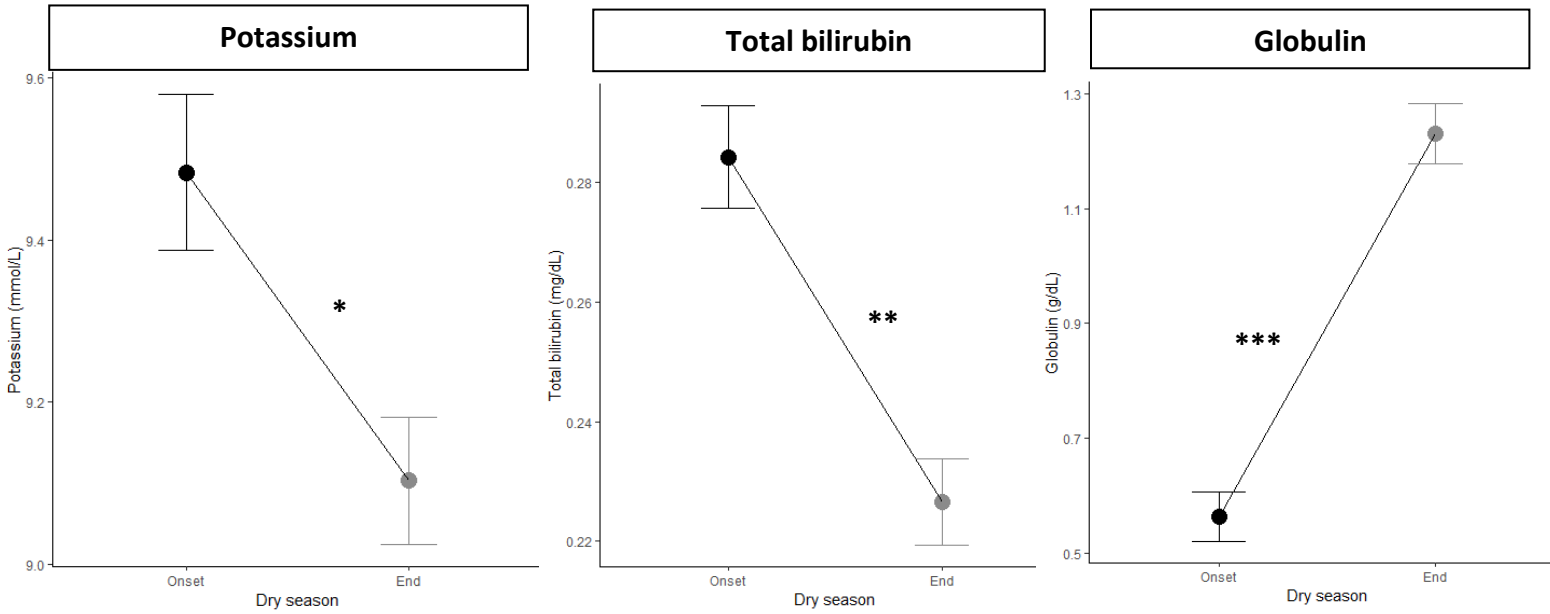

675 ***<u>Figure 5: Significant shifts in blood physiological marker</u>***

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

677

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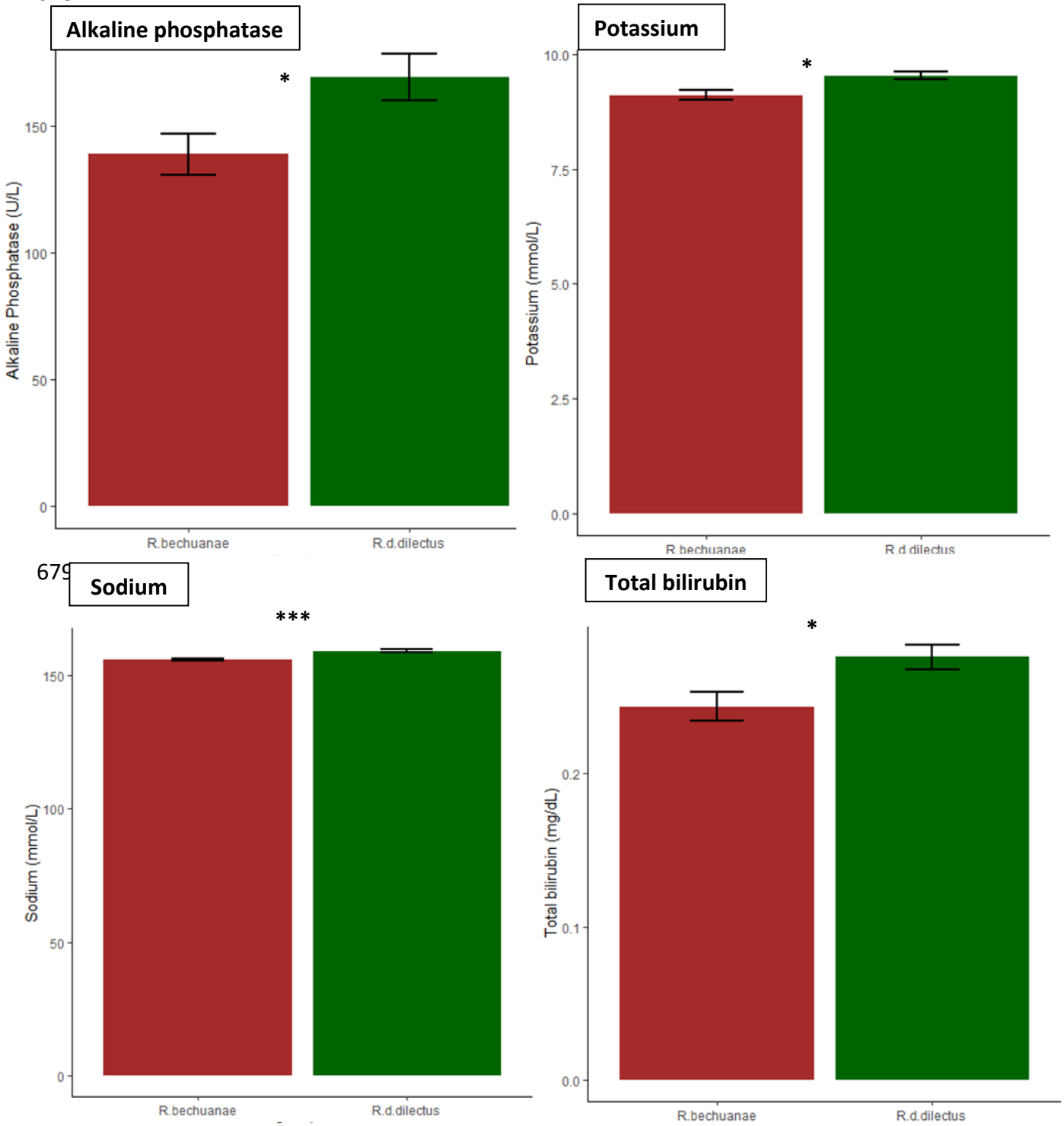

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680 ***Figure 6: Significant interspecific differences involving four blood***

681 ***physiological marker concentrations (mean ± SE)***

682

683

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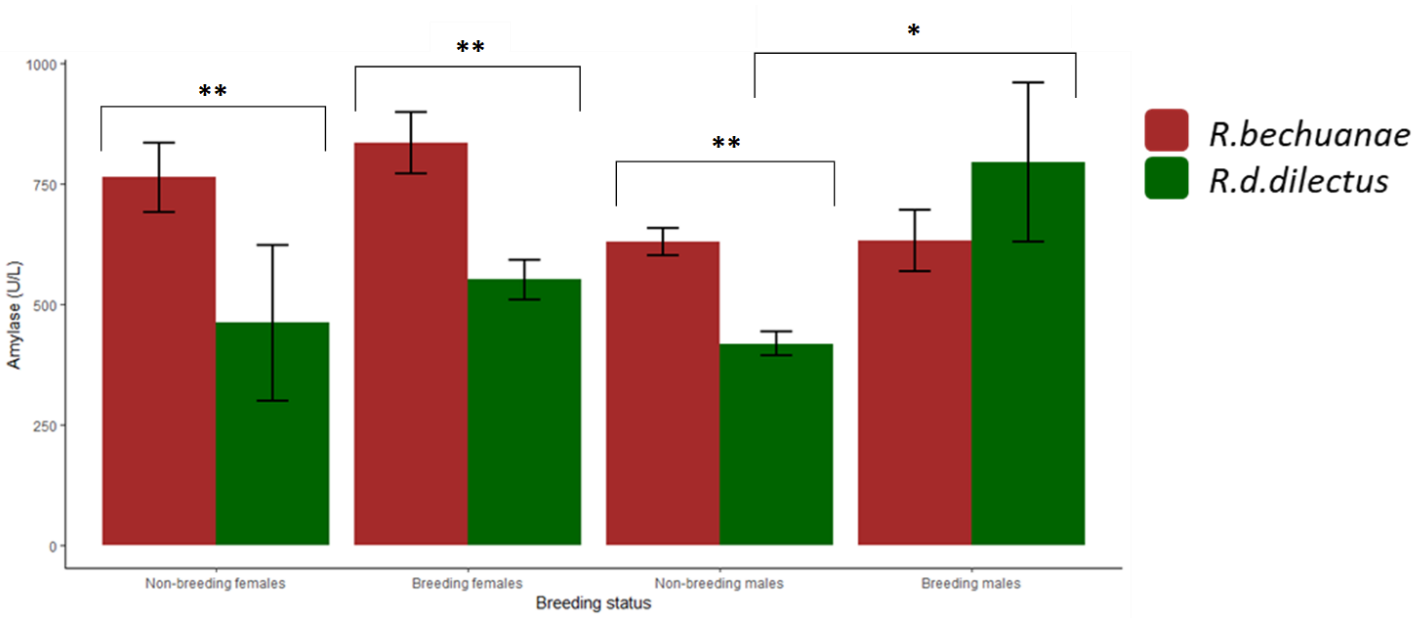

685 ***Figure 7: Variation of blood amylase concentration between species and***

686 ***breeding status (mean ± SE)***

687

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